21

Phenols and phenolic glycosides

Phenols probably constitute the largest group of plant secondary metabolites. Widespread in Nature, and to be found in most classes of natural compounds having aromatic moieties, they range from simple structures with one aromatic ring to highly complex polymeric substances such as tannins and lignins. Phenols are important constituents of some medicinal plants and in the food industry they are utilized as colouring agents, flavourings, aromatizers and antioxidants. This chapter mainly deals with those phenolic classes of pharmaceutical interest, namely: (1) simple phenolic compounds, (2) tannins, (3) coumarins and their glycosides, (4) anthraquinones and their glycosides, (5) naphthoquinones, (6) flavone and related flavonoid glycosides, (7) anthocyanidins and anthocyanins, (8) lignans and lignin. The biosynthetic origin of some of these compounds involving the shikimic acid pathway is shown in Fig. 21.2. Phenols may also have aromatic rings derived by acetate condensation (Fig. 18.9.).

SIMPLE PHENOLIC COMPOUNDS

Catechol (*o*-dihydroxybenzene) occurs free in kola seeds and in the leaves of *Gaultheria* spp. and its derivatives are the urushiol phenols of the poison oak and poison ivy (q.v.). Derivatives of resorcinol (*m*-dihydroxybenzene) constitute the narcotic principles of cannabis and the glucoside arbutin involves quinol (hydroquinone, *p*-dihydroxybenzene). The taenicidal constituents of male fern, the bitter principles of hops and the lipophilic components of hypericum (q.v.) are phloroglucinol derivatives.

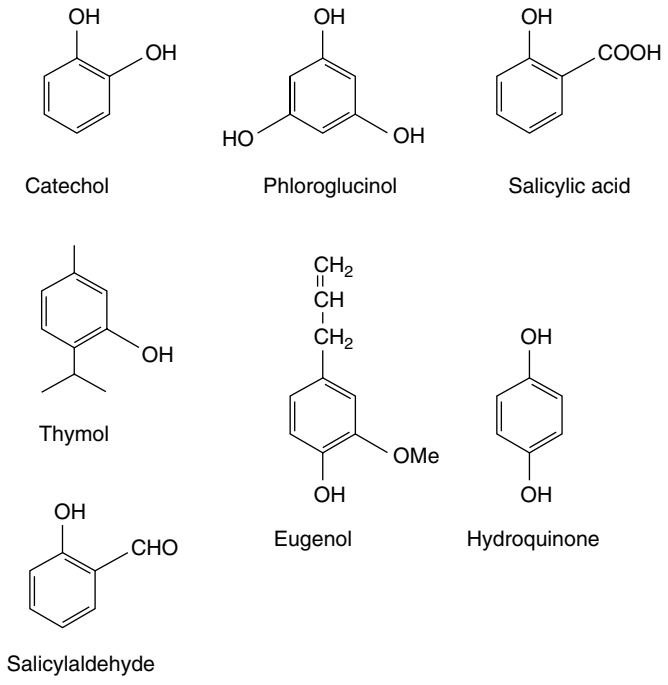


Fig. 21.1
Simple phenolic compounds.

The phenolic compounds in this group often also possess alcoholic, aldehydic and carboxylic acid groups; they include eugenol (a phenolic phenylpropane), vanillin (a phenolic aldehyde) and various phenolic acids, such as salicylic, ferulic and caffeic acids. Glycoside formation is common, and the widely distributed glycoside coniferin and other derivatives of phenolic cinnamic alcohols are precursors of lignin. Some of the best-known simple phenolic glycosides are listed in Table 21.1.

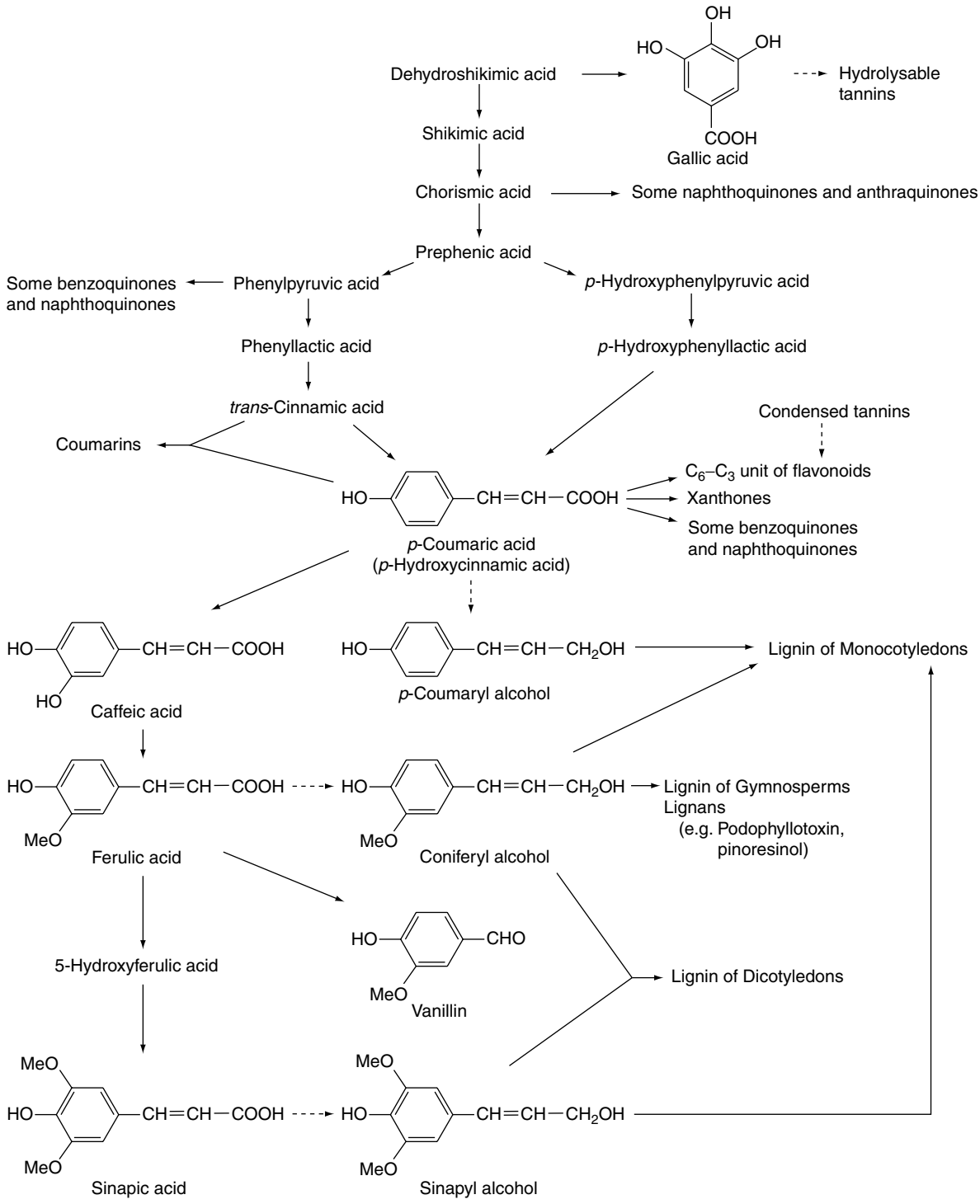


Fig. 21.2
Phenolic compounds originating from shikimic acid (see Fig. 18.8 for details of shikimic acid pathway).

MEADOWSWEET

Meadowsweet *BP*/*EP*, Filipendula *BHP* 1983 consists of the dried flowering tops of *Filipendula ulmaria* (L.) Maxim. [*Spirea ulmaria* L.], family Rosaceae.

This well-known perennial plant is found in wet meadows, marshes, by rivers, etc. throughout most of Europe, temperate Asia and as an escape in the eastern US and Canada. It is up to 120 cm in height with numerous radical longish petioled leaves. Each leaf is composed of up to five pairs of ovate serrated leaflets. Numerous aromatic cream-coloured flowers form irregular cymose panicles, which are particularly dense on the terminal branches of the leafy stems.

The commercial chopped drug occurs as clumps of broken leaflets dark green on the upper surface, paler and tormentose on the lower. Also brown fragmented flowers, unopened flower buds and small, more or less spirally twisted fruits containing brown seeds. Angular,

Table 21.1 Examples of phenolic glycosides.

Name	Examples of sources	Products of hydrolysis
Salicin	*Salix* and *Populus* spp. *Viburnum prunifolium*	Salicyl alcohol, glucose
Populin (benzoyl-salicin)	*Populus tremula*	Salicyl alcohol, benzoic acid, glucose
Arbutin	Ericaceae and Rosaceae	Hydroquinone, glucose
Phloridzin	Rosaceae, including spp. of *Malus*	Phloretin, glucose
Trilobatin	*Malus*, *Spiraea*	Phloretin, glucose
Coniferin	Coniferae	Coniferyl alcohol, glucose
Gaultherin	*Gaultheria*, *Betula* and *Monotropa*	Methyl salicylate, primeverose
Syringin	Particularly in Oleaceae	Methoxyconiferyl alcohol, glucose
Glucovanillin	*Vanilla* spp. and some Gramineae	Vanillin, glucose
Gein	*Geum* spp.	Eugenol, vicianose (glucose + arabinose)
Glucogallin	*Rheum* spp.	Gallic acid, glucose
Hamamelitannin	*Hamamelis virginiana*	Gallic acid (2 mols), hamamelose

greenish-brown longitudinally ridged hollow stems up to 5 mm in diameter constitute a considerable portion of the drug.

Among the complex mixture of structures in the powder the following can be noted: leaves and sepals having lower epidermis with slightly sinuous anticlinal walls, anomocytic stomata and cluster crystals of calcium oxalate up to 40 µm diameter in the mesophyll; papilose epidermis of petals; pollen grains with three pores and a smooth to slightly pitted exine; numerous trichomes, occasionally glandular with a one- to three-celled stalk and multicellular head with brown contents but principally clothing trichomes of various size, often twisted together; vascular tissue of the stem and veins.

Constituents. The *BP*/*EP* requires a minimum concentration of 0.1% for the steam volatile fraction of Meadowsweet; the flowers have recorded higher values. The major component of the oil (up to *ca* 70%) is salicylaldehyde (Fig. 21.1) together with methyl salicylate, benzaldehyde, benzyl alcohol, and smaller amounts of other components such as vanillin. In 1839, Löwingand and Weidmann, working on meadowsweet, were the first to report salicylic acid as a natural product. Other constituents of the drug are the phenolic glycosides gaultherin (Table 21.1) and spiraein (salicyl alcohol + primerose), various flavonoids, e.g. hyperoside (Fig. 21.18), tannins and mucilage.

The pharmacopoeial TLC test for identity indicates the required presence of methyl salicylate and salicylaldehyde in the test sample. The permitted maximum for stems with a diameter greater than 5 mm is 5% and for foreign matter, 3%.

Action and uses. The *BP*/*EP* cites meadowsweet as a diuretic; traditionally it has also been used for its anti-inflammatory, astringent and stomachic properties.

Oil of wintergreen

Natural oil of wintergreen was formerly obtained from the leaves of *Gaultheria procumbens* (Ericaceae), but is now distilled from the bark of *Betula lenta* (Betulaceae). Gaultheria oil of the *Indian Pharmacopoeia* is obtained from the fresh plant of *Gaultheria fragrantissima* and contains not less than 98% of esters calculated as methyl salicylate.

WILLOW BARK

Various species of *Salix* which include *S. purpurea* L., (purple willow) *S. daphnoides* Vill. and *S. fragilis* L. (crack willow) are sources of the official drug (*BP*/*EP*, *BHP*, *ESCOP*, *Complete German Commission E*). There are about 300 species of *Salix* showing much hybridization and unusual forms. They are distributed in all parts of the North Temperate Zone, the Arctic Zone and the South Temperate Zone. Identification can present difficulties. Species range from tall trees to tiny shrubs. The commercial drug is obtained principally from S.E. Europe but also from Britain and other European countries.

The commercial drug occurs as thin, channelled pieces of varying length, about 1.5 cm wide and 1.5 mm thick. It easily fractures longitudinally and, transversely, shows an inner inconspicuous fibrous fracture. The outer surface is brown, grey or greenish, glossy and smooth or dull and rugged; the inner surface is lightish brown and finely longitudinally striated. The powder is characterized by cork cells, parenchymatous cells containing cluster crystals of calcium oxalate and lignified fibre groups with crystal sheaths of calcium oxalate.

Willow bark is a source of salicin (Table 21.1), a phenolic glycoside now seldom used but generally regarded as the natural forerunner of aspirin. The composition of the glycoside mixture is variable in the bark depending on species, age of bark and time of collection. The latter is usually made in spring when the bark is easily removed from the branches. Other phenolic glycosides are salicortin (an ester of salicin), acetylated salicin (fragilin) and salicortin. Salicin is easy to prepare (see 15th edition of this book) and is a suitable compound with which to introduce students to this class of glycoside.

Flavonoids of the bark (to over 4%) include the 5- and 7-glucosides of naringenin, isoquercitrin and chalcone (see Fig. 21.18). Tannins are of the condensed types (q.v.).

The *BP* requires the dried drug to contain a minimum of 1.5% total salicylic acid derivatives, calculated as salicin. Liquid chromatography with spectrophotometric determination at 270 nm is used for the assay.

Willow is employed as an anti-inflammatory in the treatment of rheumatism, arthritis and muscular pains.

Black haw bark

The root bark of *Viburnum prunifolium* (Caprifoliaceae) was formerly official in most pharmacopoeias, but its use for dysmenorrhoea, threatened abortion and asthma has gradually decreased. It contains about 0.2% of salicin, volatile oil and isovaleric acid, tannin and resin.

HOPS

Hops are the dried strobiles of *Humulus lupulus* L. (Cannabinaceae). Only the pistillate plants are cultivated, large quantities being produced in England (particularly Kent), Germany, Belgium, France, Russia and

California. The strobiles are collected, dried in kilns and pressed into bales known as 'pockets'. They are sometimes exposed to the fumes of burning sulphur, which modifies the sulphur components already in the hops but which is said to stabilize the aroma and colour.

Hops are included in the *EP*, *BP*, *BHP* and in monographs of the *British Herbal Compendium*, ESCOP and German Commission E.

The hop strobiole consists of external and internal sessile bracts which overlap one another and enclose the ovary. Together they form a petiolate greenish-yellow inflorescence 2–5 cm in length. The odour is characteristically aromatic.

On the fruits and bases of the bracts are numerous shining glands. These, when separated, constitute the drug lupulin. The commercial product is generally very impure, owing to the fact that it is obtained by sieving the sweepings of the hop room floors. It occurs as a granular, reddish-brown powder with a characteristic odour and bitter aromatic taste.

The bracts and stipules of the hop contain tannin but the odour and taste of the drug are mainly due to the very complex secretion contained in the lupulin glands. On distillation the fruits yield 0.30–1.0% of an oil composed of well over 100 components and containing terpenes, sesquiterpenes including humulene (Fig. 21.3) and compounds such as 2-methyl-but-3-ene-2-ol and 3-methylbutanoic acid. The two latter, and related substances, increase significantly during processing of the fresh hops. The bitterness is due to crystalline phloroglucinol derivatives known as α-acids (e.g. humulone), β-acids (e.g. lupulone) and also about 10% of resins. 2,3,4-Trithiapentane, *S*-methylthio-2-methylbutanoate, *S*-methylthio-4-methyl-pentanoate and 4,5-epithiocaryophyllene have been isolated from the volatile oil of unsulphurated hops.

There has been considerable recent interest in the wide-ranging biological activities of the constituents of hops. Thus prenylated compounds such as xanthohumol and the recently isolated acylphloroglucinol-glucopyranosides have been variously reported to have cytotoxic effects on human cancer cell lines together with antiproliferative, antioxidant and oestrogenic properties. For details, see L. R. Chadwick *et al.*, *J. Nat. Prod.*, 2004, **67**, 2024; G. Bohr *et al.*, *J. Nat. Prod.* 2005, **68**, 1545. The mildly sedative properties of hops are ascribed, in part, to 2-methyl-3-buten-2-ol; their principal use is as an aromatic bitter in the preparation of beer.

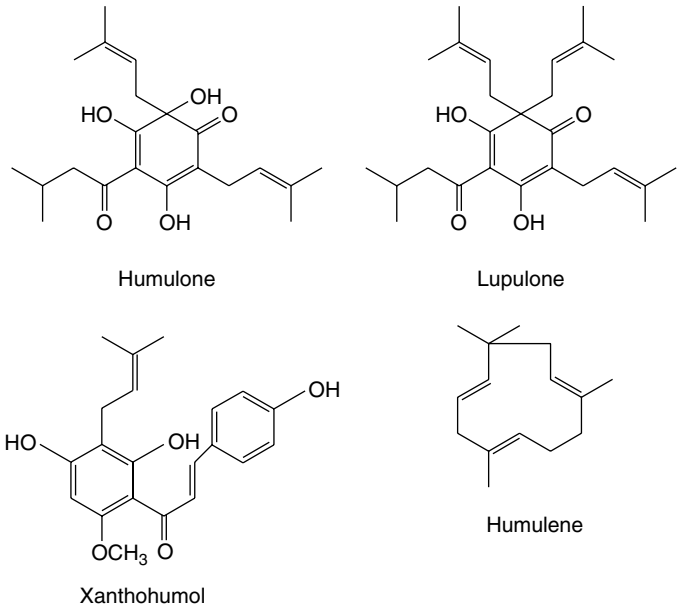


Fig. 21.3
Constituents of hops.

Further reading

Zanoli P, Zavatti M 2008 Pharmacognostic and pharmacological profile of *Humulus lupulus* L. Journal of Ethnopharmacology 116: 383–396

Male fern. Male fern (*Filix Mas*) consists of the rhizome, frond bases and apical bud of *Dryopteris filix-mas* agg. (Polypodiaceae). The taxonomy of the genus is complicated and the aggregate is composed of a complex of three related species—*D. filix-mas* (L.) Schott. *s. str., D. borrei* Newm. and *D. abbreviata* (Lam and D.-C.) Newm. Other ferns may also be involved in extracts produced globally.

Male fern samples that have not deteriorated in activity due to long storage, etc. should have an internal green colour. The active constituents are an interesting range of phloroglucinol derivatives, which have been thoroughly investigated.

Extracts of male fern were traditionally employed as taenicides, particularly for tape worms, but safer drugs are now available and used in preference.

A full account involving history, characters, constituents and allied drugs is given in the previous edition of this book pp. 214–217.

Kamala

Kamala consists of the trichomes and glands separated from the fruits of *Mallotus philippinensis* (Euphorbiaceae), a tree found in India, Pakistan and the East Indies. It occurs as a dull reddish-brown powder without odour or taste. Under the microscope it is seen to consist of very characteristic globular glands containing red resin, and radiating groups of unicellular curved trichomes. It contains the anthelminthic phloroglucinol derivatives rottlerin and isorottlerin, resins and wax. It is used in India for the treatment of tapeworm infestation; also for treating poultry.

Tar (*Pix Liquida*)

Wood tar is known in commerce as Stockholm tar. It is prepared by the destructive distillation of various trees of the family Pinaceae. In addition to the tar, an aqueous distillate is obtained from which acetic acid, methyl alcohol and acetone are prepared. A residue of wood charcoal remains in the retorts. Wood tar is a blackish semiliquid with a characteristic odour and taste.

The constituents include the following phenols and phenolic ethers: phenol, C_6H_5OH; cresols, $C_6H_4(CH_3)OH$; methyl cresols; catechol or pyrocatechin, $C_6H_4(OH)_2$; guaiacol (methyl catechol) and its homologues. Also the hydrocarbons benzene, toluene (methylbenzene), xylenes (dimethylbenzenes), mesitylene and pseudocumene (trimethylbenzenes), styrene (phenylethylene), naphthalene ($C_{10}H_8$), retene (*m*-methylisopropylphenanthrene), chrysene ($C_{18}H_{12}$) and paraffins.

Pine tar is characterized by the large amount of guaiacol and its homologues which are present. Other tars, such as those of the birch and beech, show considerable differences in composition. Wood tar is acid in reaction, whereas coal tar, which is also official, is alkaline and in light petroleum gives a blue fluorescence. Creosote is obtained from wood tar by distillation. Tar is mainly used externally, in the form of ointment or tar parogen, as a stimulating antiseptic in certain skin diseases.

Wood tar, when shaken with water, gives an aqueous layer that is acid to litmus (cf. coal tar below) (*BP* test for identity).

COAL TAR

Coal tar is prepared by the destructive distillation of bituminous coal; it is a nearly black viscous liquid and when shaken with water gives

an aqueous alkaline solution. A petroleum spirit extract has a blue fluorescence enhanced by UV light. The upper ash limit for the *BP* product is 2.0%.

Both coal tar and wood tar are used in the treatment of psoriasis.

VANILLA AND VANILLIN

Vanilla (*Vanilla Pods*) consists of the carefully cured fully grown but unripe fruits of *Vanilla fragrans* (Salis.) Ames (*syn. V. planifolia* Andrews) (Orchidaceae) (Mexican or Bourbon vanilla) and of *V. tahitensis* (Tahiti vanilla). The fruits of other species, such as *V. pompona* (West Indian vanilla), are also used but to a much more limited extent.

Vanilla fragrans is grown, in a semi-wild state, in the woods of eastern Mexico, its natural home. Vanilla is cultivated in Réunion (or Bourbon), Mauritius, Seychelles, Madagascar, Java, Ceylon, Tahiti, Guadeloupe, Martinique and Indonesia. China and India are now major producers and due to oversupply prices have fallen dramatically over the past few years.

History. Vanilla was found in Mexico by the Spaniards, where it was used for flavouring chocolate, a use to which it is still put. It found a place in the *London Pharmacopoeia* of 1721.

Cultivation. Vanilla requires a warm and fairly moist climate. Propagation is simple: cuttings 1–3 m long are attached to trees (e.g. *Casuarina equisetifolia*), where they soon strike roots on the bark. The plant is an epiphyte. It flowers at the end of 2 or 3 years and continues to produce fruit for 30–40 years. The flowers are usually pollinated by women and children, a pointed stick being introduced into one flower after another. Clonal propagation of the vanilla plant has been described together with *in vitro* multiplication using axillary bud explants (P. S. George and G. A. Ravishankar, *Plant Cell Rep.*, 1997, **16**, 490).

Collection and curing. The fruits are collected when the upper part of the pod changes in colour from green to yellow. The characteristic colour and odour of the commercial drug are only developed as a result of enzyme action during the curing. The details of the latter process vary somewhat in different countries, but frequently it consists of slow drying in sheds which are kept at carefully regulated temperatures.

Packing and grading. Before grading, any pods showing a tendency to mould are picked out. The remainder are sorted to size and packed in bundles of 50 pods. Traditionally, these were packed in tin cases or boxes holding about 10–12 kg, soldered up and packed in wooden cases. On arrival in London the tins were opened and the pods were examined. UK supplies now arrive via France or Germany, with some from Madagascar. During storage crystals frequently develop on the surface of the pods.

Characters. Vanilla pods are 15–25 cm long, 8–10 mm diameter and somewhat flattened. The surface is longitudinally wrinkled, dark brown to violet-black in colour, and frequently covered with needle-like crystals of vanillin ('frosted'). The fruits are very pliable and have a very characteristic odour and taste.

Constituents. Green vanilla contains glycosides, namely glucovanillin (vanilloside) and glucovanillic alcohol. During the curing these are acted upon by an oxidizing and a hydrolysing enzyme which occur in all parts of the plant. Glucovanillic alcohol yields on hydrolysis glucose and vanillic alcohol; the latter compound is then by oxidation converted into vanillic aldehyde (vanillin). Glucovanillin, as its name implies, yields on hydrolysis glucose and vanillin (Fig. 21.4).

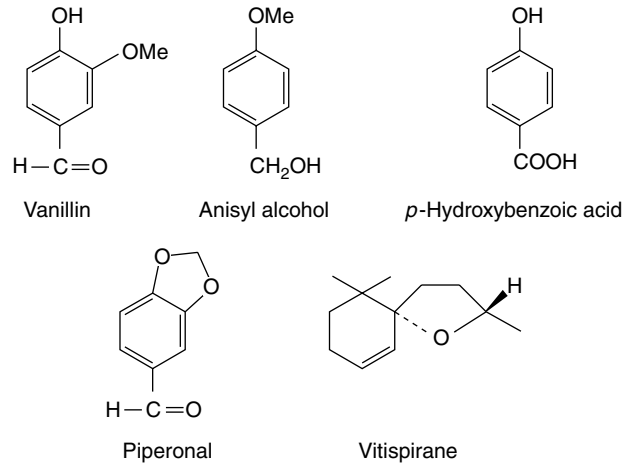


Fig. 21.4
Constituents of vanilla.

The three species given above differ in their relative contents of anisyl alcohol, anisaldehyde (also anisyl ethers and anisic acid esters), piperonal and *p*-hydroxybenzoic acid. These minor components, together with the two diastereoisomeric vitispiranes, add to the flavour of the pods.

Vanillin *BP/EP*. Vanillin *BP* is the aldehyde corresponding to methyl-protocatechuic acid and has been synthesized in a number of ways. Large quantities of it are prepared from eugenol isolated from oil of cloves (q.v.) or from guaiacol (methyl catechol). It can also be produced by microbial oxidation of eugenol. In the plant glucovanillin is biosynthesized *via* ferulic acid (see Fig. 21.2). Synthesis begins when elongation of the fruit ceases, which is about 8 months after pollination; before this, other phenolic glycosides predominate.

Adulteration. Extracts of Mexican origin may be adulterated by coumarin, probably arising from the use of tonka beans (q.v.). A capillary GC assay has been described for such products (see R. J. Marles *et al*., *Economic Bot*., 1987, **41**, 41).

Uses. Vanilla pods are widely used in confectionery and in perfumery. They have been replaced to some extent, but by no means completely, by synthetic vanillin. About 0.07 parts of vanillin are approximately equivalent to 1 part of the bean, but an essence so prepared fails to represent the odour and flavour of the whole pods.

For a review of natural vanillin, covering biosynthesis, biotechnological production, cell and organ culture and metabolic engineering, see N. J. Walton *et al*., *Phytochemistry*, 2003, **63**, 505–515.

BEARBERRY LEAVES

(*Uva ursi*)

Bearberry leaf *EP/BP/BHP* consists of the dried leaves of *Arctostaphylos uva-ursi*, Ericaceae; ESCOP and German Commission E monographs on the drug are also available.

A. uva-ursi is a small evergreen shrub found in central and northern Europe and in North America. The leaves are dark green to brownish-green, 2–3 cm long, obovate or spathulate, gradually narrowing to a very short petiole, apex obtuse or retuse. They are coriaceous in texture and almost glabrous. The upper surface is shiny and marked with sunken veinlets; the lower surface is lighter and marked with a network of dark veinlets. The drug is odourless but has an astringent and somewhat bitter taste.

Microscopical features include: an upper epidermis of polygonal cells with a thick cuticle; lower epidermis with anomocytic stomata and surrounded by 5–11 subsidiary cells; scars of trichome bases, occasional conical trichomes, crystal fibres.

Bearberry contains the glycosides arbutin (Table 21.1) and methylarbutin, about 6–7% of tannin, (+)-catechol, ursone and the flavone derivative quercetin. Some 14 phenolic acid constituents, including gallic and ellagic acids, have been recorded.

The pharmacopoeial drug is required to contain at least 7.0% of hydroquinone derivatives calculated as arbutin. These are assayed by liquid chromatography of an aqueous extract of the leaves with arbutin as a reference and absorbance measurement at 280 nm. The official TLC chromatographic test for identity distinguishes arbutin, gallic acid and hydroquinone. Bearberry is diuretic and astringent and during excretion it exerts an antiseptic action on the urinary tract.

Propolis or bee glue

This is the material with which the honey bee seals cracks and crevices, and varnishes surfaces within the hive. Its composition varies according to geographical source. It is collected by worker bees from the leaf buds and is enriched by wounded plant exudates such as mucilages, gums and resins; bee secretions and enzymes are then mixed in. Like honey, the composition varies according to geographical source.

Propolis has a long history, it being used by the Egyptians in the embalming process (antiputrefactive), by the Greeks and Romans in wound treatment (antiseptic), by the Incas (antipyretic) and by inclusion in the London pharmacopoeias of the 17th century. Today it is used by medical herbalists and has become a popular medicament (S. Castaldo and F. Capasso, *Fitoterapia*, 2002, **73**, S1). It also features in apitherapy—an old tradition that has experienced a recent revival.

Over 160 compounds have been shown to be involved and one analysis gave phenolics (58%), beeswax (24%), flavonoids (6%), terpenes (0.5%), lipids and wax (8%) and bioelements, e.g. Mn, Cu, Zn (0.5%). In temperate regions of Europe the resinous coating of poplar buds (*Populus nigra*, *P. italica*, *P. tremula*) forms a major collection source for the bees and the natural phenolic content of the resin, e.g. esters of caffeic and ferulic acids, vanillin, eugenol, flavonoids, etc., can be used to identify the natural source.

Latterly there have been numerous reports concerning the analysis and biological activity of propolis originating from various regions and especially from Latin American countries. In these areas species of *Araucaria* (Araucariaceae), *Baccharis* (Compositae) and *Clusia* (Guttiferae) have been established as biological sources. In addition to the constituents listed previously, prenylated cinnamic acid and chromane derivatives, diterpenoid acids, lignans and components of the volatile oil have been identified.

Notwithstanding the differences in chemical composition of propolis depending on geographical source, a pronounced antibacterial property is common to all. In temperate regions flavonoid and phenolic esters have been shown to exert bacterial activity. New polyisoprenylated benzophenones have recently been reported as antibacterial agents in propolis of Venezuelan origin (B. Trusheva *et al.*, *Fitoterapia*, 2004, **75**, 683), and similar compounds (propolones) have been found in that of Cuban origin together with garcinelliptone and hyperibone (I. M. Hernández *et al*., *J. Nat. Prod*., 2005, **68**, 931). Neoflavonoids with anti-nitric oxide production activity occur in propolis from Nepal (S. Awale *et al*., *J. Nat. Prod*., 2005, **68**, 858).

Readers requiring further information on this interesting substance can refer to the references on p. 219 in the 15th edition of this book, and to *Fitoterapia*, Supplement 1, 2002, **73**, S1–S64, devoted entirely to propolis; V. Bankova, *J. Ethnopharmacol*., 2005, **100**, 114; Y. Lu *et al*., *Fitoterapia*, 2004, **75**, 267.

CAPSICUM

The *BP*/*EP* drug (*Chillies*; *Red Peppers*) consists of the dried, ripe fruits of *Capsicum annuum var. minimum* (Miller) Heiser, and small-fruited varieties of *C. frutescens* L. (Solanaceae). In commerce the description given applies to various African commercial varieties (principally from Zimbabwe and Malawi) and these are sold in England as chillies, while the larger but less pungent Bombay and Natal fruits are known as capsicums. Very large *Capsicum* fruits, resembling tomatoes in texture and practically non-pungent, are widely grown in southern Europe as vegetables.

History. Capsicums appear to be of American origin and were referred to in 1494 by Chanca, a physician who accompanied Columbus on his second voyage to the West Indies. The plants were introduced into India at a very early date, possibly by the Portuguese. 'Ginnie Pepper' was well known in England in 1597 and was grown by Gerarde.

Macroscopical characters. *African Chillies* are oblong-conical in shape, 12–25 mm long and up to 7 mm wide. The five-toothed calyx and straight pedicel are together about 20–30 mm long. The pericarp is glabrous, shrivelled and orange-red; the Sierra Leone and Zambian chillies usually have a better colour than those from Zanzibar.

Internally the fruits are divided into two cells by a membranous dissepiment to which the seeds were originally attached. The latter, usually about 10–20 in each fruit, are of a flattened reniform shape and are about 3–4 mm long. Like other solanaceous seeds, they have a coiled embryo and oily endosperm. African chillies are very sternutatory and have an intensely pungent taste.

Constituents. In 1876, Thresh extracted the drug with petroleum, treated the extract with aqueous alkali, and by passing carbon dioxide through the alkaline liquid precipitated crystals of an intensely pungent compound, capsaicin. As may be inferred from the method of preparation, capsaicin is of phenolic nature.

The pungent phenolic fraction of capsicum also contains a proportion of 6,7-dihydrocapsaicin. The capsaicin content of fruits varies appreciably in a range up to 1.5% and is much influenced by environmental conditions and age of the fruit. It occurs principally in the dissepiments of the fruits—for example, entire fruit 0.49, pericarp 0.10, dissepiment 1.79, seed 0.07. The pungency of capsicum is not destroyed by treatment with alkalis (distinction from gingerol, which also contains the vanillyl group) but is destroyed by oxidation with potassium dichromate or permanganate. Chillies also contain ascorbic acid (0.1–0.5%), thiamine, red carotenoids such as capsanthin and capsorubin (see 'Carotenoids') and fixed oil (about 4–16%). They yield about 20–25% of alcoholic extract (capsicin) and about 5% (official limit 10.0%) of ash. Hungarian capsicums or 'Paprika' are derived from a mild race of *C. annuum* and are a convenient source of ascorbic acid. According to Bennett and Kirby, the pungent principle of *C. annuum* is composed of capsaicin 69%, dihydrocapsaicin 22%, nordihydrocapsaicin 7%, homocapsaicin (C_{11} acid) 1% and homodihydrocapsaicin 1%. A number of minor components of this class have been recorded.

In a study of the water-soluble constituents of the fruits of three varieties of *C. annuum*, Izumitani *et al.* (*Chem. Pharm. Bull.*, 1990, **38**, 1299) isolated twelve novel acyclic glycosides (geranyllinalool derivatives) named capsianosides A–F (dimeric esters of acyclic diterpene glycosides) and capsianosides I–V (monomeric compounds of acyclic diterpene glycosides). Further capsianosides have now been reported by J.-H. Lee *et al.*, (*Chem. Pharm. Bull.*, 2006, **54**, 1365). T. Ochi *et al.*, (*J. Nat. Prod.*, 2003, **66**, 1094) record a dimeric capsaicin having almost the same antioxidant activity as capsaicin but with no pungent taste (Fig. 21.5).

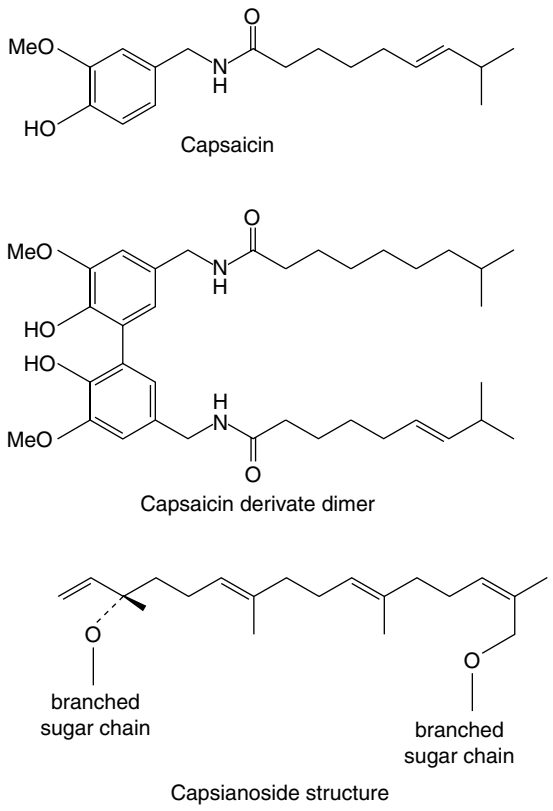


Fig. 21.5
Some constituents of capsicum.

Biogenesis of capsaicin. Work by Leete and Louden on *C. frutescens* and by Bennett and Kirby on *C. annuum* demonstrated that phenylalanine is incorporated into the C_6–C_1 vanillyl unit of capsaicin, the C-3 of phenylalanine giving the methylene group of the vanillylamine residues; the incorporation probably proceeds via cinnamic, *p*-coumaric, caffeic and protocatechuic acids. Tyrosine did not appear to be a probable precursor. Leete's feeding experiments with [U-^{14}C]-valine gave incorporations consistent with the hypothesis that the C_{10} isodecanoic acid is formed from isobutyryl coenzyme A and three acetate units. More recent work showed that the homo derivatives (C_{11} acid) are formed from leucine and isoleucine.

The ontogenetic formation of capsaicinoids in the fruits of *C. frutescens* involves a prior active accumulation of *p*-coumaroyl, caffeoyl and 3,4-dimethoxycinnamoyl glycosides, 3-*O*-rhamnosylquercetin and 7-*O*-glucosylluteolin. When the fruit ceases to increase in length the amount of these compounds falls and capsaicinoid synthesis commences together with that of the glycosides of vanillic acid and *p*-hydroxybenzaldehyde (see N. Sukrasno and M. M. Yeoman, *Phytochemistry*, 1993, **32**, 839).

Cell cultures. The biogenetic potential for capsaicin production is reported (1991) as 10 times greater in immobilized cell cultures (alginate entrapment) than in control suspension cultures.

Tests and standards. The official TLC test for identity establishes the presence of capsaicin and dihydrocapsaicin in the sample. The synthetic equivalent of capsaicin, nonivamide (pelargonyl vanillylamide), a commercial product used as a flavour in the food industry and in medicine as a topical rubifacient, is limited by a liquid chromatographic assay to a maximum of 5% of the total capsaicinoid content. Liquid chromatography is also used to determine the total capsaicinoid content (minimum 0.4%). A number of colorimetric assays can be used for the quantitative determination of capsaicin (see Table 16.5); the *BPC* 1973 utilized ultraviolet absorption at 248 and 296 nm for the ointment and oleoresin. Foreign matter should not exceed a maximum of 2%; fruits of *C. annuum* L. var. *longum* (Sendtn.) (see 'Bombay capsicums' below) should be absent.

Allied drugs. *Japanese Chillies* are probably derived from *C. frutescens* and are about 3–4 cm long. They possess about one-quarter of the pungency of the African Chillies, but are now no longer commercially relevant.

Bombay Capsicums are ascribed to *C. annuum* L. The pericarp is thicker and tougher than in the chillies, and the pedicel is frequently bent. They are much less pungent than African chillies.

Natal Capsicums are larger than the Bombay variety, being up to 8 cm long. They have a very bright red, transparent pericarp. They are much less pungent than chillies.

Uses. Capsicums are used as a condiment under the name of Cayenne pepper. The drug is given internally in atonic dyspepsia and flatulence. It is used externally as a counter-irritant, in the form of ointment, plaster, medicated wool, etc., for the relief of rheumatism, lumbago, etc. Capsaicin creams are available for the relief of pain in osteoarthritis, post-herpetic neuralgia and painful diabetic neuropathy (*Pharm. J.*, 1998, **260**, 692).

Further reading

De AK (ed), Hardman R (series ed) 2003 Medicinal and aromatic plants, Vol 34, Capsicum, the genus *Capsicum*. CRC Press/ Taylor and Francis, Andover, UK. *An overall coverage of the subject, 800 references.*

TANNINS

The term 'tannin' was first applied by Seguin in 1796 to denote substances present in plant extracts which were able to combine with protein of animal hides, prevent their putrefaction and convert them into leather. On this basis a tannin is a substance which is detected qualitatively by a tanning test (the goldbeater's skin test) and is determined quantitatively by its adsorption on standard hide powder. This definition excludes simpler phenolic substances, often present with tannins, such as gallic acid, catechins and chlorogenic acid, although they may under certain conditions give precipitates with gelatin and be partly retained by hide powder. Such substances of relatively low molecular weight are called 'pseudo-tannins'. Most true tannins have molecular weights of from about 1000 to 5000. To be effective for tannage the polyphenol molecule most be neither so large as to be unable to enter the interstices between the collagen fibrils of the animal skin nor so small that it is unable to cross-link between the protein molecules of adjacent fibrils at several points. Many tannins are glycosides. The definition of a tannin as given above is an old, essentially practical one which may be purely fortuitous and, in the light of further research, could prove misleading from the point of view of plant metabolism and plant biochemistry. Indeed, modern authors often treat tannins not as a specific phytochemical group but as examples of polyphenols illustrating particular aspects of gallic acid and flavan-3-ol phytochemistry. The characteristic properties of tannins derive from the accumulation within a moderately sized molecule of a substantial number (1–2 per 100 mol. wt.) of phenolic groups many of which are associated with *o*-dihydroxy and *o*-trihydroxy orientation within a phenyl ring.

The above tannin-protein co-precipitation is important not only in the leather industry but also in relation to the physiological activity of herbal medicines, taste of foodstuffs and beverages, and in the nutritional value of feeds for herbivores. Environmental factors affecting this process have been studied by H. Kawamoto and F. Nakatsubo (*Phytochemistry*, 1997, **46**, 479).

Two main groups of tannins are usually recognized; these are the hydrolysable tannins and the condensed tannins (proanthocyanidins).

Hydrolysable tannins

These may be hydrolysed by acids or enzymes such as tannase. They are formed from several molecules of phenolic acids such as gallic and hexahydroxydiphenic acids which are united by ester linkages to a central glucose molecule. A simple tannin illustrating this point is one derived from a species of sumac (*Rhus*), with a possible structure as shown in Fig. 21.6. Like gallic acid their solutions turn blue with iron salts. They were formerly known as pyrogallol tannins, because on dry distillation gallic acid and similar components are converted into pyrogallol. Two principal types of hydrolysable tannins are gallitannins and ellagitannins which are, respectively, composed of gallic acid and hexahydroxy-diphenic acid units. Ellagic acid (the depside of gallic acid) can arise by lactonization of hexahydroxydiphenic acid during chemical hydrolysis of the tannin; thus, the term ellagitannin is a misnomer.

Ellagitannins found in plants of medicinal interest, and for which structures have been elucidated include geraniin (Herb Robert and American cranesbill) and tellimagrandins 1 and 2 (Oak bark, Pomegranate and Meadowsweet); Fig. 21.6.

Modern methods of analysis have made considerable advances in the study of tannin chemistry of medicinal plants as evidenced by the work of Okuda on oriental drugs. In 1982 agromoniin, the first of a new class of *oligomeric hydrolysable tannins* was isolated from *Agromonia*. These tannins are composed of two, three or four monomeric units. Something less than 20 of these units including geraniin and tellimagrandins 1 and 2 are known to be involved in the production of over 150 compounds.

As an example, many plants of the Onagraceae e.g. *Oenothera* spp. contain in addition to tellimagrandin, the dimer oenothein B and trimer oenothein A; these macrocyclic ellagitannins are also produced in callus cultures of *O. lacinata* and are of interest for their anticancer and polygalacturonase-inhibiting properties (S. Taniguchi *et al.*, *Phytochemistry*, 1998, **48**, 981).

C-glucosidic ellagitannins are common in a number of families including the Myrtaceae, Hamamelidaceae, Punicaceae and Rosaceae

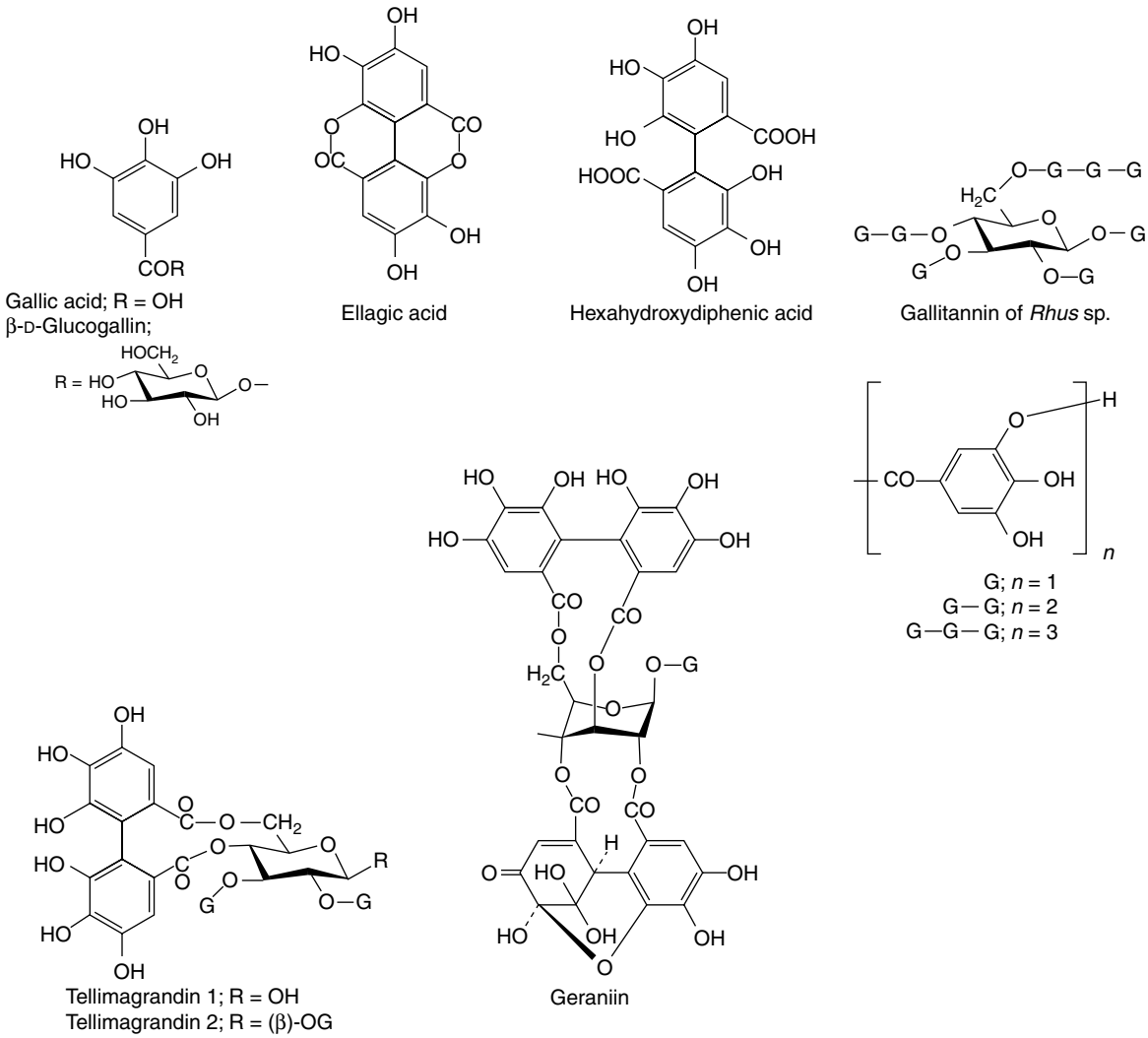


Fig. 21.6
Examples of hydrolysable tannins and their component acids.

and several have also been recorded as moieties of more than 10 oligomeric ellagitannins.

For an article on the classification of oligomeric hydrolysable tannins and the specificity of their occurrence in plants see Okuda *et al.*, *Phytochemistry*, 1993, **32**, 507.

In a series of enzymatic studies Gross and colleagues indicated the central position of β-D-glucogallin in the early stages of tannin synthesis in *Quercus robur* leaves. This compound appears to act as both donor and acceptor of the galloyl group in the enzymatic formation of 1,6-digalloyl-D-glucose; the responsible enzyme is β-glucogallin: β-glucogallin 6-*O*-galloyl-transferase.

The presumed immediate precursor of the two subclasses of hydrolysable tannins (gallotannins and ellagitannins) is 1,2,3,4,6-pentagalloylglucose, and in a continuation of their enzyme studies the above group have purified (×500) the enzyme responsible for the conversion of the precursor to the gallotannin 3-*O*-digalloyl-1,2,4,6-tetra-*O*-galloyl-β-D-glucose. The source of the enzyme was *Rhus typhina* (staghorn sumac) and its designation is β-glucogallin: 1,2,4,6-pentagalloyl-β-D-glucose galloyl-transferase (R. Niemetz and G. G. Gross, *Phytochemistry*, 1998, **49**, 327).

Examples of drugs containing hydrolysable tannins are:

Gallitannins: rhubarb, cloves, red rose petals, bearberry leaves, Chinese galls, Turkish galls, hamamelis, chestnut and maple.

Ellagitannins: pomegranate rind, pomegranate bark, myrobalans, eucalyptus leaves, kousso, some Australian kinos, chestnut (*Castanea* spp.) and oak bark.

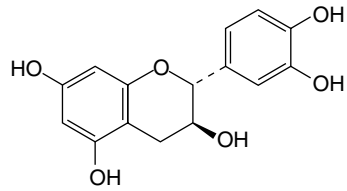
Condensed tannins (proanthocyanidins)

Unlike hydrolysable tannins, these are not readily hydrolysed to simpler molecules and they do not contain a sugar moiety. They are related to the flavonoid pigments and have polymeric flavan-3-ol structures. Catechins, which also occur with the tannins and flavan-3,4-diols (leucoanthocyanidins) are intermediates in the biosynthesis of the polymeric molecules. Stereochemical variations add to the variety of possible structures. Monomeric, dimeric and trimeric forms are illustrated in Fig. 21.7. Work by Japanese phytochemists has exploited modern techniques for separating and determining the structures of these oligomers and polymers including those of cassia bark, *Cassia fistula*, cinchona, *Quercus* and rhubarb.

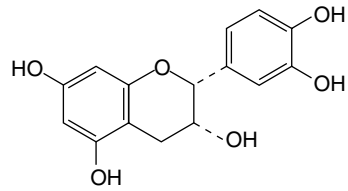
On treatment with acids or enzymes condensed tannis are converted into red insoluble compounds known as phlobaphenes. Phlobaphenes give the characteristic red colour to many drugs such as red cinchona bark, which contain these phlobatannins and their decomposition products. On dry distillation they yield catechol and these tannins are therefore sometimes called catechol tannins. Like catechol itself, their solutions turn green with ferric chloride.

Some drugs (e.g. tea, hamamelis leaves and hamamelis bark) contain both hydrolysable and condensed tannins. The following are rich in condensed tannins:

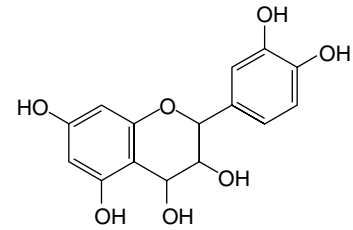
1. Barks: cinnamon, wild cherry, cinchona, willow, acacia (wattle, mimosa), oak and hamamelis
2. Roots and rhizomes: krameria (rhatany) and male fern
3. Flowers: lime and hawthorn
4. Seeds: cocoa, guarana, kola and areca
5. Fruits: cranberries, grapes (red wines), hawthorn
6. Leaves: hamamelis, hawthorn and tea, especially green tea
7. Extracts and dried juices: catechu, acacia and mangrove cutches, East Indian kino, butea gum and eucalyptus kino.



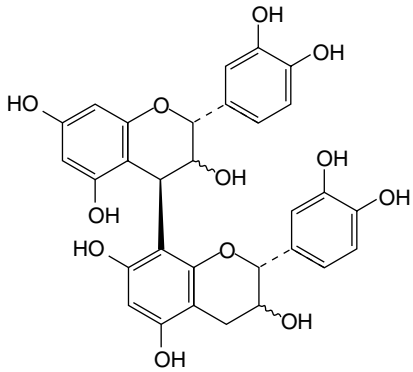
(+)-Catechin



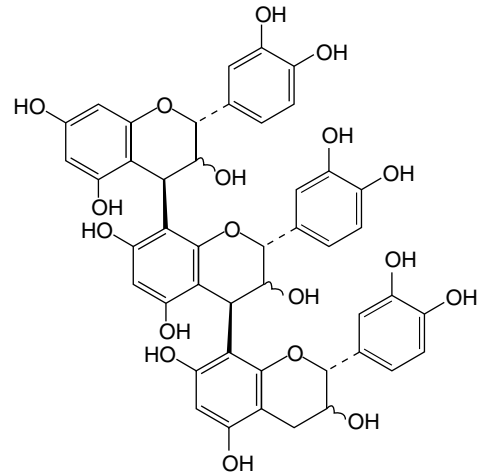
(–)-Epicatechin



Flavan-3,4-diol structure



A dimeric procyanidin



A trimeric procyanidin

Fig. 21.7
Structures associated with condensed tannins.

'Complex tannins'

This term has been applied by Okuda to a newly-discovered group of tannins which are biosynthesized from both a hydrolysable tannin (mostly a *C*-glucoside ellagitannin) and a condensed tannin. The union occurs through a C–C bond between the C-1 of the glucose unit of the ellagitannin and the C-8 or C-6 of the flavan-3-ol derivative. The monomers are also involved in oligomer formation.

To date, complex tannins have not great relevance to mainstream pharmacognosy; monomers have been isolated from the Combretaceae, Fagaceae (*Quercus*, *Castanea*), Myrtaceae, Polygonaceae (*Rheum*) and Theaceae (*Camellia*). It is anticipated that many more compounds of this group will be discovered.

Pseudotannins

As already mentioned, pseudotannins are compounds of lower molecular weight than true tannins and they do not respond to the goldbeater's skin test. Examples:

1. *Gallic acid*: rhubarb and most materials which contain gallitannins
2. *Catechins*: catechu, acacia cutch, many Australian kinos, cocoa, guarana and many other drugs containing condensed tannins
3. *Chlorogenic acid*: maté, coffee (particularly unroasted) and nux vomica (a small quantity only)
4. *Ipecacuanhic acid*: ipecacuanha

Occurrence of tannins. Tannins are of wide occurrence in plants and are usually found in greatest quantity in dead or dying cells. They exert an inhibitory effect on many enzymes due to protein precipitation and, hence, they may contribute a protective function in barks and heartwoods. Commercial tannins, as used in the leather industry, are obtained from quebracho, wattle, chestnut and myrobalans trees. Pharmaceutical tannin is prepared from oak galls (q.v.) and yields glucose and gallic acid on hydrolysis; many commercial samples contain some free gallic acid.

Some plants (clove, cinnamon, etc.) contain tannin in addition to the principal therapeutic constituents. This may complicate extraction or produce incompatibilities with other drugs (many alkaloids, for example, are precipitated by tannins).

Properties and tests. Tannins are soluble in water, dilute alkalis, alcohol, glycerol and acetone, but generally only sparingly soluble in other organic solvents. Solutions precipitate heavy metals, alkaloids, glycosides and gelatin. With ferric salts, gallitannins and ellagitannins give blue-black precipitates and condensed tannins brownish-green ones. If a very dilute ferric chloride solution is gradually added to an aqueous extract of hamamelis leaves (which contains both types of tannin), a blue colour is produced which changes to olive-green as more ferric chloride is added. Other tests are the following.

1. *Goldbeater's skin test*. Soak a small piece of goldbeater's skin in 2% hydrochloric acid; rinse with distilled water and place in the solution to be tested for 5 min. Wash with distilled water and transfer to a 1% solution of ferrous sulphate. A brown or black colour on the skin denotes the presence of tannins. Goldbeater's skin is a membrane prepared from the intestine of the ox and behaves similarly to an untanned hide (a reference sample of hide powder, as used in the *BP* assay of the tannins of Rhatany Root, may be obtained from the European Pharmacopoeia Secretariat, Strasbourg).
2. *Gelatin test*. Solutions of tannins (about 0.5–1%) precipitate a 1% solution of gelatin containing 10% sodium choloride. Gallic acid and other pseudotannins also precipitate gelatin if the solutions are sufficiently concentrated.
3. *Phenazone test*. To about 5 ml of an aqueous extract of the drug add 0.5 g of sodium acid phosphate; warm, cool and filter. To the filtrate add 2% solution of phenazone. All tannins are precipitated, the precipitate being bulky and often coloured.
4. *Test for catechin*. Catechins on heating with acids form phloroglucinol and they can, therefore, be detected by a modification of the well-known test for lignin. Dip a matchstick in the plant extract, dry, moisten with concentrated hydrochloric acid and warm near a flame. The phloroglucinol produced turns the wood pink or red.
5. *Test for chlorogenic acid*. An extract containing chlorogenic acid when treated with aqueous ammonia and exposed to air gradually develops a green colour.

In practice, these tests have to some extent been superseded by the use of TLC, particularly for the identification of crude drugs.

Medicinal and biological properties. Tannin-containing drugs will precipitate protein and have been used traditionally as stypics and internally for the protection of inflamed surfaces of mouth and throat. They act as antidiarrhoeals and have been employed as antidotes in poisoning by heavy metals, alkaloids and glycosides. In Western medicine their use declined after World War II when it was found that absorbed tannic acid can cause severe central necrosis of the liver. Recent studies have concentrated on the antitumour activity of tannins (M. Ken-ichi *et al*., *Biol. Pharm. Bull*., 1993, **16**, 379) and it has been shown that, to exhibit a strong activity, ellagitannin monomer units having galloyl groups at the *O*-2 and *O*-3 positions on the glucose core(*s*), as in the tellimagrandins (Fig. 21.6) are required. Anti-HIV activity has also been demonstrated.

Proanthocyanidins (condensed tannins) are associated with the beneficial effects of various herbs and infusions produced from them. The antitumour activity of green and black tea has been extensively researched in recent years with positive findings. Of the components of tea, epigallocatechin-3-gallate, specifically, has been shown to prevent angiogenesis in mice. Cranberry juice has long been used for reducing bacterial infections of the bladder and these claims have now been supported by a randomized, double-blind, placebo-controlled trial carried out on 153 elderly women (J. Avorn *et al.*, *J. Amer. Med. Assoc*., 1994, **271**, 751). Fructose has been implicated in this activity but recently, proanthocyanidins prepared from cranberries by reverse-phase and adsorption chromatography were shown to inhibit the adherence of P-fimbriated *E. coli* to uroepithelial-cell surfaces; other *Vaccinium* spp., including blueberries had similar bioactivity, suggesting their contribution to the salutary effects in urinary tract infections (A. Howell *et al.*, *New Engl. J. Med.*, 1998, **339**, 1085).

Further reading

Beecher GR 2004 Proanthocyanidins: biological activities associated with human health. Pharmaceutical Biology 42 (Supplement): 2–20

Waterman PG, Mole S 1994 Analysis of phenolic plant metabolites. Methods in ecology. Blackwell Scientific Publications, Oxford, UK

OAK BARK

Oak bark is the cut and dried bark from the fresh young branches of *Quercus robur* L. (English oak, Common oak), *Q. petraea* Liebl. (Sessile or Durmast oak) and *Q. pubescens* Willd. (Downy oak), family Fagaceae. The three species are recognized by the *BP*/*EP* and the first two by the *BHP*. The distribution of the species is widespread in Europe and W. Asia. *Q. alba* L. (White oak) is used in the USA.

The commercial bark, obtained principally from E. and S.E. Europe, occurs as channelled pieces, 3–4 mm thick and of various lengths. Younger, thinner pieces have a smooth, greyish-green cork with lenticels, older pieces have a greyish-brown rhytidome and show

a fracture, granular in the outer part and fibrous and splintery in the inner part. Conspicuous features of the reddish-brown powder include cork cells, lignified fibres with crystal sheaths of calcium oxalate, pitted sclereids and cluster crystals of calcium oxalate in parenchymatous cells.

Principal constituents are phlobatannins, ellagitannins and gallic acid, a minimum of 3.0% calculated as pyrogallol [$C_6H_3(OH)_3$ (1:2:3)] being specified by the *BP*/*EP*.

Oak bark is used medicinally for its astringent properties and industrially for tanning and dyeing.

GALLS AND TANNIC ACID

Turkish galls (*Turkey Galls*; *Galla*) are vegetable growths formed on the young twigs of the dyer's oak, *Quercus infectoria* (Fagaceae), as a result of the deposition of the eggs of the gall-wasp *Adleria gallaetinctoriae*.

The dyer's oak is a small tree or shrub about 2 m high which is found in Turkey, Syria, Persia, Cyprus and Greece. Abnormal development of vegetable tissue round the larva is due to an enzyme-containing secretion, produced by the young insect after it has emerged from the egg, which by the rapid conversion of starch into sugar stimulates cell division. As starch disappears from the neighbourhood of the insect, shrinkage occurs and a central cavity is formed in which the insect passes through the larval and pupal stages. Finally, if the galls are not previously collected and dried, the mature insect or imago bores its way out of the gall and escapes. During these changes the colour of the gall passes from a bluish-grey through olive-green to almost white.

Galls are collected by the peasants of Turkey and Syria. After drying they are graded according to colour into three grades, blue, green and white, which are found on the London market.

History. Galls were well known to the ancient writers and Pliny records the use of their infusion as a test for sulphate of iron in verdigris, possibly the earliest mention of an attempt to detect adulteration by chemical means.

Characters. Aleppo galls are globular in shape and from 10 to 25 mm in diameter. They have a short, basal stalk and numerous rounded projections on the surface. Galls are hard and heavy, usually sinking in water. The so-called 'blue' variety are actually of a grey or brownish-grey colour. These, and to a lesser extent the olive-green 'green' galls, are preferred to the 'white' variety, in which the tannin is said to have been partly decomposed. White galls also differ from the other grades in having a circular tunnel through which the insect has emerged. Galls without the opening have insect remains in the small central cavity. Galls have a very astringent taste.

Sections through a gall show a very large outer zone of thin-walled parenchyma, a ring of sclerenchymatous cells, and a small, inner zone of rather thick-walled parenchyma surrounding the central cavity. The parenchymatous tissues contain abundant starch, masses of tannin, rosettes and prisms of calcium oxalate, and the rounded so-called 'lignin bodies', which give a red colour with phloroglucinol and hydrochloric acid.

Constituents. Galls contain 50–70% of the tannin known as gallotannic acid (Tannic Acid *BP*/*EP*); this is a complex mixture of phenolic acid glycosides varying greatly in composition. It is prepared by fermenting the galls and extracting with water-saturated ether. Galls also contain gallic acid (about 2–4%), ellagic acid, sitosterol, methyl betulate, methyl oleanolate, starch and calcium oxalate. Two new compounds, derivatives of ellagic acid and pentahydroxynaphthalene, isolated from the alcoholic extract of galls have been shown to have nitricoxide- and superoxide-inhibiting activity (H. Hamid *et al.*, *Pharm. Biol.*, 2005, **43**, 317). Nyctanthic, roburic and syringic acids have more recently been identified and syringic acid has been identified as the CNS-active component of the methanolic extract of galls. (For the isolation of flavonoids of oak galls see M. Ahmed *et al.*, *Fitoterapia*, 1991, **62**, 283.)

Tannic acid is a hydrolysable tannin (see above) yielding gallic acid and glucose and having the minimum complexity of pentadigalloyl glucose. Solutions of tannic acid tend to decompose on keeping with formation of gallic acid, a substance which is also found in many commercial samples of tannic acid. It may be detected by the pink colour produced on the addition of a 5% solution of potassium cyanide.

Allied drugs. Many different kinds of galls are known. They are generally produced on plants, but sometimes on animals. In addition to the large number produced by insects, particularly of the genera *Cynips* and *Aphis*, some are produced by fungi.

Chinese and Japanese galls are of considerable commercial importance. They are produced by an aphis, *Schlectendalia chinensis*, on the petioles of the leaves of *Rhus chinensis* (Anacardiaceae). These galls, which the Chinese call 'wu-pei-tzu', meaning 'five knots', are irregular in shape and partly covered with a grey, velvety down, the removal of which discloses a reddish-brown surface. They break easily and show a large, irregular cavity containing insect remains. They contain 57–77% of tannin and have been valued in China as astringents and styptics for at least 1250 years.

Crowned Aleppo galls are sometimes found in samples of ordinary Aleppo galls. They are about the size of a pea, are stalked, and bear a crown of projections near the apex. The insect producing them is *Cynips polycera*.

Hungarian galls are produced by *Cynips lignicola* on *Quercus robur* growing in former Yugoslavia. They are used in tanning. *English oak galls*, formed by *Adleria kollari* on *Quercus robur*, contain about 15–20% of tannin.

Uses. Galls are used as a source of tannic acid, for tanning and dyeing, and in the manufacture of inks. Tannic acid is used as an astringent and styptic.

HAMAMELIS LEAF

Hamamelis leaf (*witch hazel leaves*) consists of the dried leaves of *Hamamelis virginiana* L. (Hamamelidaceae), a shrub or small tree 2–5 m high, which is widely distributed in Canada and the USA. It is official in the *BP*/*EP* and is the subject of an ESCOP monograph.

Macroscopical characters. The leaves are shortly petiolate, 7–15 cm long, and broadly oval to ovate in shape; base asymmetrically cordate, apex acute. The lamina is dark brownish-green to green in colour and very papery in texture. The venation is pinnate and the margin crenate or sinuate-dentate. The veins are very conspicuous on the lower surface; they leave the mid-rib at an acute angle and run straight to the margin, where they terminate in a marginal crenation. Odour, slight; taste, astringent and bitter.

The *BP*/*EP* drug is required to contain not more than 7% of stems and not more than 2% of other foreign matter.

Microscopical characters. The drug has very distinctive microscopical characters. These include characteristic stomata present on the lower surface only; very large lignified idioblasts, crystal cells accompanying the pericyclic fibres, tannin-containing cells and, especially in young leaves, stellate hairs. The calcium oxalate is in monoclinic prisms 10–35 μm long. The stellate hairs (Fig. 42.1H) consist of 4–12 cells united at the base. Each cell is thick-walled and up to 500 μm long.

Constituents. Hamamelis contains gallitannins, ellagitannins, free gallic acid, proanthocyanidins, bitter principles and traces of volatile oil. With ferric chloride solution the gallitannins and the free gallic acid give a blue colour and the ellagitannins, green.

The pharmacopoeia requires the leaves to contain not less than 3.0% tannins; these tannins represent the difference between the total polyphenol content of the leaf and the polyphenol content not absorbed by hide powder. Reagents employed in the assay are Phosphomolybdotungstic reagent and sodium carbonate solution with absorbance measurements made at 760 nm; pyrogallol is used as a test solution. The leaves appear to contain no hamamelitannin (see 'Hamamelis Bark', below).

Volatile compounds, although present in small amounts only, have been studied by GC-MS analysis. Some 175 compounds have been distinguished and classified as homologous series of alkanes, alkenes, aliphatic alcohols, related aldehydes, ketones and fatty acid esters; distinctive monoterpenoids were evident (R. Engel *et al.*, *Planta Medica*, 1998, **64**, 251).

A procedure for the identification and assay involving TLC, HPLC, plate densitometry and spectrophotometry for the proanthocyanidins, phenolic acids and flavonoids in leaf extracts has been described (B. Vennat *et al.*, *Pharm. Acta Helv.*, 1992, **67**, 11).

Allied drugs. *Hamamelis bark* occurs in curved or channelled pieces which seldom exceed 10 cm long or 2 cm wide. The bark is silvery grey and smooth, or dark grey and scaly. The inner surface is pinkish and often bears fragments of whitish wood. Sections show a cortex containing prismatic crystals of calcium oxalate, a complete ring of sclerenchymatous cells, and groups of phloem fibres. The bark contains a mixture of hamamelitannin and condensed tannin; the former has recently been demonstrated to be a potent oxygen scavenger (H. Masaki *et al.*, *Phytochemistry*, 1994, **37**, 337). Three separate hamamelitannins, α-, β- and γ-, are now known. The most important, β-hamamelitannin, is formed from two gallic acid molecules and one molecule of the sugar hamamelose. Newer galloylhamameloses and proanthocyanidins have now been identified (C. Haberland and H. Kolodziej, *Planta Medica*, 1994, **60**, 464; C. Hartisch and J. Kolodziej, *Phytochemistry*, 1996, **42**, 191). For the fractionation of those polymeric proanthocyanidins having similar structures but different molecular weights, see A. Dauer *et al*., *Planta Med*., 2003, **69**, 89.

Uses. Hamamelis owes its astringent and haemostatic properties to the tannins. Hamamelitannin and the galloylated proanthocyanidins isolated from *H. virginiana* are reported to be potent inhibitors of 5-lipo-oxygenase, supporting the anti-inflammatory action of the drug (C. Hartisch *et al.*, *Planta Medica*, 1997, **63**, 106). The above compounds are presumably not present in Hamamelis Water or Distilled Witch Hazel, which is, however, widely used as an application to sprains, bruises and superficial wounds and as an ingredient of eye lotions. It contains safrole and other volatile components.

TORMENTIL

There are over 300 spp. of *Potentilla* family Rosaceae of which several, including *P. anserina*, (silverweed), *P. reptans* (creeping cinquefoil) and *P. erecta* (common tormentil), find medicinal use. Tormentil *BP*/*EP* consists of the whole or cut dried rhizome, freed from roots of *P. erecta* (L.) Raeusch. (*P. tormentilla* Stokes). This perennial plant is widely spread throughout central and northern Europe, favouring the acidic soils of marshes, meadows, open woods and hills. Commercial supplies come from East European countries.

Plants are up to 30 cm tall with several loosely pilosed stems bearing leaves consisting of three- to five-toothed finely haired leaflets. Yellow flowers in loose terminal cymes have long pedicels and, unusually for the genus, four petals.

The rhizomes are dark brown on the outer corky layer and white on the inside when freshly broken, but turning red on exposure to the air. The chopped dried drug consists of hard pieces of rhizome with the remains of roots attached. Depressed pale scars from the stems are visible and some remains of stems in the form of fine, branching strands, less than 1 mm in diameter, may also be attached to the rhizome. The fracture is granular, odour faint but not unpleasant and the taste strongly astringent.

Characteristic features of the powder include brown cork cells, parenchymatous tissue containing tannin, sclerenchymatous tissues, vascular elements, starch in conglomerates or as single grains up to about 20 μm in length, and abundant cluster crystals of calcium oxalate up to about 50 μm in diameter.

Constituents. The rhizome contains a mixture of both hydrolysable and condensed tannins (proanthocyanidins). Among the former is agrimoniin, a dimeric ellagitannin found also in *Agrimonia* and *Alchemilla*, and belonging to the same biosynthetic group, ellagic acid and catechol gallates. Other components are flavan-3-ols, the pseudosaponin tormentoside, quinovic acid and various phenylpropanes together with a trace of volatile oil.

Standards. *BP*/*EP* requirements are a minimum of 7.0% tannins calculated as pyrogallol for the dried rhizome. A maximum of 3% for roots, stems and rhizomes with a black fracture. Other foreign matter limited to 2%. Compliance with a TLC test for identity using catechin as a reference substance.

Uses. As an astringent; internally as an antidiarrhoeic and externally for gargles and inflamed mucous membranes.

HAWTHORN

The leaves, flowers and false fruits are all medicinally useful, the leaves and flowers being used principally for the preparation of infusions, etc. with the fruits employed in the manufacture of prepared medicaments. The dried false fruits of *Crataegus monogyna* and *C. laevigata*, family Rosaceae, together with their hybrids are official in the *EP*, *BP* and *BHP*; similarly the leaf and flower, for which there is also an ESCOP monograph.

The thorny, deciduous trees are native to Europe and have a long medical and ethnobotanical history. Commercial supplies of the dried fruits, required to contain not less than 1.0% procyanidins, originate from Eastern Europe.

Characters. Characteristic of a number of genera of the family Rosaceae, so-called hawthorn berries are false fruits (pomes, and not in the strict botanical sense berries) in which the carpels become adherent to the hollow, fleshy receptacle and the sepals, petals and stamens become situated at the upper end of the fruit. The carpels become stony so that the pome comes rather to resemble a drupe (Ch. 41). The false fruits of *C. monogyna* with one carpel contain a single stony true fruit whereas those of *C. laevigata* with two or three carpels contain two or three fruits.

The dried reddish-brown to dark red fruits have a slight odour and mucilaginous, slightly acid taste; with *C. monogyna* they are up to 10 mm in length and slightly larger for *C. laevigata*. At the upper end of the false fruit are the remains of the five reflexed sepals which surround a shallow depression from the base of which arise stiff

lignified tufts of trichomes and the remains of the style (two styles with *C. laevigata*). The base of the fruit may be either attached to a pedicel or show the scar of attachment of the latter.

In addition to the long, lignified, tapering clothing trichomes of the inner surface of the receptacle other microscopical features include: cells of the outer receptacle with red pigmentation; sclereids; calcium oxalate as clusters and in files of cells as prisms; seed fragments showing a mucilaginous testa and embryo cells containing aleurone grains and fixed oil. A more detailed description will be found in the pharmacopoeias.

Constituents. The fruits contain 1–3% oligomeric procyanidins, the structures of which appear to be only partially ascertained together with flavonoids, principally hyperoside about 1%. The leaves in contrast contain less hyperoside and more vitexin rhamnoside.

Thin layer chromatography of a methanolic extract of the drug and fluorescence visualization at 365 nm is used as a test for identity. Procyanidins are evaluated by acid hydrolysis of an alcoholic extract followed by absorbance measurements at 545 nm of the butanol-soluble procyanidins produced.

The leaves and flowers, in contrast to the fruits, contain less hyperoside and more vitexin rhamnoside. In a study of important factors for the use of monitored commercial material, W. Peschel *et al.* (*Fitoterapia*, 2008, **79**, 1) have examined the variability of total flavonoid content of the drug in relation to wild trees, age of cultivation site, sun exposure and harvest time.

Allied species. Adulteration of the readily available product is rare; other species of *Crataegus* may be detected by their having more than three seeds. *C. pinnatifida* fruits are used in Chinese medicine.

Uses. Hawthorn is widely used as a mild cardiac tonic particularly for patients of advancing age. It does not have the toxic effects of *Digitalis* and can usefully be employed before recourse is made to the digitalis cardioactive glycosides.

AGRIMONY

Agrimony *BP*/*EP*, *BHP* family Rosaceae consists of the dried flowering tops of *Agrimonia eupatoria* L.

This erect, chalk-loving perennial herb is common throughout southern Europe and is indigenous to the British Isles, except for northern Scotland. Related species are found across North America. Hungary and Bulgaria are commercial suppliers of the drug.

The leaves are compound imparipinnate, with four to six opposite pairs of leaflets and a terminal leaflet. Larger leaflets are up to 6 cm in length with coarsely serrate or serrate–dentate margins, usually densely villous and often greyish on the lower surface. The golden flowers, 5–8 mm in diameter, are arranged spirally as terminal spikes. The pendulous fruits, 4–6 mm long, are deeply grooved with small projecting hooked bristles.

Characteristic microscopical features include stiff, thick-walled trichomes (500 μm) often with spiral thickenings and abundant clusters and prisms of calcium oxalate in the leaf mesophyll. Stomata are mainly of the anomocytic, occasionally anisocytic type. Pollen grains are ovoid to subspherical (up to 60 μm × 35 μm) with three pores and a smooth, thin exine.

The *BP* drug is required to contain a minimum of 2.0% tannins, expressed as pyrogallol when assayed by the official 'determination of tannins in herbal drugs'. The TLC test of identification exploits the flavonoid content (rutin and isoquercitroside as test substances). Vitamins, triterpenes, volatile oil have also been reported as components of the drug.

Among other herbal uses, agrimony is employed as a mild astringent, internally and externally, against inflammation of the throat and for gastroenteritis.

ALCHEMILLA

The flowering and aerial parts of the lady's mantle, *Alchemilla xanthochlora* (*A. vulgaris sensu latiore*), family Rosaceae, are described in the *BP*/*EP* and *BHP* 1996. The plant is widespread in Europe, North America and Asia; commercial supplies are obtained principally from Eastern Europe. In addition to the identification by macroscopic and microscopic characters the pharmacopoeias include thin-layer chromatographic tests providing characteristic fluorescent zones.

The *BP*/*EP* drug is required to contain not less than 6.0% of tannins expressed as pyrogallol when determined by the official method (cf. Hamamelis and Rhatany). The characterized ellagitannins are pedunculagin and the dimeric alchemillin. Other constituents are flavonoids, quercetin 3-*O*-β-D-glucoside having been isolated as the major flavonoid in leaves of French origin.

Alchemilla acts as an astringent against bleeding and diarrhoea and has a long tradition of use for gynaecological conditions such as menorrhagia.

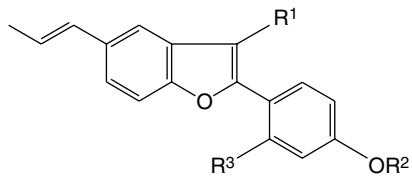
RHATANY

Rhatany of the *BP* and *EP* (*Krameria*) is the dried root of *Krameria triandra* (Krameriaceae, a small family related to the Leguminosae), a small shrub with decumbent branches about 1 m long. The drug is collected in Bolivia and Peru and is known in commerce as Peruvian rhatany.

The root has a knotty crown several centimetres in diameter and gives off numerous branch roots some of which attain a length of 60 cm. The roots are nearly cylindrical and are covered with a reddish-brown cork, which is scaly except in very young roots. A transverse section shows a reddish-brown bark which occupies about one-third of the radius and encloses a yellowish, finely radiate wood. A small, deeply coloured heartwood is sometimes present in the larger species. The bark readily separates from the wood. The former is astringent but the latter almost tasteless.

The tannins of krameria root (krameria-tannic acid) are entirely of the condensed (proanthocyanidin) type having a 'polymeric' flavin-3-ol structure. In this instance there is a procyanidin:propelargonidin ratio of 35:65 as determined by acid hydrolysis. Astringency of the root is due to compounds with a degree of polymerization of more than five. (For further details see E. Scholz and H. Rimpler, *Planta Med.*, 1989, **55**, 379). A phlobaphene (krameria-red), starch and calcium oxalate are also present. Stahl and Ittel (1981) reported the isolation of two benzofuran derivatives, ratanhiaphenols I and II, from the root. Both compounds are effective u.v. light filters and could be useful in sun-protection preparations. The *BP* and *EP* include an assay for tannins (polyphenols) of not less than 5.0% based on the colour reaction involving alkaline sodium phosphomolybdotungstate (absorbance measured at 760 nm). Polyphenols not adsorbed by hide powder, also determined with the same reagent, are excluded from the calculation.

The drug is used as an astringent and the significant antimicrobial activity of the extract gives rational support for its use in mouth and throat infections.



Ratanhiaphenol I: R^1 = H; R^2 = Me; R^3 = OH
Ratanhiaphenol II: R^1 = Me; R^2 = R^3 = H

Allied species. The roots of several other species are occasionally encountered in commerce, but the Peruvian drug is the only one generally available. *Krameria cystisoides* of Mexican origin has indigenous medicinal uses. It contains over 20 compounds of the lignan, neolignan and norneolignan type. Similar constituents are reported for *K. lanceolata*; see H. Achenbach *et al.*, *Phytochemistry*, 1987, **26**, 1159; 1989, **28**, 1959.

Pomegranate rind

The pomegranate fruit is one of the oldest known to man and has featured in mythology, and as a food and medicine from ancient civilizations of the Middle East to its present wide cultivation in India and surrounding countries, Turkey, southern Europe and California.

Pomegranate rind consists of the dried pericarp of the fruit of *Punica granatum* (Punicaceae). It occurs in thin, curved pieces about 1.5 mm thick, some of which bear the remains of the woody calyx or a scar left by the stalk. The outer surface is brownish-yellow or reddish. The inner surface bears impressions left by the seeds. Pomegranate rind, used in India as a herbal remedy for non-specific diarrhoea, is very astringent and contains about 28% of tannin (ellagitannins) and colouring matters. It should be distinguished from the root bark, which contains alkaloids.

For a discussion of the biochemistry, health effects, commercialization, plant growth and improvement of the pomegranate fruit, see N. P. Seeram *et al.* (eds), R. Hardman (series ed.) 2006 Medicinal and aromatic plants – industrial profiles, Vol 43. Pomegranate. CRC Press, Taylor and Francis Group. Boca Raton, FL., 244 pp.

Aspidosperma barks

The bark of *Aspidosperma quebracho-blanco* (Apocynaceae), which is used as a tanning material, was formerly official in several pharmacopoeias.

Myrobalans

Myrobalans are the dried fruits of *Terminalia chebula* (Combretaceae), a tree common in India. The immature fruits are black, ovoid and about 1–3 cm long. They contain about 20–40% of tannin, β-sitosterol, anthraquinones and a fixed oil containing principally esters of palmitic, oleic and linoleic acids. The tannin and anthraquinone constituents make the drug both astringent and cathartic in action. Microbiological tests support the Indian use of an aqueous extract of the fruit as an anticaries agent (A. G. Jagtap and S. G. Karkera, *J. Ethnopharmacology*, 1999, **68**, 299).

CATECHU

Gambir or pale catechu of the *BP* 1989; *BP* (Veterinary), 2007 is a dried, aqueous extract prepared from the leaves and young twigs of a climbing shrub, *Uncaria gambir* (Rubiaceae). It must be carefully distinguished from black catechu or cutch. The plant is a native of Malaya and it is largely cultivated for the production of the drug in Indonesia and Malaya for marketing through Singapore.

History. The catechu described by Barbosa (1514) was black catechu or cutch, and the first account of gambir appears to be that of a Dutch trader in 1780. In addition to the cube gambir used in pharmacy, large blocks of the extract are imported for use in dyeing and tanning. Other forms are used in the East for chewing with betel leaf.

Preparation. The preparation of catechu in Johore differs only slightly from the procedure adopted in Indonesia. It consists of extracting the leaves and young twigs with boiling water, evaporating the extract to a pasty consistency and dividing it into cubes, which are then sun-dried. Fuller details of the preparation are given in the 10th edition.

Many different forms of catechu are used in the East, and the drug for the Eastern market frequently has 20–50% of fine rice husks added as the liquid coagulates in the tubs. Such catechu is, of course, unofficial, and contains starch.

Macroscopical characters. Catechu occurs in cubes, which are very friable and may be broken up in transit or, if incompletely dried, may be more or less agglutinated. Of the samples available, those from Indonesia measure 17–22 mm and have a reddish-brown surface, often stamped with a maker's mark, while those from Johore measure 24–29 mm and have a blackish exterior and the faces of the cube are depressed. Internally, both varieties are cinnamon-brown and porous. Odourless; taste, very astringent and at first somewhat bitter, afterwards sweetish.

Microscopical characters. When mounted in water, catechu shows minute, acicular crystals of catechin, many of which are branched and interlacing. They dissolve on warming and a considerable amount of vegetable debris is left. The leaves, particularly the stipules, bear simple, unicellular hairs up to about 350 μm long, with smooth, moderately thick, lignified walls. The twigs have lignified pericyclic fibres, wood fibres, and spiral, annular and pitted vessels. Minute starch grains are commonly present, particularly in the Indonesian drug, but the amount should be strictly limited. Rice husks have been observed in some samples.

Chemical tests

1. *For gambir-fluorescin*. Extract a little of the powdered drug with alcohol and filter. To the filtrate add solution of sodium hydroxide. After shaking, add a few millilitres of light petroleum, shake again and allow to stand. The petroleum spirit layer shows a strong green fluorescence.
2. *For catechin*. This is a modification of the usual test for lignin. Phloroglucinol is formed from catechin and with hydrochloric acid turns a matchstick red.

Constituents. Gambir contains about 7.33% of catechins, 22–50% of catechutannic acid, catechu red, quercitin and gambir-fluorescin.

BP (Vet.) 2007 standards include a loss on drying of not more than 15.0% and a water-insoluble matter of not more than 33.0% with reference to the dried material.

Catechin forms white, acicular crystals which are soluble in hot water and alcohol and give a green colour with ferric salts. Catechutannic acid is an amorphous phlobatannin which appears to be formed from catechin by loss of the elements of water. It readily yields the phlobaphene catechu-red. If the drug is carefully prepared, it will contain a high proportion of catechin and correspondingly smaller amounts of catechutannic acid and catechu-red.

Allied drug. *Cutch* or *black catechu* is an extract prepared from the heartwood of *Acacia catechu* (Leguminosae). Cutch occurs in black, somewhat porous masses. The taste resembles that of gambir. Microscopical examination of the water-soluble residue shows wood fibres and large vessels and sometimes fragments derived from the leaves on which the drug is spread.

Cutch contains 2–12% of catechins, 25–33% of phlobatannin, 20–30% of gummy matter, quercitrin, quercitin, moisture, etc. It yields 2–3% of ash. The catechin (acacatechin) is not identical with that in gambir.

The drug may be distinguished from gambir as it gives no reaction for gambir-fluorescin.

Uses. Catechu is used in medicine as an astringent.

Kinos

The name 'kino' has been applied to a number of dried juices, rich in phlobatannins and formerly used for their astringent properties. They include Malabar kino from *Pterocarpus marsupium* (Leguminosae), butea gum or Bengal kino from *Butea frondosa* (*B. monosperma*) (Leguminosae) and eucalyptus kino or red gum from *Eucalyptus rostrata* (Myrtaceae).

Croton lechleri

The bark of this and related euphorbiaceous trees yield, when slashed, a blood-red sap commonly known in South American folk medicine as Sangre de Grado, Sangre de Drace, or dragon's blood (not to be confused with the dragon's blood obtained from species of *Daemonorops* palms, q.v.). It is used locally for its anti-infective, antitumour and wound-healing properties. Cai *et al.* (*Phytochemistry*, 1991, **30**, 2033; 1993, **32**, 755) have shown proanthocyanidins to be the principal constituents (*c.* 90%) which vary from monomers to heptamers. These polyphenols possess oxygen free-radical scavenging activity and may assist in wound healing (C. Desmarchelier *et al.*, *J. Ethnopharmacology*, 1997, **58**, 103). Minor components isolated are phenols, alcohols, sterols and four diterpenoids, two of the latter being of the clerodane type. An alkaloid, tapsine, has been ascribed as the wound healing constituent; it could also account for the antitumour activity claimed for the latex (Z. Chen *et al.*, *Planta Medica*, 1994, **60**, 541).

Further reading

For various aspects of the chemotaxonomy, chemistry, biosynthesis, enzymology and health factors of tannins and related polyphenols, see D. Ferreira *et al* (eds) *Phytochemistry* 2005, **66**, 1969–2120, (Pt 1); 2124–2291 (Pt 11)

COUMARINS AND GLYCOSIDES

Derivatives of benzo-α-pyrone such as coumarin (the lactone of *O*-hydroxycinnamic acid), aesculetin, umbelliferone and scopoletin are common in plants both in the free state and as glycosides. Not all are phenolic but they are included here with the phenolic derivatives for convenience. Some 1000 natural coumarins have been isolated. Coumarin itself has been found in about 150 species belonging to over 30 different families, although it is probably present in the undamaged plant as *trans-O*-glucosyloxycinnamic acid. Enzyme activity in the damaged tissue leads to a loss of glucose and a *trans* → *cis* isomerization followed by ring closure. Coumarin gives a characteristic odour of new-mown hay and occurs in many Leguminosae such as sweet clover, melitot and tonco beans; the latter contain about 1–3% of coumarin. It is also recorded in woodruff, *Asperula odorata* (Rubiaceae) and cassia oil.

In ammoniacal solution these compounds have a blue, blue–green or violet fluorescence, which has long been used as a qualitative test for certain umbelliferous resins such as asafoetida and galbanum. The fluorescence is, of course, more marked if examined in filtered ultraviolet light and is used for the chromatographic visualization of the compounds.

The substitution patterns of some common hydroxy and methoxy coumarins are given in Table 21.2. Structurally more complex coumarins such as the calanolides and inophyllums have received recent attention as potent HIV-1-RT inhibitors (see Chapter 32).

The *furanocoumarins* are closely related to the above and occur particularly in the Rutaceae and Umbelliferae. For example, celery fruits contain rutaretin and its dehydrated derivative apiumetin. Bergapten occurs in bergamot oil and in the Chinese root-drug derived from *Peucedanum decursivum* (Umbelliferae) which also contains the less-common *pyranocoumarins*. Marmesin derivatives (Fig. 13.2) and archangelicin have a reduced furanocoumarin structure consisting of coumarin and a C_5 sub-unit. Other prenylated compounds are the 3-iso-prenylcoumarins, as illustrated by rutamarin of the genus *Ruta*; for recent research on *R. graveolens* see S. D. Srivastava *et al.*, *Fitoterapia*, 1998, **69**, 80. A wide range of biological activities has been demonstrated for these metabolites (see R. H. Galán *et al.*, *Phytochemistry*, 1990, **29**, 2053).

Furanocoumarins are responsible at least in part for the unpredictable and variable effects on drug availability resulting from the consumption of grapefruit juice. Two components of the juice (6′, 7′-dihydroxybergamottin and FC26) inactivate cytochrome P450 enzymes (specifically CYP3A4 and CYP3A5) resulting in an increased oral bioavailability of various drugs used to treat cancer, hypertension, heart disease and allergies. However, unnamed constituents of the juice have recently been shown to activate the efflux pump controlling P-glycoprotein-mediated drug transport which secretes absorbed drugs back into the gut. *In vitro* studies have demonstrated reduced absorption of vinblastine, cyclosporin, losartan, digoxin and fexofenadine. The two effects are therefore antagonistic and explain the unpredictable action of grapefruit juice on drug bioavailability. For reports on this research see *The Lancet*, 1999, **353**, 1335; *Pharm. J.*, 1999, **262**, 573; *HerbalGram*, 1998, No. 43, 22.

Table 21.2 Hydroxy and methoxy coumarins.

Compound	Additional groupings	Occurrence
Umbelliferone	HO at 7 (above)	Belladonna and stramonium (Solanaceae): *Daphne mezereum* (Thymeliaceae); *Ferula* species yielding asafoetida and galbanum, and many other Umbelliferae, chicory leaves (Compositae)
Herniarin	CH_3O at 7	*Lavandula spica* (Labiatae), *Ruta graveolens* (Umbelliferae) and certain Compositae
Aesculetin	HO at 6, HO at 7	Horse-chestnut (Hippocastanaceae), certain Rosaceae and *Fraxinus* (Oleaceae)
Scopoletin	CH_3O at 6, HO at 7	Roots of gelsemium, oat, jalap, scammony, scopolia and belladonna; leaves of tobacco, stramonium, chicory and many others
Fraxin	CH_3O at 6, HO at 7, *O*-glucose at 8	*Fraxinus* spp. (Oleaceae)
Chicoriin	CH_3O at 6, *O*-glucose at 7	*Cichorium intybus* herb

Ammi species contain furanomethoxycoumarins but are more important for their content of furanobenzo-γ-pyrones (q.v. under 'Flavones').

Bicoumarins are formed from two coumarin moieties and the linkage may occur in a number of ways. Dicoumarol is formed at C3–C3′ through a methylene group and was, in 1941, the first of the series to be isolated. It is a constituent of fermenting hay and is formed by microbial action of coumarin. It is a powerful anticoagulant and haemorrhagic and can cause the death of animals consuming the spoiled fodder.

Further reading

Estévez-Braun A, González RG 1997 Coumarins (1995–1996). Natural Product Reports 14(5): 465–476

Murray RDH 1995 Coumarins (1988–1994). Natural Product Reports 12(5): 477–506

ANGELICA ROOT

The root of the official drug (*BP*, *EP*, *BHP*) consists of the rhizome and root of *Angelica archangelica* L. (*A. officinalis* Haffm.) (Umbelliferae), whole or cut and carefully dried. It is required to contain not less than 0.2% of volatile oil. The North American root is derived from *A. atropurpurea* and the Chinese from a number of species under the name *man-mu* or *tangkuei*.

The rhizomes are vertical and up to 5 cm in diameter, greyish-brown or reddish-brown in colour, bearing leaf and stem scars at the apex. Entwined, longitudinally furrowed, roots occur on the lower surface. The fracture is uneven and the transverse surface shows brown spots, indicating secretory cells, situated in the spongy, radiate, off-white bark. Microscopy of the powder shows, among other features, numerous simple starch grains 2–4 μm, yellowish-brown secretory canals, cork cells and lignified reticulately thickened vessels.

Considerable recent work on the genus has resulted in the isolation of a number of furanocoumarins and their glycosides; the formulae of bergapten, angelicin, archangelicin (a diester) and apterin are given in Fig. 21.8, and those of marmesin and psoralen in Fig. 13.2. These compounds are reported to have potent coronary vasodilator effects and are calcium antagonists. Monoterpenes constitute the major components (80–90%) of the volatile oil.

There are official limits for foreign matter, loss on drying (⩾10%), total ash (⩾10%) and acid-insoluble ash (⩾2.0%).

In herbal medicine the root is indicated in the treatment of bronchitis associated with vascular deficiency, and dyspeptic conditions.

MELILOT

Melilot *BP*/*EP*, *BHP* 1996 consists of the dried flowering tops of *Melilotus officinalis* (L.) Lam, (common melilot, ribbed melilot, king's clover, yellow sweet clover), family Leguminosae/Papilionaceae. It is found throughout Europe and eastwards to western China, N. America, except the far north, and elsewhere often as a weed of cultivation, probably introduced into Britain, together with other melilots (of which there are three common species), in the 16th century. Habitats include fields, hedgerows and waste places.

Melilot is an erect or decumbent branched biennial up to 100 cm tall. The finely ridged glabrous stems bear alternate stalked trifoliate leaves with two stipules joined to the base of the petiole. The leaflets of the upper leaves are oblong–elliptic, each with acute apex and base, margin entire. The yellow papillionaceous flowers occur in racemes up to 5 cm in length and give rise to almost straight glabrous pods, brown when ripe and transversely rugose. Seeds are wrinkled giving the 'ribbed' of the common name.

Features of the powdered drug include numerous anisocytic stomata with between three and six subsidiary cells on both epidermi; uniseriate covering trichomes composed of two small basal cells and a longer, bent, somewhat warty terminal cell; a few glandular trichomes with a two- to three-celled stalk and biseriate head of four cells; prismatic crystals of calcium oxalate associated with the vascular tissue; papillose epidermal cells of the petals; lignified fibrous anther fragments; spherical–ovoid pollen grains about 25 μm across with three pores and a smooth exine.

Constituents. Coumarin derivatives occur in melilot although coumarin itself is not present to any extent in the living plant. It arises when the plant is crushed, or the dried material treated with water, by the action of a β-gluconidase enzyme specific to *cis-o*-hydroxycinnamic acid glucoside giving first the unstable hydrolytic product coumarinic acid, which then cyclizes to coumarin producing the well-known 'new-mown hay' odour. The *trans*-isomer remains unchanged and is isolated as melilotoside (see F. Bourgoud *et al.*, *Phytochem. Anal.*, 1994, **5**, 127; P. Bradley *British Herbal Compendium*,

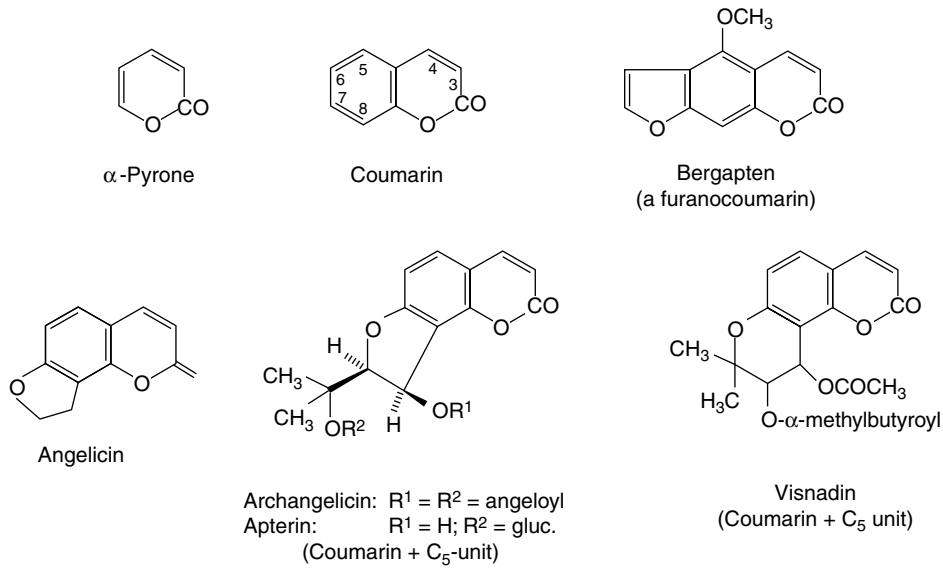
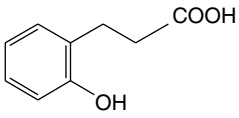
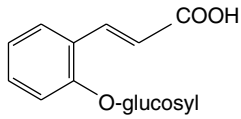


Fig. 21.8
Coumarin and derivatives.



Dihydro-*o*-coumaric acid
(Melilotic acid)



Melilotoside

Vol. 2, 2006, p. 270). Other acids isolated from melilot include dihydro-*o*-coumaric acid (melilotic acid), caffeic acid and other minor acids. Various oleanene saponins, volatile compounds and flavonoids have also been reported.

The pharmacopoeial TLC identification test for melilot indicates the presence of coumarin and possibly *o*-coumaric acid in the genuine drug. Assay of the coumarin content, minimum 0.3%, involves absorbance measurements at 275 nm on a boiled methanolic extract of the powder.

The various traditional medical uses of the drug have yet to be firmly established by clinical trials.

Tonco seed

Tonco seed or Tonquin beans are the dried seeds of *Dipteryx odorata* Willd. (*Coumarouna odorata* Aubl.) and *Dipteryx oppositifolia* (Leguminosae). The former plant is a native of Guiana and Brazil and is extensively cultivated in Venezuela, while the latter is found in Guiana and northern Brazil. Both are large trees bearing single-seeded fruits about 3–5 cm long.

The fruits are collected when ripe (May and June), they are opened and the seeds are dried in the sun. If sold without further treatment, they are known as 'black' beans. The seeds produced in Venezuela and near its border are larger than those produced in northern Brazil and parts of Guiana. The former, which are more highly valued, are known as 'Angostura' and the latter as 'Para' beans. Large quantities of both Angostura and Para beans are sent to Trinidad, where they are macerated for 24 h in rum and dried in the open air. This treatment causes a crystalline deposit of coumarin to be formed on the testa and the seeds are said to be 'frosted'. Angostura and Para beans thus occur in commerce both black and frosted.

Tonco beans are up to 40 mm long, 10 mm wide and 5 mm thick. They are rounded at one end and bluntly pointed at the other. The surface is black and deeply wrinkled longitudinally, a crystalline encrustation being present in the frosted variety. A transverse section shows a very thin, black testa and two yellowish-brown, planoconvex, oily cotyledons. Odour, very fragrant; taste, aromatic and bitter.

Tonco beans contain 1–3% of coumarin, 25% of fat (containing unsaponifiable sitosterin and stigmasterin) and a larger amount of starch. Ash about 3.5%. Tonco beans are used in tobacco manufacture and in perfumery. Synthetic coumarin has, to some extent, replaced the natural product.

Celery fruit. *Apium; Apii Fructus*

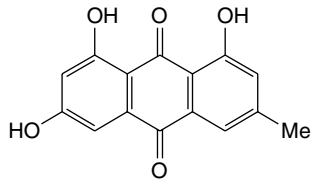
The drug consists of the dried ripe fruits of *Apium graveolens* (Umbelliferae).

The cremocarp is brown, subspherical and about 1–1.5 mm long. The mericarps are mostly separate in the drug and each shows five straight primary ridges. A transverse section is almost pentagonal and shows 6–9 vittae, two on the commissural surface and four to seven in the grooves of the dorsal surface. Odour and taste, aromatic. Celery fruits contain 2–3% of oil consisting of terpenes with smaller quantities of the anhydride of sedanonic acid, the lactone of sedanolic acid and phenols. The fruits also contain a number of coumarins, furanocoumarins and coumarin glycosides.

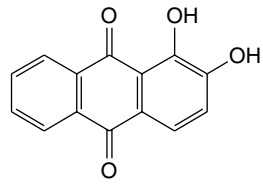
Celery fruits are official in the *BHP* and have a long-standing use in the treatment of rheumatic diseases; the therapeutic action appears to be potentiated by Taraxacum (q.v.).

ANTHRAQUINONES AND GLYCOSIDES

Long before anything was known of their chemistry, rhubarb, aloes, senna and cascara were recognized as forming a natural group of purgative drugs. Also certain vegetable and animal dyestuffs such as madder and cochineal were of great economic importance before the introduction of synthetic dyestuffs. Later the chemical similarity of these purgative drugs and dyestuffs became apparent, as illustrated by the formulae for emodin (the aglycone of a number of purgative glycosides of *Rhamnus* spp.) and alizarin (the aglycone of a dyestuff of the madder plant).



Emodin



Alizarin

Substances of the anthraquinone type were the first to be recognized, both in the free state and as glycosides. Further work showed that natural products also contained reduced derivatives of the anthraquinones (oxanthrones, anthranols and anthrones) and compounds formed by the union of two anthrone molecules (i.e. the dianthrones).

Because glycosides are often easily hydrolysed, the earlier workers tended to isolate products of hydrolysis rather than the primary glycosides. The following aglycones have long been established: chrysophanol or chrysophanic acid from rhubarb and cascara; aloe-emodin from rhubarb and senna; rhein from rhubarb and senna; emodin or frangula-emodin from rhubarb and cascara. Improved extraction methods, developed by Stoll and his colleagues, led to the isolation of the main senna glycosides, sennosides A and B, in 1941. Since this date many new glycosides including *C*-glycosides and various stereoisomers have been isolated and their structures determined.

In monocotyledons, anthraquinone derivatives are found only in the Liliaceae, in the form of the unusual *C*-glycoside barbaloin. Among dicotyledons they occur in the Rubiaceae, Leguminosae, Polygonaceae, Rhamnaceae, Ericaceae, Euphorbiaceae, Lythraceae, Saxifragaceae, Scrophulariaceae and Verbenaceae. They appear to be absent from the Bryophyta, Pteridophyta and Gymnosperms but occur in certain fungi and lichens. The fungal anthraquinone pigments are nearly all chrysophanol or emodin derivatives.

As indicated in Chapter 18, natural anthraquinones are synthesized either via the acetate–malonate pathway or they are derived from shikimate and mevalonate. The medicinally important purgative anthraquinones are formed by the former route and all have a 1,8-dihydroxy substitution. Conversely, compounds such as alizarin which have one of the rings unsubstituted arise by the second pathway. The relationships between the oxidized and reduced forms of the anthraquinone nucleus are shown in Fig. 21.9.

Modern research indicates that the 1,8-dihydroxyanthraquinone derivatives frequently occur with 1,8-dihydroxynaphthalene glycosides.

Anthraquinones

The derivatives of anthraquinone present in purgative drugs may be dihydroxy phenols such as chrysophanol, trihydroxy phenols such as emodin or tetrahydroxy phenols such as carminic acid. Other groups are often present, for example, methyl in chrysophanol, hydroxymethyl in aloe-emodin and carboxyl in rhein and carminic acid.

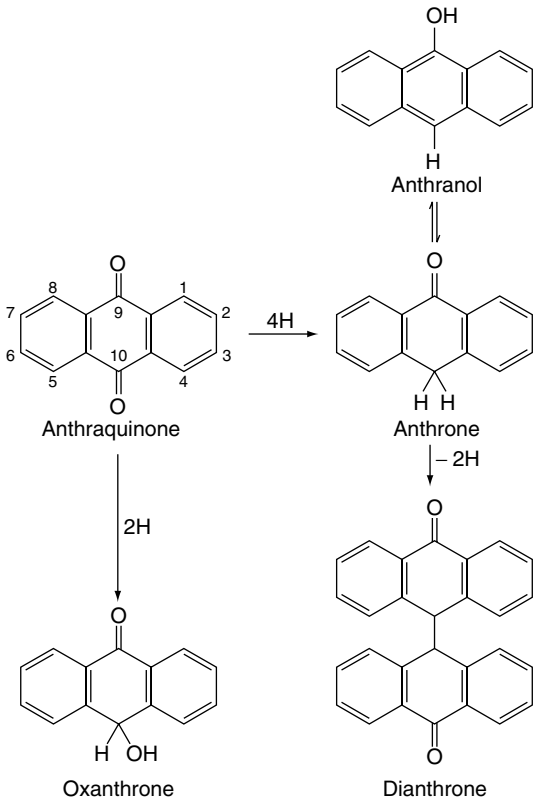


Fig. 21.9
Interrelationship of anthraquinone derivatives.

When such substances occur as glycosides, the sugar may be attached in various positions. See formulae for carminic acid and glucofrangulin. Some examples of anthraquinone derivatives are given in Table 21.3.

Anthraquinone derivatives are often orange-red compounds, which may sometimes be observed *in situ* (e.g. in the medullary rays of rhubarb and cascara). They are usually soluble in hot water or dilute alcohol. Bornträger's test is often used for their detection. The powdered drug is macerated with an immiscible organic solvent, ether is recommended, and after filtration aqueous ammonia or caustic soda is added, when a pink, red or violet colour in the aqueous layer after shaking indicates the presence of free anthraquinone derivatives. If glycosides only are present, the test should be modified by first hydrolysing with alcoholic potassium hydroxide solution or 2 M acid. When alkali is added to powdered drugs or to sections, the red colour produced serves to locate the anthraquinone derivatives in the tissues (e.g. in the medullary rays of cascara bark). If the drug being tested contains either very stable anthraquinone glycosides or reduced derivatives of the anthranol type, Bornträger's test will be negative.

Anthraquinones containing a free carboxylic acid group (e.g. rhein) can be separated from other anthraquinones by extraction from an organic solution with sodium bicarbonate solution.

Anthranols and anthrones

These reduced anthraquinone derivatives occur either free or combined as glycosides. They are isomeric and one may be partially converted to the other in solution. The parent substance, anthrone, is a pale yellow, non-fluorescent substance which is insoluble in alkali; its isomer, anthranol, is brownish-yellow and forms a strongly fluorescent solution in alkali. Anthranol derivatives, such as are found in aloes, have similar properties, and the strong green fluorescence which aloes give in borax or other alkaline solution has long been used as a test for its identification. Anthranols and anthrones are the main constituents of chrysarobin, a mixture of substances prepared by benzene extraction from the material (araroba) found in the trunk cavities of the tree *Andira araroba*. If a little chrysarobin is treated on a white tile with a drop of fuming nitric acid, the anthranols are converted into anthraquinones. A drop of ammonia allowed to mix gradually with the acid liquid produces a violet colour. This modification of Bornträger's test had been used as a test for identity before the underlying chemistry was known.

Oxanthrones

The formula given shows that these are intermediate products between anthraquinones and anthranols. They give anthraquinones on oxidation and Fairbairn's modification of the Bornträger test accomplishes this by means of hydrogen peroxide. An oxanthrone has been reported as a constituent of cascara bark.

Table 21.3 Anthraquinone glycosides and aglycones.

			Aglycone		
Glycoside	Aglycone	Sugar	OH Groups	Other groups	Occurrence
Ruberythric acid	Alizarin	Primeverose	1,2	–	*Rubia tinctorum*
Rubiadin primeveroside	Rubiadin	Primeverose	1,3	2-methyl	*Rubia tinctorum*
Rubiadin glucoside	Rubiadin	Glucose	1,3	2-methyl	*Rubia tinctorum*
Chrysophanein	Chrysophanol	Glucose	1,8	3-methyl	*Rheum and Rumex* spp.
Rheochrysin	Physcion	Glucose	1,8	3-methyl 6-methoxy	*Rheum* spp.
Glucorhein	Rhein	Glucose	1,8	3-carboxylic acid	*Rheum, Rumex* and *Cassia* spp.
Gluco aloe-emodin	Aloe-emodin	Glucose	1,8	3-hydroxymethyl	*Rheum* and *Cassia* spp.
Glucochrysaron	Chrysarone	Glucose	1,2,7	6-methyl	*Rheum rhaponticum*
Glucofrangulin A	Emodin	Glucose, rhamnose	1,6,8	3-methyl	*Rhamnus* spp.
Frangulin	Emodin	Rhamnose	1,6,8	3-methyl	*Rhamnus* spp.
Morindin	Morindone	Primeverose	1,5,6	2-methyl	*Morinda* spp. (Rubiaceae)
–	Islandicin	–	1,5,8	6-methyl	*Penicillium islandicum*
Carminic acid	–	Glucose	1,3,4,6	5-carboxylic acid 8-methyl	Cochineal

Dianthrones

These are compounds derived from two anthrone molecules, which may be identical or different; they readily form as a result of mild oxidation of the anthrone or mixed anthrones (e.g. a solution in acetone and presence of atmospheric oxygen). They are important aglycones in species of *Cassia*, *Rheum* and *Rhamnus*; in this group the sennidins, aglycones of the sennosides (see formula), are among the best-known examples. Reidin A, B and C which occur in senna and rhubarb are heterodianthrones, i.e. composed of unlike anthrones, and involve aloe-emodin, rhein, chryophanol or physcion.

It will be noted that two chiral centres (at C-10 and C-10′) are present in the dianthrones, and for a compound having two identical anthrone moieties, e.g. sennidin A, two forms (the 10*S*, 10′*S* and 10*R*, 10′*R*) are possible together with the *meso* form (sennidin B). These compounds also occur in the plant as their 1,1′-diglucosides.

Aloin-type or *C*-glycosides

The aloin obtained from species of *Aloe*, although one of the first glycosides to be isolated, was a problem for investigators for a long time. It is strongly resistant to normal acid hydrolysis but may be oxidized with ferric chloride. A study of its degradation products and infrared spectrum indicated a sugar-like chain and the structure shown, in which the sugar is joined to the aglycone with a direct C–C linkage (a *C*-glycoside). Two aloins (A and B) are known and arise from the chiral centre at C-10; their separation by high-speed countercurrent chromatography (see Chapter 17) has been recently described (C. XueLi *et al.*, *J. Chrom. and Rel. Technol.*, 2007, **30**, 12).

Pharmacological action

The action of the anthraquinone laxatives is restricted to the large bowel; hence their effect is delayed for up to 6 h or longer. The nature of the peristaltic initiation is not known for certain but it has been suggested that the common anthraquinone and anthranol derivatives influence the ion transport across colon cells by inhibition of Cl^- channels (J. Hönig *et al.*, *Planta Med.*, 1992, **58** (Suppl. 1), A586).

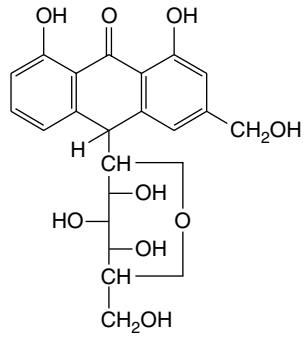
SENNA LEAF

Senna (*Sennae Folium*) consists of the dried leaflets of *Cassia senna* L. (*C. acutifolia* Delile), which are known in commerce as Alexandrian or Khartoum senna, and of *Cassia angustifolia* Vahl, which are known in commerce as Tinnevelly senna. The senna plants are small shrubs of the family Leguminosae, about 1 m high, with paripinnate compound leaves. *C. senna* is indigenous to tropical Africa and is cultivated in the Sudan (Kordofan, Sennar). *C. angustifolia* is indigenous to Somaliland, Arabia, Sind and the Punjab, and is cultivated in South India (Tinnevelly). The botanical validity for distinguishing between the above two plants has been called in question (Brenan, *Kew Bull.*, 1958, 231), but Fairbairn and Shrestha (*Lloydia*, 1967, **30**, 67) reinvestigated the well-established character differences between the two commercial types (see below) and concluded that the distinction remains valid; any further investigation on the two varieties grown under identical conditions does not appear to have been reported.

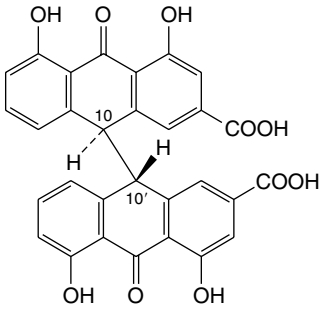
History. Senna appears to have been used since the ninth or tenth century, its introduction into medicine being due to the Arabian physicians, who used both the leaves and the pods. It was formerly exported through Alexandria, from where the name of the Sudanese drug is derived.

Collection and preparation. Alexandrian senna is collected mainly in September, from both wild and cultivated plants. The branches bearing leaves and pods are dried in the sun and conveyed to Omdurman. Here the pods and large stalks are first separated by means of sieves (see 'Senna Fruit'). That which has passed through the sieves is then 'tossed' in shallow trays, the leaves working to the surface and heavier stalk fragments and sand to the bottom. The leaves are then graded, partly by means of sieves and partly by hand-picking into (1) whole leaves, (2) whole leaves and half-leaves mixed, and (3) siftings. The whole leaves are those usually sold to the public, while the other grades are used for making galenicals. The drug is packed, somewhat loosely, in bales and sent by rail to Port Sudan, from where it is exported.

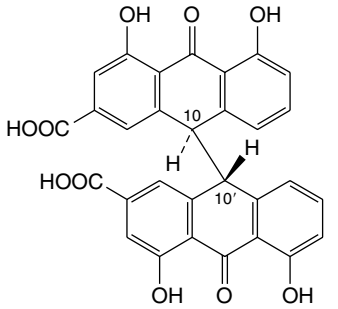
Tinnevelly senna is obtained from cultivated plants of *Cassia angustifolia* grown in South India, N.W. Pakistan and Jammu, where the plants are more luxuriant than those found wild in Arabia. It may be grown either on dry land or in wetter conditions as a successor to rice. Being a legume, it usefully adds nitrogen to the soil. Owing to the careful way in which the drug is collected and compressed into bales, few leaflets are usually broken.



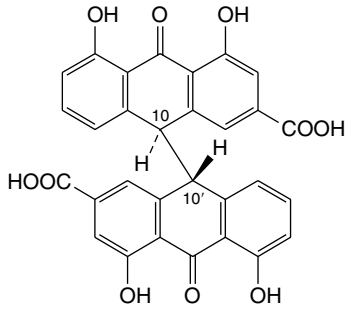
Aloin



Sennidin A (10*S*, 10′*S*)



Sennidin A (10*R*, 10′*R*)



Sennidin B (*meso*)

Macroscopical characters. Senna leaflets bear stout petiolules. The lamina has an entire margin, an acute apex, and a more or less asymmetric base. The surfaces are pubescent. Odour, slight but characteristic; taste, mucilaginous, bitterish and unpleasant.

Typical senna leaflets are shown in Fig. 21.10. The main differences between the two varieties are given in Table 21.4.

Microscopical characters. Senna leaflets have an isobilateral structure (see Fig. 41.4). The epidermal cells have straight walls, and many contain mucilage. Both surfaces bear scattered, unicellular, non-lignified warty hairs up to 260 μm long (Fig. 21.10D, G). The stomata have two cells with their long axes parallel to the pore and sometimes a third or fourth subsidiary cell (Fig. 21.10E, F). The mesophyll, consisting of

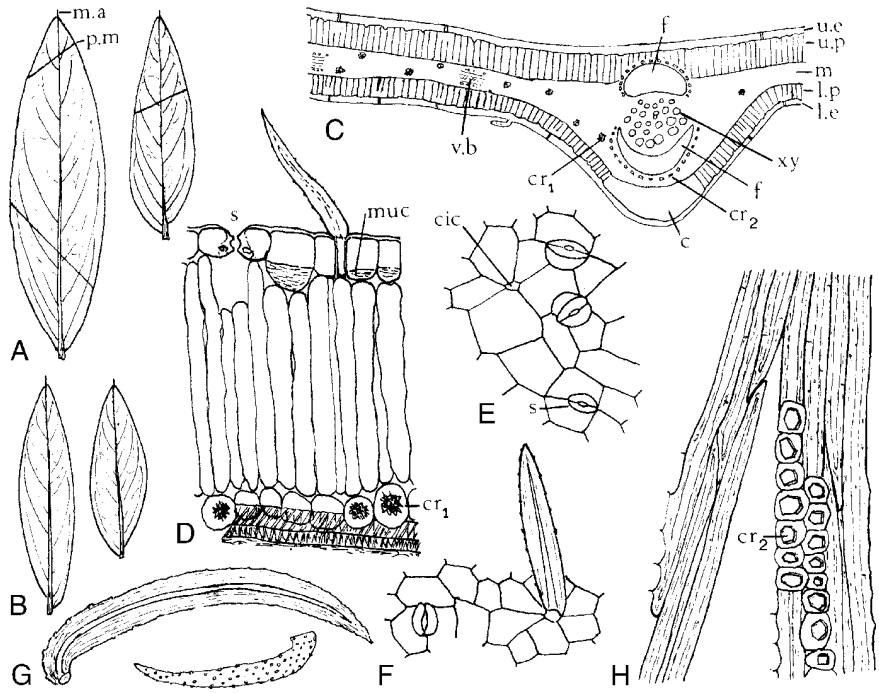


Fig. 21.10
Senna leaflets. A, Indian senna; B, Alexandrian senna (both ×1); C, transverse section of leaflet (×80); D–H, elements of the powder (all ×200); D, leaflet fragment in transverse section; E, F, epidermal fragments in surface view; G, isolated trichomes; H, portion of fibre group with crystal sheath, c, collenchyma; cic, cicatrix; cr_1, cr_2, calcium oxalate crystals of the cluster and prismatic type respectively; f, fibre groups; l.e, lower epidermis; l.p, lower palisade layer; m, mesophyll; muc, mucilage; m.a, mucronate apex; p.m, press mark; s, stoma (paracytic type); u.e, upper epidermis; u.p, upper palisade layer; xy, xylem.

Table 21.4 Comparison of Alexandrian and Indian senna leaves.

Alexandrian senna	Tinnevelly senna
Macroscopical characters	
Seldom exceed 40 mm in length	Seldom exceed 50 mm in length
Greyish-green	Yellowish-green
More asymmetric at base	Less asymmetric at base
Rather more broken and curled at the edges	Seldom broken and usually flat owing to compression
Few press markings	Often shows impressions due to the midvein of other leaflets
Microscopical characters	
Hairs more numerous, the average distance between each being about three epidermal cells	Hairs less numerous, the average distance between each being about six epidermal cells
Most of the stomata have two subsidiary cells only	The stomata having two or three subsidiary cells respectively are in the ratio of about 7:3
Vein-islet number 25–29.5	Vein-islet number 19.5–22.5
Stomatal index 10.0–15.0, usually 12.5	Stomatal index 14.0–20.0, usually 17.5
Chemical tests*	
Ether extract of hydrolysed acid solution of drug gives with methanolic magnesium acetate solution: a pink colour in daylight, a pale greenish-orange in filtered ultraviolet light	The same test: an orange colour in daylight, a yellowish-green in filtered ultraviolet light
TLC test for distinctive naphthalene derivatives†	
6-Hydroxymusizin glycoside present	Tinnevellin glycoside present

*For full details, see Nandy and Santra, *J. Ind. Pharm. Manuf.*, 1968, **6,** 235.
†See Lemli *et al.*, *Planta Med.*, 1983, **49,** 36.

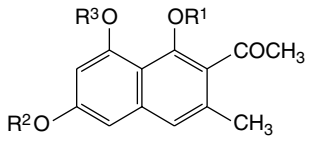
upper and lower palisade layers and median spongy mesophyll, contains cluster crystals about 15–20 µm in diameter. The midrib is biconvex. Below the midrib bundle is a zone of collenchyma. The midrib bundle and larger veins are almost surrounded by a zone of lignified pericyclic fibres and a sheath of parenchymatous cells containing prisms of calcium oxalate 10–20 µm long (Fig. 21.10 C).

Vein-islet numbers and stomatal indices can be used to distinguish the two species (see Table 21.4) and the *BP*/*EP* utilizes stomatal index.

Constituents. Since Tutin first isolated aloe-emodin and rhein in 1913, many other compounds based on these two have been obtained. Stoll *et al.* (1941) isolated two active crystalline glycosides, sennoside A and sennoside B. They both hydrolyse to give two molecules of glucose and the aglycones sennidin A and B. Sennidin A is dextrorotatory and B is its mesoform formed by intramolecular compensation (Fig. 21.12).

The activity of senna was still not fully explained by the isolation of these constituents, and later work, notably by Fairbairn, Friedrich, Friedmann, Lemli and their associates demonstrated the presence of many other (some pharmacologically active) components. These include: sennosides C and D, which are the glycosides of heterodianthrones involving rhein and aloe-emodin; palmidin A (see 'Rhubarb'); aloe-emodin dianthrone-diglycoside, rhein-anthrone-8-glycoside, rhein-8-diglucoside, aloe-emodin-8-glucoside, aloe-emodin-anthrone-diglucoside, possibly rhein-1-glucose, and a primary glycoside having greater potency than sennosides A and B and distinguished from them by the addition of two glucose molecules. A new anthraquinone glycoside, emodin-8-*O*-sophoroside (a diglucoside), has been isolated in 0.0027% yield from dried Indian senna leaves (J. Kinjo *et al.*, *Phytochemistry*, 1994, **37**, 1685).

Two naphthalene glycosides isolated from senna leaves and pods (Lemli *et al.*, *Planta Med.*, 1981, **43**, 11) are 6-hydroxymusizin glucoside and tinnevellin glucoside. The former is found in Alexandrian senna and the latter in Indian senna; this difference has been used as a



6-Hydroxymusizin glucoside:
$R^1 = R^2 = H$; R^3 = β-D-glucopyranosyl
Tinnevellin glucoside:
$R^1 = H$; R^2 = β-D-glucopyranosyl; R^3 = Me

distinguishing feature of the commercial varieties, see Table 21.4. Senna also contains the yellow flavonol colouring matters kaempferol (3,4′,5,7-tetrahydroxyflavone), its glucoside (kaempferin) and isorhamnetin; also a sterol and its glucoside, mucilage, calcium oxalate and resin. The structures of water-soluble polysaccharides and a lignan have been reported.

Although senna is not noted for its volatile components, Tutin in his 1913 publication had observed the 'strongly aromatic dark-coloured essential oil'. Over 80 years later W. Schulz *et al.* (*Planta Medica*, 1996, **62**, 540) have again examined the volatiles of senna leaf and recorded (GC-MS) more than 200 components afforded by aqueous distillation. 122 constituents were identified including monoterpenes, phenylpropanes, fatty acids and esters, etc. Hexadecanoic acid was a significant component in addition to many of the more common constituents of volatile oils.

Formation and distribution of anthraquinone derivatives. In young senna seedlings chrysophanol is the first anthraquinone formed, then aloe-emodin appears and finally rhein; this ontogenetic sequence is in keeping with the expected biogenetic order, which involves the successive oxidation of the 3-methyl group of chrysophanol (Table 21.3). In the presence of light glycosylation follows and later the glycosides are translocated to the leaves and flowers. During fruit development the amounts of aloe-emodin glycoside and rhein glycoside fall markedly, and sennosides accumulate in the pericarp.

Lemli and Cuveele (*Planta Med.*, 1978, **34**, 311) considered that fresh leaves of *Cassia senna* contain anthrone glycosides only. By drying between 20 and 50°C these are enzymatically converted to dianthrone forms (sennosides). However, Zenk and coworkers (*Planta Med.*, 1981, **41**, 1) maintained that sennoside formation is not entirely an artefact arising through drying but that these compounds together with the monoanthrones, and their oxidized forms (anthraquinones), are part of a redox system of possible significance to the living cell.

The distribution of sennoside B (determined by Zenk and coworkers by immunoassay) was for a *C. angustifolia* plant (sample dried at 60°C): flowers 4.3%, leaves 2.8%, pericarp 2.4%, stems 0.2%, roots 0.05%. Within the flowers the anthers and filaments contained 7.2%, carpels and ovaries 5.8%, petals 5.2%, sepals 4.7% and flower stalks 3.2%.

Evaluation. It is difficult to remove all fragments of rachis, petiole and stalk from the drug, but the amount of these structures is limited by the *BP* to 3%. In the whole drug the percentage of these is determined by hand-picking and weighing, but with the powdered drug recourse has to be made to quantitative microscopy.

C. senna is cultivated in Russia and the leaves are harvested mechanically; this leads to unavoidable mixture with petioles and stems but, because the active constituents are similar in all parts of the plant, this does not affect the quality of the glycosidal extracts.

Lack of knowledge of the precise active principles of senna coupled with the synergistic action of various compounds hampered the development of a satisfactory chemical assay for the drug. The *BP*/*EP* determines the total senna leaf glycosides in terms of sennoside B (not less than 2.5%). This involves extraction of the glycosides and free anthraquinones from the leaves, removal of the free aglycones and hydrolysis and oxidation of the remaining sennosides and other glycosides to give rhein and some aloe-emodin, which are then determined spectrophotometrically. Chromatographic tests for the leaf are given in the *BP* and *EP*.

The leaves are officially required to give an acid-insoluble ash of not more than 2.5%.

Allied drugs. *Bombay*, *Mecca* and *Arabian Sennas* are obtained from wild plants of *C. angustifolia* grown in Arabia. Some of the leaflets are shipped to Port Sudan and are graded like the Alexandrian drug, while some are sent to Bombay and frequently arrive in England with shipments of the Tinnevelly.

The leaflets resemble those of Tinnevelly senna but are somewhat more elongated and narrower, and of a brownish or brownish-green colour. Levin (1929) states that they may be distinguished microscopically from other sennas by their vein islet number.

Dog senna, a variety formerly much esteemed and still used in France, is derived from *Cassia obovata*. The plant is indigenous to Upper Egypt, but was cultivated in Italy in the sixteenth century. The leaves are obovate and quite different in appearance from the official leaflets. When in powder they may be distinguished by the papillose cells of the lower epidermis. Maurin found them to contain 1.0–1.15% of anthraquinone derivatives.

Palthé senna, derived from *Cassia auriculata*, has been found in Indian senna. It may be distinguished by the long hairs, the crimson

colour given when boiled with chloral hydrate solution or treated with 80% sulphuric acid and the absence of anthraquinone derivatives. The leaves of other parts of the plant are widely used in Ayurvedic medicine for rheumatism and diabetes. The antioxidant activity of the flowers has been recently demonstrated (L. Pari and M. Latha, *Pharm. Biol.*, 2002, **40**, 512; A. Kumaran and R. J. Karunakaran, *Fitoterapia*, 2007, **78**, 46).

The leaflets of other species of *Cassia* have also been imported, but may be distinguished from the genuine drug by the characters given above.

For Nigeria, the leaves of the local *Cassia podocarpa* have been suggested as a substitute for the official senna; bioassays have given an equivalent activity (A. A. Elujoba and G. O. Iweibo, *Planta Med.*, 1988, **54**, 372).

C. alata produces anthraquinone derivatives and has been used traditionally in Thailand as a laxative. Root cultures have been studied for their anthraquinone-producing properties (N. Chatsiriwej *et al.*, *Pharm. Biol.*, 2006, **44**, 416).

Substitute. *Argel leaves*, which are derived from *Solenostemma arghel* (Asclepiadaceae), were at one time regularly mixed in a definite proportion with Alexandrian senna. The plant occurs in the Sudan, but the leaves are now seldom seen in commerce. If used to adulterate senna powder, it may be distinguished by the two- or three-celled hairs, each of which is surrounded by about five subsidiary cells.

SENNA FRUIT

Senna pods (*Sennae Fructus*) are the dried, ripe fruits of *C. senna* and *C. angustifolia* (Leguminosae), which are known as Alexandrian and Tinnevelly senna pods, respectively. Both have separate monographs in the *BP*/*EP*.

Collection. The pods are collected with the leaves and dried as described above. After separation from the leaves they are hand-picked into various qualities, the finer being sold in cartons and the inferior ones used for making galenicals.

Characters. The characteristic sizes and shapes of the two varieties are shown in Fig. 21.11. The Tinnevelly pods are longer and narrower than the Alexandrian and the brown area of pericarp surrounding the seeds is greater. The remains of the style are distinct in the Tinnevelly but not in the Alexandrian.

After soaking in water the pods are readily opened and about six wedge-shaped seeds are disclosed, each attached to the dorsal surface of the pod by a thin funicle (Fig. 21.11C). Under a lens the testas of

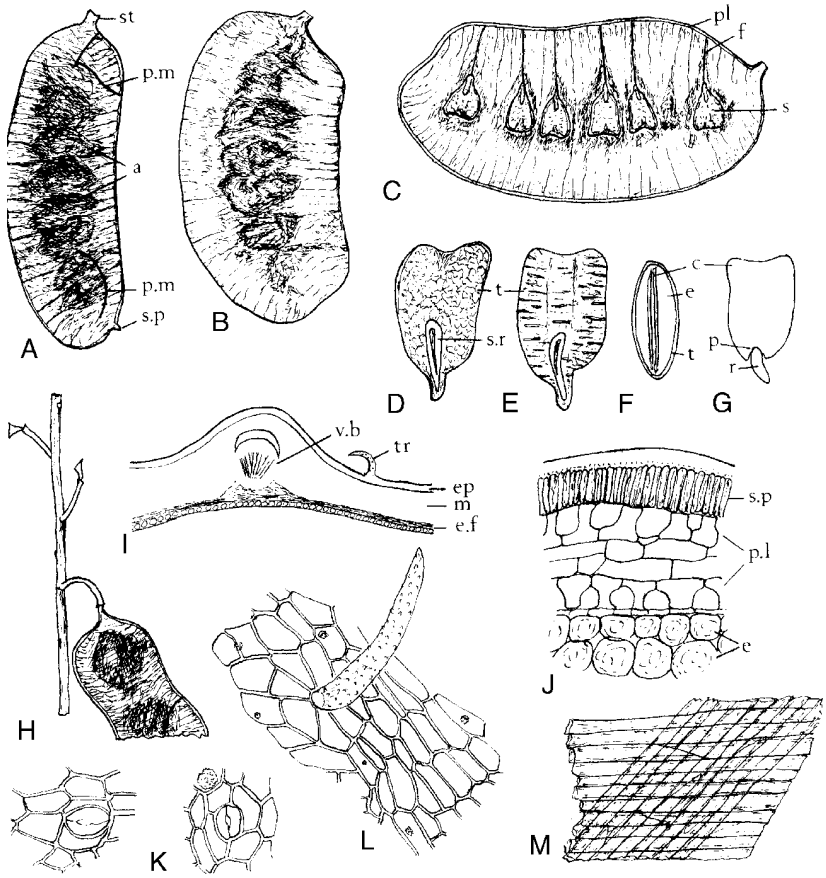
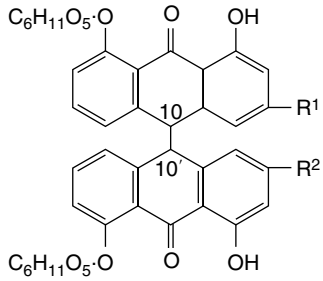


Fig. 21.11
Senna fruits. A, Tinnevelly fruit; B, Alexandrian fruit; C, Alexandrian pod opened to show seeds (all ×1); D, seed of Alexandrian fruit; E, seed of Tinnevelly fruit; F, transverse section of seed; G, isolated embryo with one cotyledon removed (all ×4); H, stem with Tinnevelly fruit attached (×1); I, transverse section of pericarp (×90;) J, transverse section of seed coat; K, fragments of epidermis with stomata; L, fragment of epidermis with trichome; M, fibrous layers from endocarp in surface view (all ×200); a, brown areas of pericarp covering seeds; c, cotyledons; e, endosperm; e.f, fibrous endocarp; ep, epicarp; f, funiculus; m, mesocarp; p, plumule; pl, placenta; p.l, parenchymatous layers of testa; p.m, press marks from other pods; r, radicle; s, seed; st, stalk; s.p, (J) subepidermal palisade; s.p, (A), stylar point; s.r, spathate ridge; tr, trichome; v.b, vascular bundle partially enclosed by fibres.



	R^1	R^2	10–10′
Sennoside A	COOH	COOH	*trans*
Sennoside B	COOH	COOH	*meso*
Sennoside C	CH_2OH	COOH	*trans*
Sennoside D	CH_2OH	COOH	*meso*
Aloe-emodin-dianthrone-diglucoside	CH_2OH	CH_2OH	*trans*
Aloe-emodin-dianthrone-diglucoside	CH_2OH	CH_2OH	*meso*

Fig. 21.12
Constituents of Senna.

the Tinnevelly show a general reticulation and wavy, transverse ridges, while the Alexandrian show a general reticulation only (Fig. 21.11D, E). The pericarp of the pod bears unicellular hairs and stomata of a type similar to those found on senna leaves; portions of the fibrous layer of the endocarp are very evident in the powder (Fig. 21.11K, L, M).

Constituents. The active constituents of the pods are located in the pericarp; they are similar to those of the leaves, together with sennoside A, which constitutes about 15% of the sennoside mixture. The seeds contain very little sennoside but Zenk's group reported the cotyledons of 3-day-old seedlings to contain amounts equivalent to those in the leaves. Sennoside content varies from about 1.2 to 2.5% in the Tinnevelly (*BP*/*EP* ⩽2.2%) and from about 2.5 to 4.5% in the Alexandrian (*BP*/*EP* ⩽3.4%). C. Terreaux *et al.* (*Planta Medica*, 2002, **68**, 349) have reported the isolation of kaempferol and tinnevellin 8-glucoside from an extract of the Tinnevelly pods together with two new carboxylated benzophenone glucosides. Preparations of the powdered pericarp, e.g. Senna Tablets *BP*, standardized in terms of sennoside B, are now commonly prescribed.

Uses. The use of laxatives is increasing and senna constitutes a useful purgative for either habitual constipation or occasional use. It lacks the astringent after-effect of rhubarb. Despite the availability of a number of synthetics, sennoside preparations remain among the most important pharmaceutical laxatives.

Cassia pods

Cassia pods are the dried ripe fruits of *Cassia fistula* (Leguminosae), a large tree thought to be indigenous to India but now widely cultivated in the tropics. The drug is chiefly obtained from the West Indies (Dominica and Martinique) and Indonesia.

The fruit is a cylindrical indehiscent pod about 25–30 cm long and 20–25 mm diameter. It is dark chocolate brown to black in colour and contains from 25 to 100 oval, reddish-brown seeds separated by membranous dissepiments. In the fresh pods the seeds are completely embedded in black pulp, which, however, gradually dries on the septa. For this reason pods which do not rattle when shaken are usually preferred. The pulp has a prune-like odour and a sweetish taste.

The pulp is dissolved from the crushed fruit by percolation with water. The percolate is strained and evaporated to a soft extract. The most important anthraquinone derivatives of the pulp appear to be rhein and combined sennidin-like compounds. Anthraquinones have been detected in non-differentiating callus cultures. Cassia pulp also contains about 50% of sugars, colouring matter and a trace of volatile oil. The leaves of *C. fistula* contain free and combined rhein, sennidins and sennosides A and B; these compounds exhibit a marked seasonable fluctuation. The heartwood is reported to contain barbaloin and rhein together with a leucoanthocyanidin.

Cassia pulp was formerly used in the form of Confection of Senna. In Ayurvedic medicine the plant is used to treat a variety of ailments. Its antifungal, antibacterial and laxative properties have been established and more recently (T. Bhakta *et al.*, *Pharm. Biol.*, 1998, **36**, 140) its antitussive activity has been demonstrated.

CASCARA BARK

Official cascara sagrada (*Sacred Bark, Chittern Bark*) is the dried bark of *Rhamnus purshianus* DC (*Frangula purshiana* (DC) A. Gray ex J. C. Cooper) (Rhamnaceae). The bark is collected from wild trees, which are 6–18 m high, growing on the Pacific coast of North America (British Columbia, Washington and Oregon). Depleted wild US sources encouraged cultivation of the tree in western Canada, the USA and Kenya but these efforts do not appear to be completely successful.

History. Cascara is a drug of comparatively recent introduction into modern medicine. According to tradition, a cascara, probably *R. californica*, was known to early Mexican and Spanish priests of California; *Rhamnus purshianus*, however, was not described until 1805 and its bark was not introduced into medicine until 1877.

The common, European buckthorn was well known to the Anglo-Saxons; its berries were official in the *London Pharmacopoeia* of 1650.

Collection and storage. The bark is collected from mid-April to the end of August, when it separates readily from the wood. Longitudinal incisions about 5–10 cm apart are first made in the trunk and the bark removed. The tree is then usually felled and the branch bark separated. The pieces are dried in the shade with the cork uppermost. Such material is referred to as 'natural' cascara but commercial supplies are now comminuted to give small even fragments known as 'evenized', 'processed' or 'compact' cascara. During preparation and storage the

bark must be protected from rain and damp, or partial extraction of the constituents may occur or the bark may become mouldy. That the bark must be kept for at least 1 year before use is no longer a *BP* requirement but the bark appears to increase in medicinal value and price until it is about 4 years old. Many companies prefer to use bark which has been stored for considerably more than 1 year. To reduce freight and handling charges on the bark, large quantities of the extract are now imported directly.

Macroscopical characters. The bark occurs in quills, or channelled or nearly flat pieces. All of these forms may attain 20 cm in length and are 1–4 mm thick, the thinner bark being most esteemed. The flat strips from the trunk are usually much wider (up to 10 cm) than the quills or channelled pieces (about 5–20 mm) obtained from the branches.

Cascara (Fig. 21.13A) bears a somewhat patchy, silvery-grey coat of lichens. Pieces bearing moss are also quite common. Between the patches of lichen may be seen a smooth, dark purplish-brown cork marked with lighter-coloured, transversely elongated lenticels. On scraping the cork, no bright purple inner cork is disclosed (distinction from *R. alnus*). The inner surface is dull purplish-brown to black, striated longitudinally and somewhat corrugated transversely. The fracture is short and granular in the outer part, but somewhat fibrous in the phloem. In the yellowish-brown cortex and phloem lighter groups of sclerenchymatous cells and phloem fibres may be seen with a lens. They may be made more distinct by staining with phloroglucinol and hydrochloric acid. The medullary rays, which tend to curve together in groups, are well seen in sections mounted in potash. Odour, slight but characteristic; taste bitter.

Microscopical characters. A transverse section of cascara bark (Fig. 21.13B–D) shows a partial coat of whitish lichen, some 10–15 layers of flattened cork cells with reddish-brown contents and a cortex composed of collenchyma, parenchyma and groups of sclereids. The collenchymatous cells show thickened pitted walls and contain chloroplasts filled with starch. Some of the parenchymatous cells also contain chloroplasts and starch; many of them contain a yellow substance coloured violet by alkalis and rosette crystals of calcium oxalate usually 6–10 µm diameter, but occasionally up to 45 µm diameter. The parenchymatous cells abutting on the groups of sclereids contain prisms of calcium oxalate. The sclereids are irregular or ovoid in

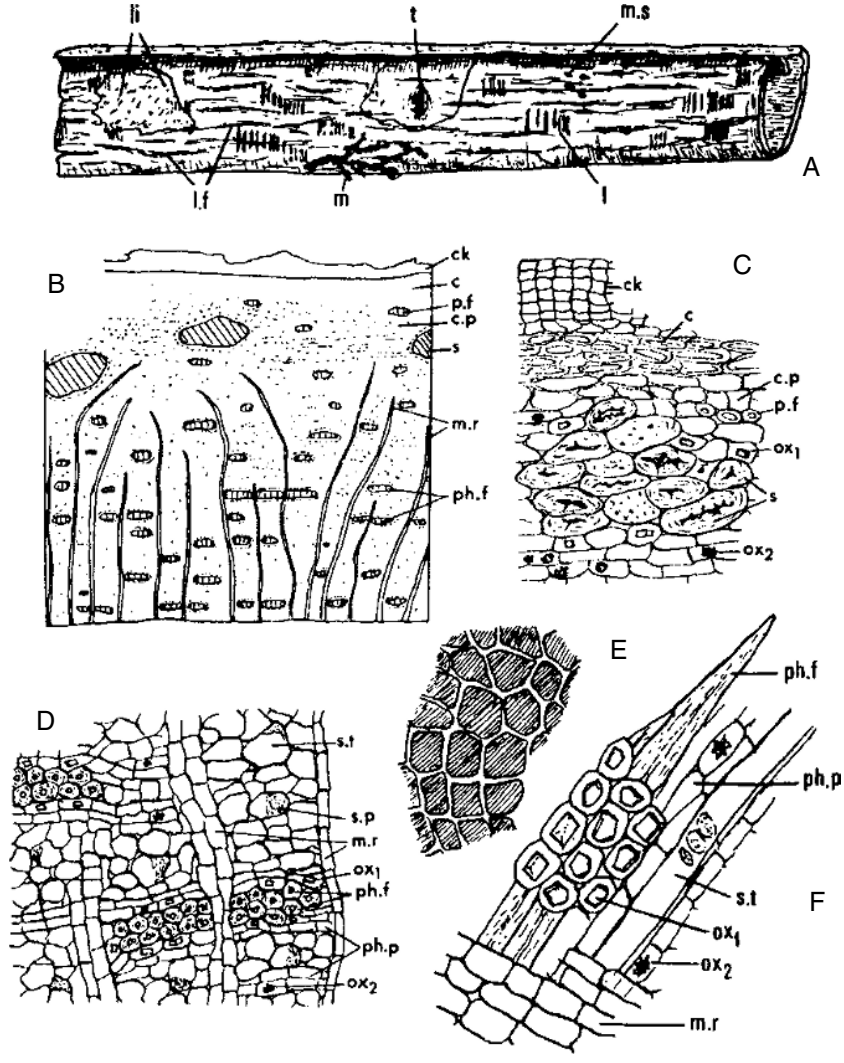


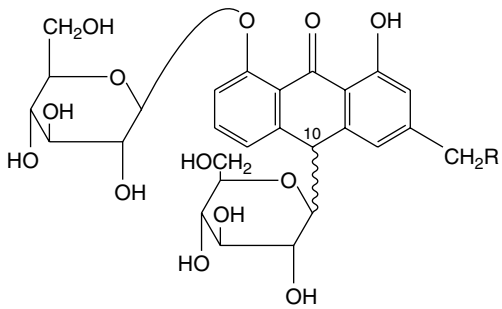
Fig. 21.13
Cascara bark. A, single quill (×0.66); B, general diagram of transverse section of bark (×20); C, transverse section of outer tissues; D, ditto of phloem (both × 200); E, cork cells in surface view; F, fragment of phloem from powder (both × 250); c, collenchyma; ck, cork; c.p, cortical parenchyma; l, lenticel; li, lichen patch; l.f, longitudinal furrows; m, moss; m.r, medullary ray; m.s, mussel scale; ox_1, ox_2, prismatic and cluster crystals of calcium oxalate respectively; p.f, pericyclic fibres; ph.f, phloem fibres; ph.p, phloem parenchyma; s, sclereids; s.p, sieve plate; s.t, sieve tube; t, scar of twig.

shape, are variable in size, and have thick lignified walls sometimes showing stratification and traversed by funnel-shaped pits. A pericycle is not clearly delimited, but the zone immediately outside the phloem in which sclereids and occasional fibres occur is regarded as representing this region. The phloem is composed of zones of tangentially elongated groups of phloem fibres, enclosed in a sheath of parenchymatous cells containing prisms of calcium oxalate which alternate with sieve tubes and phloem parenchyma (Fig. 21.13F). The individual fibres are yellow in colour, are 8–15 μm in diameter, and have thick lignified walls showing stratification and pit canals. The sieve tubes show sieve plates, each with several sieve fields, on the radial walls. The sieve plates are usually covered with a deposit of callus and can be identified after staining with alkaline solution of corallin. The phloem parenchyma resembles that of the cortex, containing plastids, starch, material coloured violet by alkali, and rosettes of calcium oxalate. The medullary rays are 1–5 cells wide and 15–25 cells deep. The medullary ray cells are parenchymatous, somewhat radially elongated and with similar contents to those of the parenchyma; their content of material stained violet by alkali is often high. Fragments of moss leaves and liverworts are usually found in the powder.

Constituents. It has long been recognized that cascara bark stored for at least 1 year gave galenicals which were better tolerated but as effective as those prepared from more recently collected bark. This is presumably due to hydrolysis or other changes during storage. It was also found at an early date that the very bitter taste of cascara is reduced by treating extracts with alkalis, alkaline earths or magnesium oxide. Proprietary extracts of this type became very popular and pharmacopoeias followed the same idea to produce such preparations.

Cascara contains about 6–9% anthracene derivatives which are present both as normal *O*-glycosides and as *C*-glycosides. The following groups of constituents are now manifest.

1. Four primary glycosides or cascarosides A, B, C and D; they contain both *O*- and *C*-glycosidic linkages. Their structures were elucidated in 1974 by Wagner *et al.* as the *C*-10 isomers of the 8-*O*-β-D-glucopyranosides of aloin and chrysophanol. A. Griffini *et al.* (*Planta Med.*, 1992, **58**, Suppl. 1, A593) described the isolation of the pure cascarosides by silica-gel chromatography and HPLC. The complete assignments of ^{1}H- and ^{13}C-NMR signals for these cascarosides were recorded by Manitto *et al.* (*J. Chem. Soc. Perk. Trans I*, 1993, 1577) and the same group (*J. Nat. Prod.*, 1995, **38**, 419) have now isolated two analogous glycosides (cascarosides E and F) derived from emodin (Table 21.3).



Cascarosides of *Rhamnus purshianus*,
Configurations; Cascaroside A = 10β, R = OH; B = 10α, R = OH;
C = 10β, R = H; D = 10α, R = H.

2. Two aloins, barbaloin derived from the aloe-emodin anthrones and chrysaloin derived from chrysophanol anthrone. These C-glycosides are probably breakdown products from (1). Also 10-hydroxyaloins A and B (H. W. Rauwaled *et al.*, *Z. Naturforsch*, Teil B, 1991, **46**, 551).
3. A number of *O*-glycosides derived from emodin oxanthrone, aloe-emodin and chrysophanol.
4. Various dianthrones, including those of emodin, aloe-emodin and chrysophanol and the heterodianthrones palmidin A, B and C (see 'Rhubarb'). These dimers are also formed during the preparation and conservation of elixirs and may constitute up to 20% of the total anthracene glycosides (see P. de Witte *et al.*, *Planta Med.*, 1991, **57**, 440).
5. Aloe-emodin, chrysophanol and emodin in the free state.

The primary glycosides are more active than the aloins whereas the free anthraquinones and dimers have little purgative activity. The cascarosides have a sweet and more pleasant taste than the aloins. The *BP*/*EP* requires the bark to contain not less than 8.0% of hydroxyanthracene glycosides of which not less than 60% consists of cascarosides, calculated as cascaroside A. A two-point spectro-photometric assay is employed with absorbance measurements at 515 nm and 440 nm.

Experiments by Betts and Fairbairn in 1964, although based on a single *fresh* plant, suggested that free anthraquinones are formed by the leaves and that they are stored in the bark mainly as *C*-glycosides, the older bark containing the most *C*-glycosides. *Rhamnus purshianus* cell suspension cultures will produce anthracene derivatives in which the accumulation of these compounds, particularly emodin, is significantly raised by a 12 h light/dark cycle; continuous illumination of the cultures suppresses anthraquinone formation.

Substitutes. Several species of *Rhamnus* have a similar geographical distribution to that of *R. purshianus*. These include *R. alnifolia*, which is too rare to be a likely substitute; *R. crocea*, whose bark bears little resemblance to the official drug, and *R. californica* Esch. The latter is so closely related to *R. purshianus* that some botanists do not divide them into separate species. The plant appears to have a much more southerly distribution than the typical *R. purshianus* and is therefore unlikely to occur in bark of Canadian origin. It has a more uniform coat of lichens and wider medullary rays than the official species, but resembles the latter in having sclerenchymatous cells. The bark of *R. fallax* has been recorded as a cascara substitute. European frangula bark, distinguished by the *BP*/*EP* TLC test, is described below.

Uses. Cascara is a purgative resembling senna in its action. It is mainly used in the form of liquid extract or elixir or as tablets prepared from a dry extract. It is also used in veterinary work.

FRANGULA BARK

Frangula bark, alder buckthorn, is obtained from *Rhamnus frangula* L. (*Frangula alnus* Mill) (Rhamnaceae), a shrub 3–5 m high and found in Britain and Europe. Commerical supplies are available from Balkan countries and a little from Russia. The plant differs from the common buckthorn, *R. cathartica*, in that it does not possess thorns; it bears dark-purple berries whose medicinal properties have long been accepted. Although much used in England, the demand decreased with the increased popularity of cascara; on the Continent, particularly in France, cascara has not replaced it to the same extent.

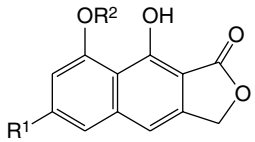
The bark, included in the *BP*/*EP*, is required to contain not less than 7.0% glucofrangulins calculated as glucofrangulin A.

The bark occurs in single or double quills which are usually of smaller size than those of cascara and about 0.5–2 mm thick. It has a purplish cork and transversely elongated, whitish lenticels. On removing the outer cork cells by scraping, a dark crimson inner cork is exposed. The transverse section closely resembles that of cascara but groups of sclerenchymatous cells are absent.

Frangula contains anthraquinone derivatives present mainly in the form of glycosides. The rhamnoside franguloside, or frangulin, was

isolated in 1857. This is now known to consist of two isomers, frangulosides A and B, formed by partical hydrolysis of the corresponding rhamnoglucosides, glucofrangulins A and B (Table 21.3). The fresh bark also contains anthranols and anthrones, which are unstable and readily oxidize to the corresponding anthraquinones; Lemli (1965, 1966) detected emodin-dianthrone, palmidin C (see 'Rhubarb'), palmidin C monorhamnoside and emodin-dianthrone monorhamnoside. Wagner *et al.* characterized frangulin B as 6-*O*-(D-apiofuranosyl)-1,6,8-trihydroxy-3-methylanthraquinone and more recently reported the new glycoside emodin-8-*O*-β-gentiobioside.

Allied drugs. The common buckthorn, *Rhamnus cathartica*, has a glossy reddish-or greenish-brown cork and does not possess sclereids. It contains frangula-emodin and a glycoside, rhamnicoside, which yields on hydrolysis rhamnicogenol (an anthraquinone derivative), glucose and xylose. Rauwald and Just (*Planta Med.*, 1981, **42**, 244) reported the isolation of the anthraquinone glycoside alaternin; 1,2,6,8-tetrahydroxy-3-methyl-anthraquinone; physcion; chrysophanol; and frangula-emodin. The bark also contains a number of blue-fluorescent substances which in the chromatograms produced by the *BP*/*EP* tests of identity for Cascara and Frangula serve to distinguish this adulterant. The fluorescent substances have recently been identified as naphtholide glycosides of the sorigenin type, as below.



α-Sorigenin glycoside: $R^1 = OCH_3$ R^2 = glucose
α-Sorigenin-primeveroside: $R^1 = OCH_3$ R^2 = glucose–xylose
β-Sorigenin-primeveroside: $R^1 = H$ R^2 = glucose–xylose

The bark of *R. carniolica* has a dull reddish cork and differs from frangula bark in that it possesses sclerenchymatous cells and has wider medullary rays. In recent years the barks of a number of Turkish species of Rhamnus have been systematically examined for their anthraquinone and flavonoid contents (see M. Koskun, *Int. J. Pharmacognosy*, 1992, **30**, 151 and references cited therein).

RHUBARB

Rhubarb (Chinese Rhubarb) consists of the dried underground parts of *Rheum palmatum* L. (Polygonaceae) or *R. officinale* Baillon or hybrids of these two species, or mixtures of these. The drug appears still to be obtained from both wild and cultivated plants grown on the high plateaux of Asia from Tibet to south-east China. The *BP/EP* drug is required to contain not less than 2.2% of hydroxyanthraquinone derivatives calculated as rhein.

Species and commercial grades. The genus *Rheum* comprises about 50 species, which may be classified into two sections, the first including *R. palmatum* and *R. officinale*, and the second *R. rhaponticum*, *R. undulatum* and *R. emodi*. A systematic study is made unusually difficult by geography and by the tendency of cultivated plants to form hybrids such as *R. palmatum* × *R. undulatum* and *R. palmatum* × *R. emodi*. The exact morphological and chemical characters of such hybrid rhizomes appear not to have been described. Formerly most of the drug was derived from *R. palmatum* L. var. *tanguticum* Maximowicz and *R. officinale* H. Br. and was traditionally known in commerce as Shensi, Canton and high-dried rhubarb. *R. palmatum* and *R. palmatum* var. *tanguticum* now appear to be the chief sources. The best grade of the present-day drug corresponds to that formerly known as Shensi rhubarb. Another present-day grade is similar to the old Canton. In addition, some inferior drug is exported, much of which fails to give the pink fracture characteristic of good-quality rhubarb.

In practice the grading system is more complex than the above might imply, and currently about a dozen grades of rhubarb are recognized by merchants. The grades commonly listed are: 'flat', 'common round', 'small round', 'extra small round', 'sticks', 'third grade' and lower qualities. The flat and round are further categorized on a percentage basis (e.g. 'flat 90%' or 'common round 80%'), depending on the pinkness and quality of the fracture. However, not all of these grades are necessarily available at any one time. Currently (2000) it is almost impossible to obtain rhubarb from China which meets *BP*/*EP* requirements for hydroxyanthraquinone derivatives. Demand for the better grades (CR & Flat 80 & 90%) is now almost exclusively for the beverage industry.

History. Chinese rhubarb has a long history. It is mentioned in a herbal of about 2700 BC and subsequently formed an important article of commerce on the Chinese trade routes to Europe. Today it still holds a place in medicine. The first international symposium on the drug was held in Chengde, China in 1990 under the title 'Rhubarb 90'.

Collection and preparation. Provided that the older accounts are still substantially correct, the rhizomes are grown at a high altitude (over 3000 m) dug up in autumn or spring when about 6–10 years old, decorticated and dried. The decorticated rhizomes are when whole roughly cylindrical ('rounds') or if cut longitudinally are in planoconvex pieces ('flats'). Pieces used often to show a hole indicating that they had been threaded on cords for drying.

The drug is exported from Shanghai to Tientsin, often via Hong Kong. The better qualities are packed in tin-lined wooden cases containing either 280 lb or 50 kg, and inferior quality in hessian bags.

Botanical characters of rhizome. The rhizomes of *R. palmatum* and *R. officinale* are similar in structure except for the size and distribution of the abnormal vascular bundles, 'star spots', of the pith. Transverse sections of both, after peeling, show phloem on the outside, cambium, radiate wood and a pith with 'star sports' (Fig. 21.14A, B). In *R. palmatum* the latter are relatively small (about 2.5 mm) and most of them are arranged in a continuous ring; in *R. officinale* the 'star spots' are larger (about 4 mm) and are irregularly scattered.

Macroscopical characters. Despite the large number of commercial grades, it is convenient to describe the various rhubarb types under three headings.

1. *High-grade, Chinghai* or *Shensi-type*. This drug occurs in rounds or flats weighing up to about 200 g and up to about 15 cm long, although usually smaller. Much of the present-day drug tends to be of small size and may therefore be obtained from younger plants than was formerly the case.

   The drug has a firm texture, non-shrunken appearance and a bright yellow surface showing whitish reticulations. These reticulations are due to the fusiform or lozenge-shaped cut ends of the closely arranged medullary rays (which are reddish-brown) seen against the white background of the phloem parenchyma. In the *palmatum* type the medullary rays are only about 6 cells deep, but in the *officinale* type they may be as much as 200 cells deep. This difference accounts for the fact that the surface of the *officinale* type gives the apperance of parallel red and white lines rather than a reticulation. In both species the appearance of the transverse surface varies according to the depth of peeling, which may extend into the radiate wood or even into the pith.

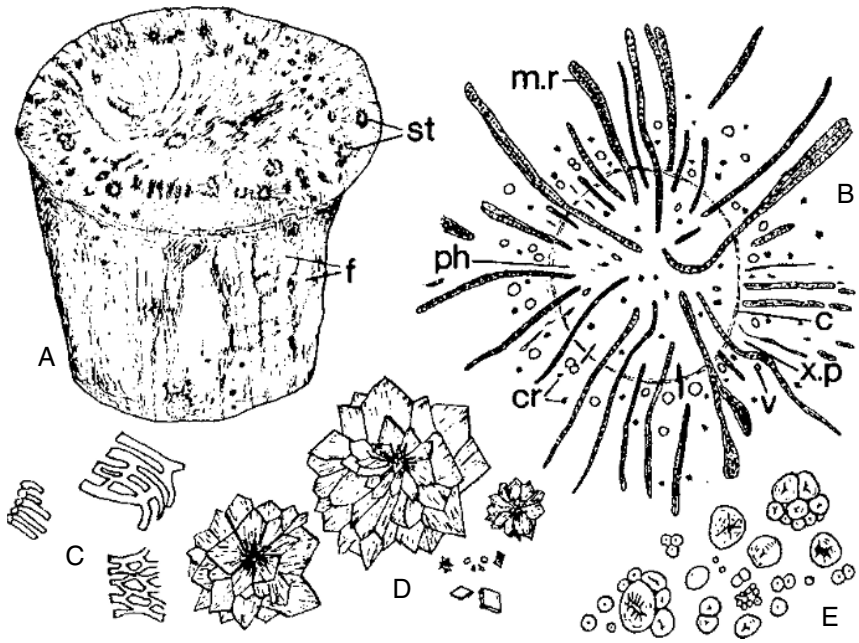


Fig. 21.14
Chinese rhubarb. A, common round (×0.5); B, star spot in transverse section (×20); C–E, fragments of the powder (×200); C, portions of reticulate vessels; D, calcium oxalate crystals; E, starch; c, cambium; cr, crystals; f, facets produced by peeling; m.r, medullary ray; ph, phloem; st, star spot; v, vessel; x.p, xylem parenchyma.

The best rhubarb breaks with a marbled or 'nutmeg' fracture, the freshly broken surface showing a bright pink colour—this is one character used in grading—see above. Such drug gives the bright yellow powder favoured by buyers. Particular attention is paid by the buyer not only to the colour of the fracture, but also to absence of signs of decay or insect attack. Odour, aromatic; taste, bitter and slightly astringent.

2. *Medium-grade or Canton-type*. This has the general characters of the drug described above but has been less carefully prepared. Some pieces are badly trimmed, greyish patches being left on the outer surface. The surface reticulations are less distinct, the fracture granular rather than marbled and the freshly fractured surface of a paler pink colour.
3. *Third grade*. This frequently consists of smaller pieces than in the higher grades. Only a small percentage of the fractured surfaces show a good pink fracture and the remainder a grey, mauve or brown one. These differences are shown in the colour of the powder.

Microscopy of powder. Powdered rhubarb (Fig. 21.14) is easily identified. It shows abundant calcium oxalate rosettes up to 200 μm in diameter; simple two to five compound starch grains; reticulate vessels and other wood elements which give no reaction for lignin. The yellow contents of the medullary ray cells (anthraquinone derivatives) become reddish-pink with ammonia solution and deep red with caustic alkalis.

Constituents. As with other anthraquinone-containing drugs, the chemical complexity of rhubarb was not fully appreciated by the earlier research workers. Free anthraquinones were the first substances to be isolated: chrysophanol, aloe-emodin, rhein, emodin and emodin monomethylether or physcion (1844–1905). Glycosides of some of the above were also separated. These substances did not account satisfactorily for the action of the drug, and modern methods of investigation have established the presence of the following types of anthraquinones in rhubarb.

1. Anthraquinones without a carboxyl group (e.g. chrysophanol, aloe-emodin, emodin and physcion). Also their glycosides (e.g. chrysophanein and glucoaloe-emodin). Kopp and coworkers (*Planta Med*., 1983, **48**, 34 and references cited) isolated a physcion diglucoside and its 8-*O*-β-D-gentiobioside from *R. palmatum*; other cytotoxic anthraquinone glycosides have recently been isolated including palmatin (1,8-dihydroxy-3-methyl-anthraquinone-1-*O*-β-7D-glucoside; see Kubo *et al*., *Phytochemistry*, 1992, **31**, 1063 with corrigendum on misspelling of names p. 4399).
2. Anthraquinones with a carboxyl group (e.g. rhein and its glycoside, glucorhein).
3. Anthrones or dianthrones of chrysophanol, or emodin or aloe-emodin, or physcion. The dianthrone glucosides of rhein (sennosides A and B) and the oxalates of these (sennosides E and F), have been isolated (1974) by Japanese workers. Sennosides A and B have been identified in callus cultures of a *R. palmatum* hybrid.
4. Heterodianthrones derived from two different anthrone molecules. For example, palmidin A from aloe-emodin anthrone and emodin anthrone; palmidin B from aloe-emodin anthrone and chrysophanol anthrone; and palmidin C from emodin anthrone and chrysophanol anthrone. Rhein anthrone occurs combined with aloe-emodin anthrone (sennidin C and as sennoside C), chrysophanol anthrone (reidin B) and physcion anthrone (reidin C). These dianthrones may be oxidized into their two components by means of ferric chloride.

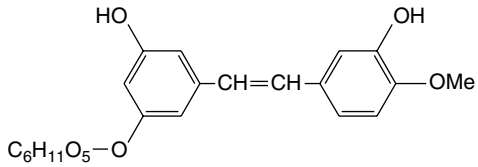
In addition to the above purgative compounds, rhubarb contains astringent compounds such as glucogallin, free gallic acid, (–)-epicatechin gallate and catechin. Other derivatives of gallic acid include glycerol gallate, gallic acid glucoside gallates and isolindleyin (a methyl *p*-hydroxyphenylpropionate derivative of a glycogallate). A new class of gallotannins has a sucrose core and chromone glucosides have also been identified (Y. Kashiwada *et al*., *Phytochemistry*, 1988, **27**, 1469; 1990, **29**, 1007).

Rhubarb also contains starch and calcium oxalate. The total ash is very variable, as the amount of calcium oxalate varies from about 5 to

40%. The acid-insoluble ash should not exceed 1%. The *BP* assay for anthraquinone derivatives is a spectrophotometric method and replaces the former standard for alcohol-soluble extractive.

Ontogenetic production of anthraquinone derivatives. As the drug is collected in autumn, variations in constituents arising from seasonable changes should present no problem. Nevertheless, considerable research has been devoted to this aspect over many years. Work by Lemli and colleagues (1982) indicated that oxidized compounds, the anthraquinones, are the major components of the anthracene mixture in the summer months and the reduced forms, the anthrones, in winter. The conversions occur within a time lapse of about 3 weeks, and just before each, the anthrone diglycoside content increases markedly. Experiments showed that the anthraquinone → anthrone conversion could be artificially induced by decreasing the ambient temperature. Earlier reports by Schmid (1951) suggested that the age of the rhizome also affected the ratio of reduced:oxidized glycosides. Chinese workers have also addressed the problem (*Chem. Abs*., 1992, **117**, 66652; 66653).

Constituents of rhapontic rhubarb. Rhapontic rhubarb contains a glycoside, rhaponticin, which is a stilbene (diphenylethylene) derivative of the formula



This substance and desoxyrhaponticin (glycoside of 3,5-dihydroxy-4′-methoxystilbene) account for the difference in fluorescence between official and rhapontic rhubarbs. Rhapontic rhubarb does contain anthraquinone derivatives, although these differ from those in the official drug. One is the glucoside glucochrysaron (see Table 21.3).

Chemical tests

1. *Test for anthraquinone derivatives*. A little powder is shaken with 10 ml of ferric chloride solution mixed with 5 ml of hydrochloric acid and heated on a water-bath for 10 min. After filtration and cooling the filtrate is extracted with 10 ml of carbon tetrachloride. The organic layer is separated, washed with 5 ml of water and shaken with 5 ml of dilute solution of ammonia. Official rhubarb gives a rose-pink to cherry-red colour. Rhapontic rhubarb also gives this test.
2. *Test for rhapontic rhubarb*. Macerate 0.5 g of powder with 10 ml of 45% alcohol for 20 min, shaking occasionally. Filter and place one drop of the filtrate on a filter paper. When examined in ultraviolet light, the spot shows no blue colour with official rhubarb but a distinct blue fluorescence if rhapontic rhubarb is present. The colour is intensified by exposure to ammonia vapour. The *BP*/*EP* test is more specific and employs TLC with ultraviolet light and phosphomolybdic acid spray for visualization of the chromatogram of a methanolic extract with rhaponticin as a standard.

Other rhubarbs

1. *Chinese rhapontic*. This is known commercially as 'Chinese Rhapontica' but has been offered under the names of 'Tai-Hwang' or 'Tze-Hwang' without indication that it is a rhapontic type. It consists of untrimmed pieces sometimes split longitudinally. The transverse surface shows a radiate structure, with concentric rings of paler and darker colour, and a diffuse ring of star spots. The centre may be hollow. The odour, which is sweetish, differs from that of official rhubarb. Rhapontic rhubarb, like the official, gives a positive test for anthraquinone derivatives. When the test for absence of rhapontic rhubarb (see below) is applied, it gives a distinct blue fluorescence, which may be further intensified by exposure to ammonia vapour.
2. *Indian rhubarb*. Indian rhubarb consists of the dried rhizome and roots of *R. australe* (formerly called *R. emodi*) and *R. webbianum*. It is found in Pakistan, Kashmir, Nepal and eastern India and large quantities of the Indian drug have been exported. It occurs in unpeeled or partly peeled pieces, which are barrel-shaped or plano-convex, shrunken and light in weight. Cork cells are present in the powder. The freshly fractured surface is dull orange to yellowish-brown and in ultraviolet light exhibits a deep violet fluorescence.

   A considerable number of the anthraquinone derivatives present in *R. palmatum* have also been reported in Indian rhubarb. L. Krenn *et al.* (*J. Nat. Prod.*, 2003, **66**, 1107; *Chem. Pharm. Bull.*, 2004, 52, 391) have identified a new sulphated anthraquinone glycoside (sulfemodin 8-*O*-β-D-glucoside) together with new 10-hydroxycascarosides C and D and, 10*R*-chrysaloin 1-*O*-β-D-glucopyranoside; some phenolic compounds have antioxidant properties.
3. *English rhubarbs*. Both *R. officinale* and *R. rhaponticum* were formerly grown as drugs but cultivation appears to have ceased. Garden rhubarb for table use is derived from *R. rhaponticum*.
4. *Japanese rhubarb*. A hybrid of *R. coreanum* and *R. palmatum*. It contains anthraquinone derivatives, naphthalene glycosides similar to those illustrated for senna, stilbene glycosides and (+)-catechin.

Uses. Rhubarb is used as a bitter stomachic and in the treatment of diarrhoea, purgation being followed by an astringent effect. The drug is suitable as an occasional aperient but not for the treatment of chronic constipation.

Further reading

Foust CM 1992 Rhubarb—the wondrous drug. Princeton University Press, Princeton, NJ. *This book gives the history of the commerce, botany and medicinal aspects of the drug*

ALOES

Aloes is the solid residue obtained by evaporating the liquid which drains from the transversely cut leaves of various species of *Aloe* (Liliaceae). The juice is usually concentrated by boiling and solidifies on cooling.

The official (*BP*, *EP*, *USP*) varieties of aloes are the Cape from South Africa and Kenya, and the Barbados (Curaçao) from the West Indian Islands of Curaçao, Aruba and Bonaire. There are separate pharmacopoeial monographs for each type. Socotrine and Zanzibar varieties are no longer official.

Plants. Of about 180 known species of *Aloe*, the drug is mainly obtained from the following: Cape variety from *Aloe ferox* and its hybrids; Curaçao variety from *Aloe barbadensis*; Socotrine and Zanzibar varieties from *Aloe perryi*. The genus *Aloe* includes herbs, shrubs and trees, bearing spikes of white, yellow or red flowers. *Aloe ferox* is an example of the arborescent type and *A. barbadensis* of the herbaceous type. Aloe leaves are fleshy, are strongly cuticularized and are usually prickly at the margins.

It has been suggested that if natural stocks of *A. ferox* became exhausted then *A. classenii* and *A. turkanensis* would be preferable for cultivation because chemical races would not be a problem and their production of sideshoots would make vegetative propagation easier. However, some problems have arisen concerning the commerce in the

African aloes because the Washington Conference of the Convention on International Trade in Endangered Species (CITES) placed all species of *Aloe*, with the exception of *A. vera* (a cultivar of *A. barbadensis*), on the protected list.

Leaf structure. Transverse sections of an *Aloe* leaf usually show the following zones: (1) a strongly cuticularized epidermis with numerous stomata on both surfaces; (2) a region of parenchyma containing chlorophyll, starch and occasional bundles of needles of calcium oxalate; (3) a central region which frequently occupies about three-fifths of the diameter of the leaf, consisting of large, mucilage-containing parenchymatous cells; (4) a double row of vascular bundles which lie at the junction of the two previous zones and have a well-marked pericycle and endodermis. The aloetic juice from which the drug is prepared is contained in the large, pericyclic cells and sometimes in the adjacent parenchyma. When the leaves are cut, the aloetic juice flows out. No pressure should be applied or the aloes will be contaminated with mucilage. The mucilage, contained in zone 3 as above is used in the cosmetic and herbal industries in 'aloe vera' preparations (see Chapter 19).

History. According to legend, Socotrine aloes was known to the Greeks as early as the fourth century BC; the Greek colonists were sent to the island by Alexander the Great solely to preserve and cultivate the aloe plant. The drug was apparently known in England in the tenth century, and from the seventeenth century records of the East India Company it would appear that they frequently purchased the whole stock of aloes of the 'King of Socotra'. Socotrine and Zanzibar aloe were for many years the only official aloes, but they have now been replaced by the Cape and Curaçao varieties. Cape aloes was first exported about 1780 and became official in Britain in 1932. Barbados aloes was produced from about 1650 and lapsed about the beginning of the present century. The production of Curaçao (also called Barbados) aloes was started by the Dutch in the Islands of Curaçao, Aruba and Bonaire about 1817; recently aloes of similar type has been exported from nearby Venezuela.

Preparation and characters of Cape aloes. Cape aloes is prepared from wild plants of *A. ferox* and its hybrids. The leaves are cut transversely near the base and about 200 of them are arranged round a shallow hole in the ground, which is lined with plastic sheeting or more traditionally a piece of canvas or a goatskin. The leaves are arranged so that the cut ends overlap and drain freely into the canvas. After about 6 h all the juice has been collected and it is transferred to a drum or paraffin tin in which it is boiled for about 4 h on an open fire. The product is poured while hot into tins, each holding 25 kg, where it solidifies. For export the tins are placed in cases holding either two, four or eight tins.

The drug occurs in dark-brown or greenish-brown, glassy masses. Thin fragments have a deep olive colour and are semitransparent. The powder is greenish-yellow, and when pieces of the drug have rubbed against one another, patches of powder are found on the surface. The drug has a very characteristic, sour odour (the so-called rhubarb or apple-tart odour), which is particularly noticeable if one breathes on the drug before smelling. Taste, nauseous and bitter. The powder when examined under the microscope in lactophenol is usually amorphous.

Preparation and characters of Barbados (Curaçao) aloes. Curaçao aloes is produced from cultivated plants of *A. barbadensis*. The cut leaves are stacked in V-shaped troughs arranged on a slope so that the juice flows from a hole at one end of the trough into a collecting vessel. When sufficient juice has been collected, it is evaporated in a copper vessel. The temperature used is generally lower than in the case of Cape aloes and the product is, therefore, usually opaque, although some which is semi-transparent may be produced and is known in commerce as 'Capey Barbados'. Originally Barbados and Curaçao aloes were packed in gourds, now seen only in museums. The present-day drug is exported in cases each holding about 58.5 kg.

Typical Barbados aloes varies in colour from yellowish-brown to chocolate-brown, but poorer qualities that have been overheated may be almost black. The drug is opaque and breaks with a waxy fracture. The semi-transparent 'Capey Barbados' becomes more opaque on keeping. Curaçao has a nauseous and bitter taste and a characteristic odour recalling iodoform. Mounted in lactophenol, it shows small acicular crystals.

Substitutes and adulterants. Socotrine and Zanzibar aloes are now rare in the British market, and Natal aloes from *A. candelabrum* is no longer imported. Socotrine is yellowish-brown to blackish-brown, opaque and breaks with a porous fracture. Zanzibar is similar but has a waxy fracture and may be packed with leaves or skins (so-called 'monkey-skin aloes'). All may be distinguished from official aloes by chemical tests.

Chemical tests

1. General. For the following tests boil 1 g of drug with 100 ml of water, add a little kieselguhr and filter. Use separate portions of the filtrate for the following tests.
   (1) *Borax reaction*. To 5 ml of solution of aloes add 0.2 g of borax and heat until dissolved. Pour a few drops of the liquid into a test-tube nearly full of water. A green fluorescence is produced the origin of which is discussed below.
   (2) *Bromine test*. To 2 ml of solution of aloes add 2 ml of freshly prepared solution of bromine. A pale yellow precipitate of tetrabromaloin is produced. This test is not specific for aloes.
2. Special.
   (1) *Nitric acid test*. To 5 ml of solution of aloes add 2 ml of nitric acid. Cape aloes gives a brownish colour rapidly changing to green; Barbados a deep brownish-red; Socotrine a pale brownish-yellow; Zanzibar a yellowish-brown colour. Nitric acid may be applied direct to the powdered drugs with similar results.
   (2) *Nitrous acid test*. To an aqueous solution of aloes add a few small crystals of sodium nitrite and a little acetic acid. A rich pink to carmine is given by Barbados and a lesser pink by Cape; Socotrine and Zanzibar show little change in colour.
   (3) *Klunge's isobarbaloin test*. To 20 ml of an aqueous 1 in 200 solution of aloes add a drop of saturated copper sulphate solution, following by 1 g of sodium chloride and 10 ml of alcohol 90%. With Barbados aloes a wine-red colour is developed, which persists for at least 12 h. With Cape aloes a lesser coloration may develop, which, however, rapidly fades to yellow. Zanzibar and Socotrine aloes give no colour. The appearance of the red colour may be hastened by warming.
   (4) *Modified Bornträger's test*. As mentioned under 'Constituents', small quantities of aloe-emodin may occur in aloes, but the amounts are usually too small for them to form the basis of a reliable test. Therefore, a modified Bornträger's test which employs ferric chloride and dilute hydrochloric acid to bring about *oxidative hydrolysis*, can be used. The anthraquinones liberated are extracted with carbon tetrachloride and give a rose-pink to cherry-red colour when their solution is shaken with dilute ammonia.

A chromatographic test is included in the *BP*/*EP* together with assays for hydroxyanthracene derivatives. Of the latter Barbados aloes should contain not less than 28% and Cape aloes not less than 18% calculated as barbaloin.

Constituents. Aloes contain *C*-glycosides and resins. The crystalline glycosides known as 'aloin' were first prepared by T. and H. Smith of Edinburgh, UK, from Barbados aloes in 1851; Aloin (*BP*, 1988) contains not less than 70% anhydrous barbaloin. The main crystalline glycoside, barbaloin, is found in all the commercial varieties (Leger, 1907). Leger showed that on heating to about 160°C barbaloin is partly converted into amorphous β-barbaloin. This substance is said to be absent from the Barbados variety, but present to the extent of about 8% in the Cape.

Barbaloin is a *C*-glycoside—a 10-glucopyranosyl derivative of aloe-emodin-anthrone. Unlike *O*-glycosides, it is not hydrolysed by heating with dilute acids or alkalis. It can, however, be decomposed by *oxidative hydrolysis*, with reagents such as ferric chloride, when it yields glucose, aloe-emodin anthrone and a little aloe-emodin. It will be seen from the formula of barbaloin that stereoisomerism is possible at C-10; in 1979 both isomers were obtained by HPLC of a methanolic extract of *Aloe ferox* and in 1980 Auterhoff *et al*. separated commercial aloin into its stereoisomers. The absolute configuration of the two aloins was independently elucidated by Rauwald *et al*. (*Angew. Chem., Int. Ed. Engl*., 1989, **28**, 1528) and Manitto *et al*. (*J. Chem. Soc., Perk. Trans. I*, 1990, 1297); aloin A is (10*S*)-barbaloin and aloin B the (10*R*)-epimer (Fig. 21.15). The two are interconvertible via the corresponding anthranol form. All varieties of aloes give a strong greenish fluorescence with borax, a characteristic of anthranols, which are readily formed from anthrones by isomeric change. This has long been used as a general test for aloes.

Small quantities of aloe-emodin are sometimes present in aloes, and Cape aloes also contains aloinosides A and B, which are *O*-glycosides of barbaloin; aloinoside B has rhamnose attached via an oxymethyl group at C-3. In *A. barbadensis* free and esterified 7-hydroxyaloins A and B are characteristic 10-*C*-glucosyl-anthrones. These compounds are responsible for the violet-purple colours given in various specific tests for Barbados aloes (see H. W. Rauwald *et al.*, *Planta Med*., 1991, **57**, Suppl. 2, A129).

The resin of aloes, reputed to have a purgative action, has been periodically investigated from the end of the nineteenth century onwards. In South African spp. (e.g. *A. ferox*) aloesin (now often referred to as aloeresin B) was identified in 1970 by Haynes *et al.*, and was the first *C*-glucosyl-chromone to be described. Other 5-methylchromones isolated from Cape aloes include aloeresin A and C which are *p*-coumaroyl derivatives linked via a hydroxyl of the glucose. Two non-glucosylated 5-methylchromones present in smaller amounts than the aloesins were reported in 1997. A glycosidic 6-phenylpyran-2-one derivative (aloenin A) was isolated and characterized from *A. arborescens* leaves in 1974 by Japanese workers. Aloenin B has now been obtained from Kenya aloes (see formulae). (For research on these and related constituents see G. Speranza *et al.*, *Phytochemistry*, 1993, **33**, 175; *J. Nat. Prod*., 1992, **55**, 723; 1993, **56**, 1089; 1997, **60**, 692. Two aloesol derivatives (Fig. 21.14) have been isolated: 8-*C*-β-D-glucopyranosyl-7-*O*-methyl-(R)-aloesol (L. Duri *et al.*, *Fitoterapia*, 2004, **75**, 520) from a commercial sample (Kenya) and the 10*S* diastereoisomer from *A. vera* (N. Okamura *et al.*, *Phytochemistry*, 1996, **43**, 495).

Three new naphtho[2,3-*c*]furan derivatives have recently been isolated from a commercial sample of Cape aloes (J. Koyama *et al.*, *Phytochemistry*, 1994, **37**, 1147).

As with other anthraquinone-producing plants, in *Aloe* species the content of anthraquinones is subject to seasonal variation, and these compounds are implicated in the active metabolism of the plant. McCarthy and coworkers in South Africa have shown that the anthraquinone derivatives are confined to the leaf juices and that aloin reaches a maximum concentration in the dried leaf juices of *A. ferox* and *A. marlothi* in the summer (24.1% in November) and is lowest in winter (14.8% in July).

Uses. Aloes is employed as purgative. It is seldom prescribed alone, and its activity is increased when it is administered with small quantities of soap or alkaline salts, while carminatives moderate its tendency to cause griping. It is an ingredient of Compound Benzoin Tincture (Friars' Balsam).

There appears to be little variation of the major constituents of the leaf exudate of *A. ferox* depending on geographical location of the plant but selection of high-yielding strains giving a high production of aloin (25%) is recommended for commercial cultivation (B.-E. van Wyk *et al.*, *Planta Medica*, 1995, **61**, 250).

'Aloe vera' products See Chapter 20.

Further reading

Reynolds T (ed), Hardman R (series ed) 2004 Medicinal and aromatic plants – industrial profiles, Vol 38. Aloes – the genus Aloe. CRC Press, Taylor and Francis Group, Boca Raton, FL, 408 pp.

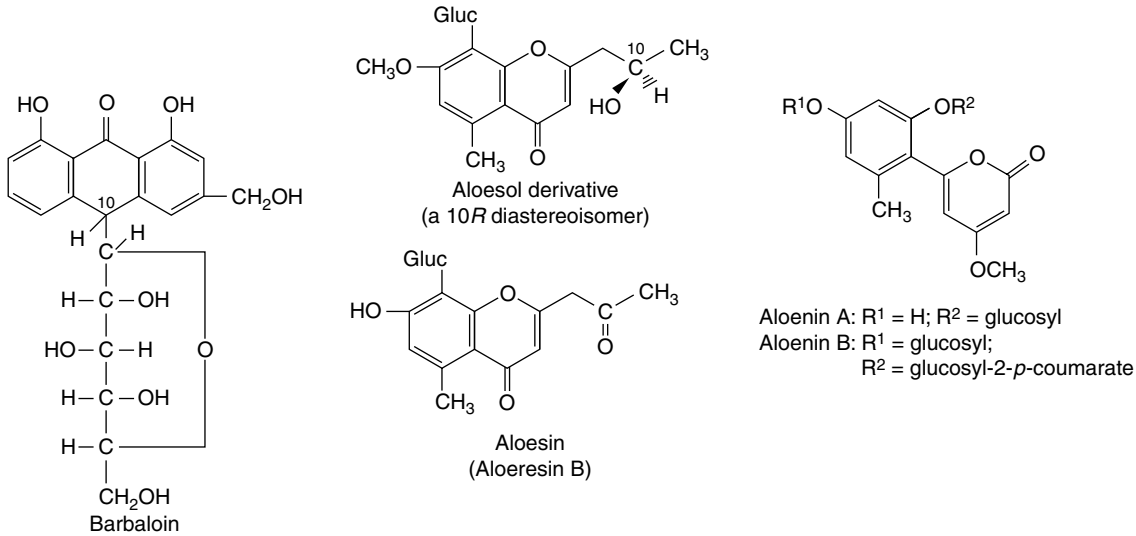


Fig. 21.15
Constituents of aloes

Chrysarobin

Chrysarobin is a mixture of substances obtained from araroba or Goa powder by extraction with hot benzene. Araroba is extracted from cavities in the trunk of *Andira araroba* (Leguminosae). Chrysarobin contains chrysophanol anthranol, the corresponding anthrone and other similar constituents; it gives a strong green fluorescence in alkaline solution. Chrysarobin was formerly much used for skin diseases and is still occasionally prescribed.

Madder

The root of *Rubia tinctorum* (Rubiaceae) was formerly grown in large quantities as a dyestuff, but has been almost completely replaced by synthetic dyes. It contains several anthraquinone glycosides, the chief of which, ruberythric acid (Table 21.3) yields on hydrolysis alizarin and primeverose. Twenty compounds have been isolated from the roots and their mutagenicity studied (Y. Kawasaki *et al.*, *Chem. Pharm. Bull*., 1992, **40**, 1504), and three hydroxymethylanthraquinone glycosides have been described (N. A. El-Emary and E. Y. Backheet, *Phytochemistry*, 1998, **49**, 277).

HYPERICUM—ST JOHN'S WORT

Hypericum consists of the dried aerial parts of *Hypericum perforatum*, family Hypericaceae (Clusiaceae) gathered usually at the time of flowering or shortly before. Commercial extracts are standardized on their naphthodianthrone content, expressed as hypericin.

The plant is abundant throughout Europe in grassland, woodlands and hedges, extending to the Himalayas and Central and Russian Asia, except in Arctic regions. It was introduced into N.E. America and Australia at an early stage of colonization where it has since become a noxious weed. It is a herbaceous perennial, usually forming a colony with a spreading root system. The bright yellow flowers are in handsome terminal corymbs.

History. The plant was known in ancient Greece for its medicinal attributes and since the Middle Ages has been used for its anti-inflammatory and healing properties. It also became highly regarded for the treatment of mental illness. The generic name derives from the Greek *hyper*—above, and *icon* (eikon)—picture, referring to the ancient practice of hanging the plant above religious pictures to ward off evil spirits. The common name St John's wort is attributed to the fact, among others, that it comes into flower around St John's Day (June 24th).

The drug is now included in the *BP*/*EP*, a number of European pharmacopoeias, the *British Herbal Pharmacopoeia*, the *American Herbal Pharmacopoeia*, and as monographs for the German Commission E and ESCOP.

Collection. Collection is from wild and cultivated plants and increased demand has meant that farmers in the US and Australia who battled to eradicate it as a weed now harvest it as a viable crop. Care should be taken during collecting as contact photosensitivity has been reported. Drying at 70° for 10 hours is recommended.

Macroscopy. The drug consists of green leaf fragments and stems, unopened buds and yellow flowers. Oil glands are visible in the leaves as transparent areas, hence the specific name *perforatum*, and as small black dots on the lower surface. The opposite, sessile leaves are 1.5–4.0 cm in length, elliptical to ovate in outline, glabrous with an entire margin. Pieces of hollow stem are cylindrical with two faint ribs on either side.

The odour is distinct and the taste slightly sweet and astringent.

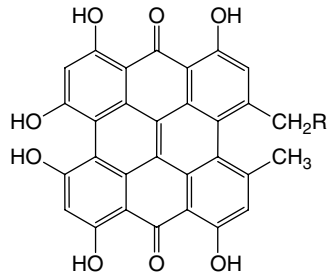
Microscopy. The upper epidermal cells of the leaf are sinuous in outline with beaded anticlinal walls; the lower epidermis possesses anomocytic and paracytic stomata. The mesophyll has large hypericin-containing oil glands, some with red contents, and these are also found in the petals and sepals. Pollen grains are ellipsoidal, 20–25 μm in diameter, with three pores and a smooth exine. Trichomes and calcium oxalate are absent.

In an innovative study, Rapisarda *et al.* (*Pharm. Biol*., 2003, **41**, 1) have used scanning electron microscopy and image analysis involving size and shape parameters of leaf epidermal cells to provide a quantitative morphological analysis of the three Italian *Hypericum* spp.—*H. perforatum* L., *H. hircinum* L. and *H. perfoliatum*. The markers obtained provided key factors for the identification and selection of these species and their hybrids.

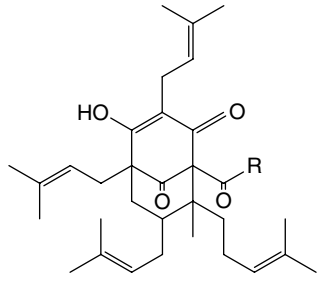
Constituents. Hypericum contains a variety of constituents with biological activity.

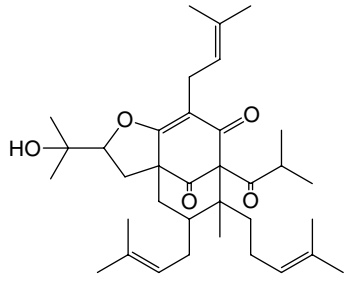
Anthraquinones. Principally hypericin and pseudohypericin; also iso-hypericin and emodin-anthrone. The *BP*/*EP* requires not less than 0.08% of total hypericins expressed as hypericin calculated with reference to the dried drug. The extracted hypericins are assayed by absorption measurement at 590 nm.

Prenylated phloroglucinol derivatives. Hyperforin (2.0–4.5%), adhyperforin and furohyperforin (L. Verotta *et al.*, *J. Nat. Prod*., 1999, **62**, 770), the latter at concentrations of about five per cent of the hyperforin content. These phloroglucinols constitute the principal components of the lipophilic extract of the plant and are considered to be the most important active constituents regarding antibiotic and antidepressant properties. Unfortunately, they are very prone to oxidative transformations and a number of such degradation products have been identified, see L. Verotta *et al.*, *J. Nat. Prod*., 2000, **63**, 412; V. Vajs *et al.*, *Fitoterapia*, 2003, **74**, 439. For an article, with many



Hypericin, R = H
Pseudohypericin, R = OH





Hyperforin, R = $CH(CH_3)_2$
Adhyperforin, R = $CH(CH_3)CH_2CH_3$

Furohyperforin

Fig. 21.16
Hypericins and phloroglucinols of hypericum.

references, on the wide-ranging aspects of hyperforin, see L. Beerhues, *Phytochemistry*, 2006, **67**, 2201.

The involvement of branched-chain amino acids in the biosynthesis of hyperforin and adhyperforin has been demonstrated with shoot cultures of *H. perforatum*: L-[U-$^{13}C_5$] valine and L-[U-$^{13}C_6$] isoleucine, when fed to the shoots, were incorporated respectively into the side-chains of hyperforin and adhyperforin. Production of the former was not increased by the administration of unlabelled L-valine, whereas the latter was enhanced by the feeding of the unlabelled L-isoleucine (K. Karppinen *et al*., *Phytochemistry*, 2007, **68**, 1038). Two phloroglucinols, hyperfirin and adhyperfirin, previously reported to be precursors of hyperforin and adhyperforin, respectively, have now been detected in the plant (E. C. Tatsis *et al.*, *Phytochemistry*, 2007, **68**, 383).

Flavonoids. These include flavonols such as kaempferol, luteolin and quercetin, the flavanol glycosides quercitrin, isoquercitrin and hyperoside. The biflavonoid amentoflavone (Fig. 21.18) is confined principally to the flowers (A. Umek *et al.*, *Planta Medica*, 1999, **65**, 388).

Selected formulae for the above are shown in Figs 21.16 and 21.18.

Volatile oil. Up to 0.35% consisting principally of saturated hydrocarbons including alkanes and alkanols in the range C_{16}–C_{29}.

Other constituents. Many other components of hypericum have been reported including various plant acids (caffeic, chlorogenic, etc.), amino acids, vitamin C, tannins and carotenoids.

Many reports have appeared concerning the distribution of the above constituents in different organs of the plant and generally on a weight for weight basis it is the flowers, particularly the petals, that possess the highest concentrations. According to an Australian report (I. A. Southwell *et al.*, *Phytochemistry*, 1991, **30**, 475) it is the narrow-leaved varieties, both in Europe and Australia, that possess a relatively high proportion of oil glands and give a higher yield of hypericin compared with the broad-leaved forms. Hypericin concentrations determined on twelve samples of herb collected throughout Oregon varied widely (0.01–0.38%) (G. H. Constantine and J. Karchesy, *Pharm. Biol*., 1998, **36**, 365). B. Buter *et al*. (*Planta Medica*, 1998, **64**, 431) have suggested that a key factor for successful future field production will rest in the selection of genetically superior strains giving increased secondary metabolite production, together with improvements in agrotechnological methods. In this connection R. J. Percifield *et al*. (*Planta Medica*, 2007, **73**, 1525), studying 50 *Hypericum* accessions, have demonstrated the value of amplified fragment-length polymorphism analysis for the characterization of closely related samples.

Cell culture of *Hypericum* spp. and their chemotypes has proved extremely variable in naphthadianthrone yields with pseudohypericin production exceeding that of hypericin (T. Kartnig *et al.*, *Planta Medica*, 1996, **62**, 51). Flavonoids, completely different to those of the intact plant and including the new compound 6-C-prenylluteolin, have been identified in callus cultures of *H. perforatum* (A. P. C. Dias *et al.*, *Phytochemistry*, 1998, **48**, 1165).

Other *Hypericum* spp. The genus contains some 400 spp.; most of the more common ones have lower or nil hypericin contents and are often distinguishable in the dried condition by the nature of the ridges on the stem. *H. maculatum* (Imperate St John's wort) is similar in constituents to *H. perforatum* but contains less; it may be distinguished by the slightly quadrangular stem and larger leaves. *H. hirsutum*, *H. tetrapterum* and *H. montana* are other common European species.

The essential oils of two Turkish species (*H. hyssopifolium* and *H. lysimachioides*) are rich in sesquiterpene hydrocarbons, but unlike some other species investigated, poor in monoterpene hydrocarbons. Caryophyllene oxide is a major component. Both oils possess antimicrobial activities (Z. Toker *et al.*, *Fitoterapia*, 2006, **77**, 57). Eight species from S. Brazil showed no detectable amounts of hypericin or pseudohypericin (A. Ferraz *et al.*, *Pharm. Biol*., 2002, **40**, 294). *H. chinense* finds use in Japanese folk medicine for the treatment of female disorders. It contains acyl phloroglucinols and spirolactones; six new xanthones have been reported (N. Tanaka and Y. Takaishi, *Chem. Pharm. Bull*., 2007, **55**, 19).

Action and uses. An explosion in the popularity of St John's wort related to its unregulated availability for the treatment of mild to moderate depression. In the USA, for the first eight months of 1999, it ranked second to ginkgo as the best-selling product of the herbal mainstream market, with retail sales valued at over 78 million (M. Blumenthal, *HerbalGram*, 1999, **47**, 64). In Germany, it represented 25% of all antidepressant prescriptions. It was described as 'nature's Prozac', without the disadvantageous side-effects of the latter. However, a cautionary warning was struck by two reports (S. Piscitelli *et al*., *Lancet*, 2000, **355**, 547; F. Ruschitzka *et al*., *Lancet*, 548). In the first, St John's wort was observed to lower plasma concentrations of the protease inhibitor indinavir. In the second report, heart transplant rejection, as a result of the lowering of ciclosporin plasma concentrations below therapeutic levels, followed St John's wort therapy. It has subsequently transpired that St John's wort will adversely affect the performance of a number of common drugs by causing their rapid elimination from the body, either by enhanced metabolism or as a result of increased action of the drug transporter *P*-glycoprotein. Among common drugs so affected are anticoagulants such as warfarin, digoxin, tricyclic antidepressant agents, simvastatin and others.

In the UK, there are currently (2007) no specific restrictions on the sale of St John's wort as a herbal preparation but it is recommended that professional advice be sought if it is to be taken in conjunction with other medicines.

Further reading

Ernst E (ed), Hardman R (series ed) 2003 Medicinal and aromatic plants – industrial profiles, Vol 31. Hypercium: The genus *Hypericum*. CRC Press, Taylor and Francis Group, Boca Raton, FL

COCHINEAL

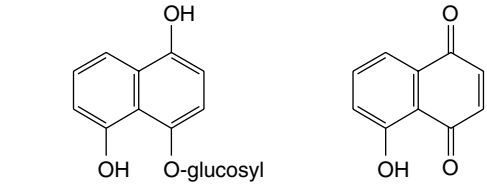
Cochineal is an important colourant and indicator and consists of the dried female insects, *Dactylopius coccus*, containing eggs and larvae. It contains about 10% of carminic acid which is a *C*-glycoside anthraquinone derivative (Table 21.3). The insects are described in detail in Chapter 33.

NAPHTHOQUINONES AND GLYCOSIDES

The nature of these compounds was indicated earlier; they are produced by higher plants, fungi and actinomycetes and exhibit a broad range of biological actions including fungicidal, antibacterial, insecticidal, phytotoxic, cytostatic and anticarcinogenic. In plants they commonly occur in the reduced and glycosidic forms as illustrated by the 4β-D-glucoside of α-hydrojuglone, a constituent of walnut tree leaves (*Juglans regia*, Juglandaceae).

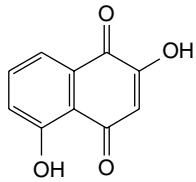
On extraction and work-up, or in the soil, the compounds are oxidatively converted to the coloured naphthoquinone. In some heart-woods, e.g. *Diospyros* spp. (Ebenaceae) napthoquinones occur as monomers, complex dimers and trimers. In addition to timber usage (ebony) many species of *Diospyros* are used world-wide in the traditional medicine of countries where they grow. S. Ganapaty *et al*. (*Phytochemistry*, 2006, **67**, 1950) have reported on the antiprotozoal properties of various naphthoquinones, *viz* two naphthaldehydes, diospyrin, 8′-hydroxydiospyrin and plumbagin isolated from the roots of *D. assimilis*. *Plumbago zeylanica* (Plumbaginaceae) grows throughout tropical Africa and Asia

and the root is used in Indian medicine; it contains juglone in addition to pentacyclic triterpenes.

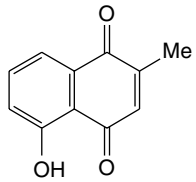


4β-D-Glucoside of α-hydrojuglone

Juglone



Lawsone



Plumbagin

Naphthoquinones have been shown to be biosynthesized via a variety of pathways including acetate and malonate (plumbagin of *Plumbago* spp.), shikimate/succinyl CoA combined pathway (lawsone) and shikimate/mevalonate combined pathway (alkannin).

Henna

Henna consists of the dried leaves of *Lawsonia inermis* (Lythraceae), a shrub cultivated in north Africa including Egypt, India and Ceylon. The leaves are greenish-brown to brown and about 2.5–5 cm long. The apex is mucronate, the margin entire and revolute, and venation pinnate. Henna contains a colouring matter, lawsone (a hydroxynaphthoquinone), various phenolic glycosides, coumarins, xanthones, quinoids, β-sitosterol glucoside, flavonoids including luteolin and its 7-*O*-glucoside, fats, resin and henna-tannin. (For a report on new glucosides see Y. Takeda and M. O. Fatope, *J. Nat. Prod.*, 1988, **51**, 725.) Henna is commonly used as a dye for the hair, and wool washed in a dilute solution of ammonia and boiled in a decoction of the drug should be dyed Titian red.

The astringent stem-bark of *L. inermis* is traditionally used in India for the treatment of jaundice, enlargement of the liver and spleen, and for various skin diseases. Isoplumbagin, exhibiting significant anti-inflammatory activity, has been isolated from the bark in 0.05% yield (M. Ali and M. R. Grever, *Fitoterapia*, 1998, **69**, 181). For a recent report on the hepatoprotective activity of the bark see S. Ahmed *et al*., *J. Ethnopharmacology*, 2000, **69**, 157.

Lithospermums

The genus *Lithospermum* (60 spp.) (Boraginaceae) contains plants with hormonal activity. The seeds of the European *L. officinale* (gromwell) were formerly official in several pharmacopoeias.

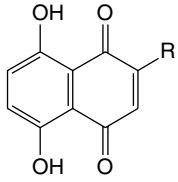
The reported constituents of the herb are shikonin, a naphthoquinone derivative; scyllitol, a cyclitol; a cyanoglucoside-lithospermocide; caffeic, chlorogenic and ellagic acids; and catechin-type tannins. Shikonin, the enantiomer of alkannin (found in Anchusa Root, see below) is also a constituent of *L. erythrorhizon* root and is produced for the cosmetic and pharmaceutical industries in Japan by cell culture of the plant. Among the many publications on this subject, Tani *et al*. (*Phytochemistry*, 1992, **31**, 690) reported on the structure of an endogenously produced oligogalacturonide necessary for the induction of shikonin biosynthesis in the culture. For investigations relating to the biosynthesis of shikonin from p-hydroxybenzoic acid and geranyl pyrophosphate in *L. erythrorhizon* see T. Okamoto *et al.*, *Phytochemistry*, 1995, **38**, 83.

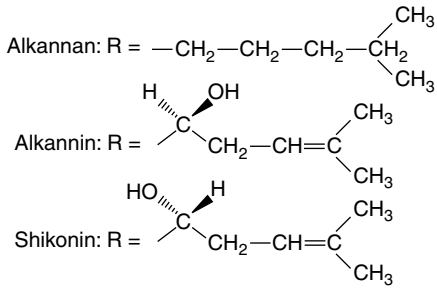
In the Far East, preparations of the purple roots have long been used for the treatment of burns, inflammations, wounds and ulcers. In Europe, alkanna root has been similarly employed and it has now been shown in laboratory tests that shikonin and alkannin have no significant difference in anti-inflammatory activity. The occurrence of these naphthoquinones is of interest, since similar compounds occur in the related families Rubiaceae (*Galium*, *Rubia*), Verbenaceae and Bignoniaceae. *Lithospermum arvense* is used as an oral contraceptive in Central Europe, as it suppresses the oestrus cycle. The North American *Lithospermum ruderale* has similar hormonal activity.

Alkanna root

Alkanet or Anchusae Radix is the dried root of *Alkanna tinctoria* (Boraginaceae), a herb found in Hungary, southern Europe and Turkey. It consists of reddish-purple roots about 10–15 cm long and 1–2 cm diameter near the crown. The surface is deeply fissured and readily exfoliates. Attached to the crown are the remains of leaves having whitish, bristly hairs. Alkanna is used for colouring oils and tars and in the form of a tincture for the microscopical detection of oils and fats. The pigments are naphthoquinone derivatives of the formulae below.

Alkannin itself may be an artefact arising from various esters. Most of the pigment compounds produced in cell culture appear to give alkannin on KOH hydrolysis (TLC, R_f values) and root cultures give pigments identical to those extracted from normal roots (G. Mita *et al.*, *Plant Cell Rep*., 1994, **13**, 406).





Other members of the Boraginaceae—for example, *Macrotomia cephalotes* (Syrian Alkanet)—produce similar red naphthoquinones.

CHROMONES AND XANTHONES

These compounds are structural derivatives of benzo-γ-pyrone and although not of great pharmaceutical importance a few compounds are worthy of mention.

Chromones are isomeric with the coumarins. A simple derivative is eugenin (Fig. 21.17) found in the clove plant, *Syzygium aromaticum*. More complex are the furanochromones, the active constituents of the fruits of *Ammi visnaga* (q.v.).

Xanthones are found mainly in the Gentianaceae and Guttiferae, otherwise scattered sporadically throughout the plant kingdom as in the Moraceae and Polygalaceae. The characteristic oxygenation pattern of these compounds derived from higher plants indicated that they were of mixed shikimate–acetate origin whereas xanthones derived from fungi show a characteristic acetate derivation. An important step in their biosynthesis appears to be the oxidative coupling of hydroxylated benzophenones. Simple oxygenated derivatives, such as gentisin which contributes to the yellow colour of fermented Gentian Root (q.v.), are found in both the Gentianaceae and Guttiferae. More highly oxygenated compounds and *O*-glycosylxanthones are found in the former family whereas prenylated xanthones, several of which have antimicrobial properties,

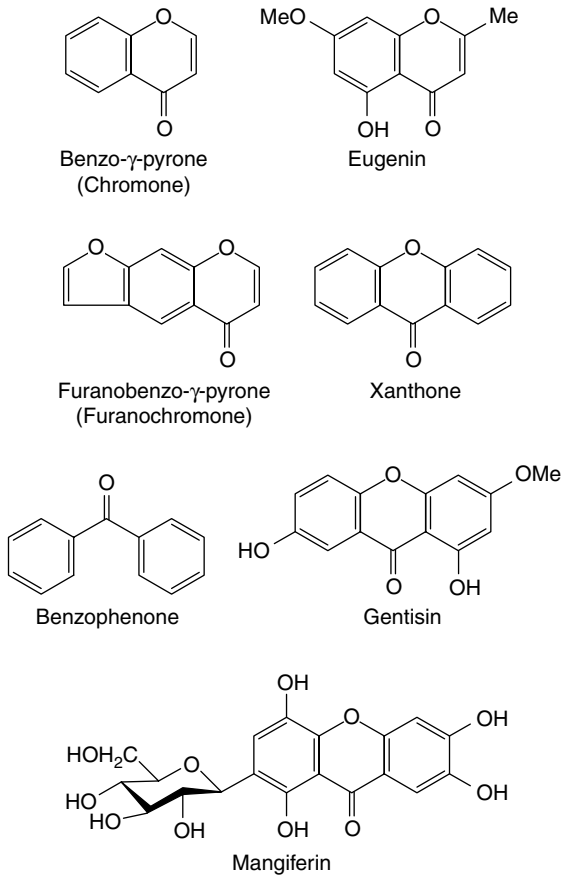


Fig. 21.17
Chromones and xanthones.

are widely distributed in the latter. For studies on the antifungal xanthones from the roots of *Marila laxiflora*, Guttiferae, see J.-R. Ioset, *Pharm. Biol*., 1998, **36**, 103. The *C*-glycosyl xanthone mangiferin (Fig. 21.17) is found in several species of *Hypericum* and in *Cratoxylem pruniflorum* and Chiretta (*Swertia chirata*). Mangiferin has anti-inflammatory, antihepatotoxic and antiviral properties. In contrast to its CNS-stimulant properties other xanthones exhibit CNS depressive properties in rats and mice.

The mycotoxin pigments of *Claviceps purpurea* (ergot) are complex xanthones called secalonic acids. They contribute, with the ergot alkaloids, to the toxic properties of the whole drug.

Further reading

Bennett GJ, Lee H-H 1989 Xanthones from Guttiferae. Phytochemistry 28(4): 967–998. *165 refs*

Peres V, Nagem TJ 1997 Trioxygenated naturally occurring xanthones. Phytochemistry 44(2): 191–214. *Review with 262 refs and 181 listed compounds including 55 fungal and lichen metabolites*

FLAVONE AND RELATED FLAVONOID GLYCOSIDES

The flavonoids which occur both in the free state and as glycosides are the largest group of naturally occurring phenols. More than 2000 of these compounds are now known, with nearly 500 occurring in the free state. They are formed from three acetate units and a phenylpropane unit as has already been outlined (Fig. 18.11) and are typed according to the state of oxygenation of the C_3 unit, i.e. C-2,3,4 (see Fig. 21.18 and Table 21.5). Examples given in this section all have a γ-pyrone moiety with the exception of the chalcones, which although not strictly flavonoids are biosynthetically related. The anthocyanins are described later.

The flavones and their close relations are often yellow (Latin *flavus*, yellow). They are widely distributed in nature but are more common in the higher plants and in young tissues, where they occur in the cell sap. They have been used extensively as chemotaxonomic markers and are abundant in the Polygonaceae, Rutaceae, Leguminosae, Umbelliferae and Compositae (see Table 21.5).

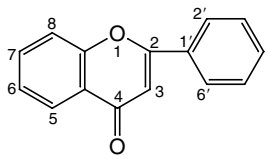
They occur both in the free state and as glycosides; most are *O*-glycosides but a considerable number of flavonoid *C*-glycosides are known. Dimeric compounds with, for example, a 5′-8-carbon–carbon linkage are also known (biflavonoids). The glycosides are generally soluble in water and alcohol, but insoluble in organic solvents; the genins are only sparingly soluble in water but are soluble in ether. Flavonoids dissolve in alkalis, giving yellow solutions which on the addition of acid become colourless.

Although the original high hopes for the therapeutic usefulness of flavonoids were not immediately realized, recent researches have demonstrated their involvement in the medicinal action of drugs such as liquorice root, Roman chamomile and gingko. It is very probable that a number of herbal remedies, whose constituents are as yet unknown, will be shown to contain active flavonoids. Of the 84 drugs described in the *BHP*, Vol 1, 1991, some 36 contain flavonoids but not necessarily as the active constituents. A number of flavonoid-containing herbs have now been included in the *BP*/*EP*, examples are Birch Leaf, Calendula Flower, Elder Flower, Horsetail, Lime Flower, Motherwort and Passiflora. The group is known for its anti-inflammatory and antiallergic effects, for antithrombitic and vasoprotective properties, for inhibition of tumour promotion and as a protective for the gastric mucosa. Some of these pharmacological properties can be explained on the basis of antioxidant activity as has recently been shown for tiliroside (see Lime Flower) and the related gnaphaliine isolated from the aerial parts of *Helichrysum italicum* (G. R. Schinella *et al.*, *Fitoterapia*, 2007, **78**, 1). Many flavonoid-containing plants are diuretic (e.g. buchu and broom) or antispasmodic (e.g. liquorice and parsley). Some flavonoids have antitumour, antibacterial or antifungal properties. E.-A. Bae *et al.* (*Planta Medica*, 1999, **65**, 442) have recently investigated the *in vitro* anti-*Helicobacter pylori* activity of a number of flavonoids (hesperidin, hesperetin, naringin, naringenin, diosmin, diosmetin) and suggest that even if not potent inhibitors of, they may contribute to the prevention of gastritis. Others, e.g. fustic (from the wood of *Morus tinctoria*) and sumac (leaves of *Rhus* spp.) are colouring and tannin materials.

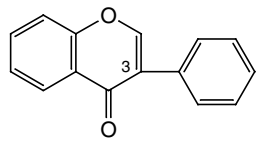
Pure flavone, which is colourless, occurs on the surface of some species of *Primula*. As shown in Table 21.5, many flavones are phenolic or methoxyl derivatives and form sap-soluble glycosides. The intensity of their yellow colour increases with the number of hydroxyl groups and with increase of pH.

Isoflavonoids. Isoflavones are found in the heartwood of species of *Prunus* and in species of *Iris*, and are particularly abundant in the Leguminosae (e.g. in dyer's broom, *Genista tinctoria*). The latter contains genistin (not to be confused with the gentisin of gentian), the 7-glucoside of genistein. Rotenone contained in the roots of *Derris* and *Lonchocarpus* species (see Chapter 40) is an isoflavonoid in which the 2,3 double bond of an isoflavone is reduced.

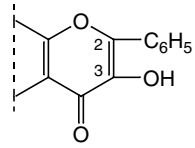
Phyto-oestrogens. Isoflavones, along with coumestans (also flavonoids) and lignans (q.v.), belong to a class of substances known as non-steroidal phyto-oestrogens. Both structurally and functionally they are similar to oestradiol (see Fig. 23.4) and related sex hormones



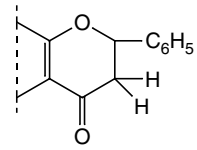
Flavone
(2-phenyl-γ-chromone)



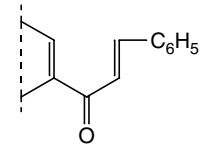
Isoflavone
(3-phenyl-γ-chromone)



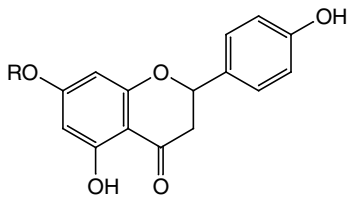
Flavonol



Flavanone

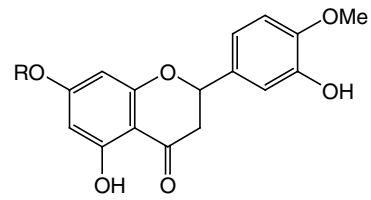


Chalcone



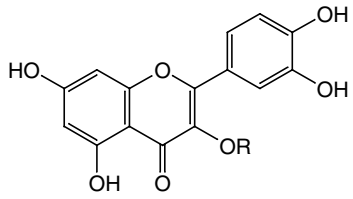
Naringenin; R = H

Naringin; R = rhamno-glucosyl
(Flavanones)



Hesperetin; R = H

Hesperidin; R = rhamno-glucosyl
(Flavanones)

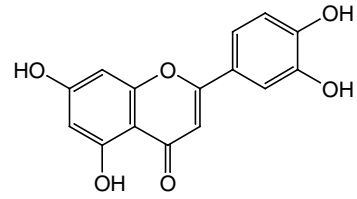


Quercetin; R = H

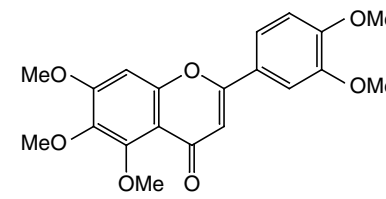
Hyperoside; R = galactosyl

Isoquercitrin; R = glucosyl

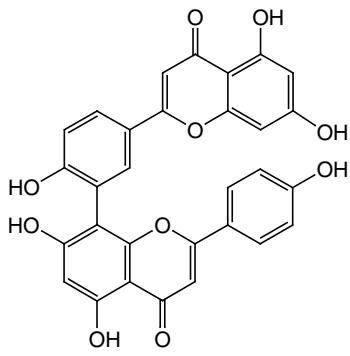
Rutin; R = rhamno-glucosyl
(Flavonols)



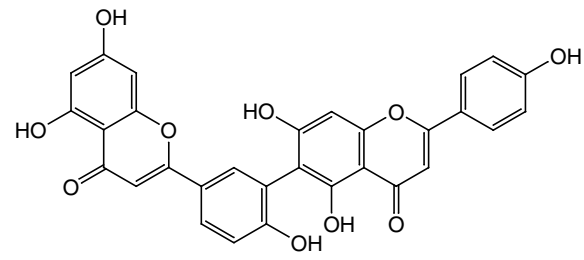
Luteolin
(Flavone)



Sinensetin
(Flavone)

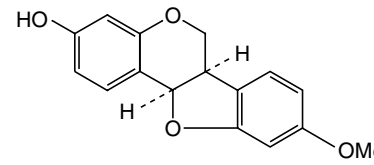


Amentoflavone



Robustaflavone

(Biflavonols)



Medicarpin—a pterocarpan
(isoflavone derivative)

Fig. 21.18
Structural types of flavonoids, aglycones and glycosides.

and exert weak oestrogenic effects. They are present in certain foods and herbal remedies and are well-documented as producing infertility in animals as, for example, clover disease in sheep grazing on clovers containing a high phyto-oestrogen content.

Studies provoking much medical and general press attention have centred on the role of phyto-oestrogens as dietary constituents having positive effects in the prevention of cancer, heart disease and post-menopausal symptoms.

Foods containing appreciable quantities of isoflavones are soya beans, soy products and other legume crops; they are also present in the herbs Red Clover Flower *BHP* (*Trifolium pratense*) and broomtops (*Cytisus scoparius*). In the plant they occur free or in the glycosidic form, in the latter case being hydrolysed by colonic bacteria to give the active aglycone; genistein and daidzein are the principal examples, the latter being formed from formononetin (Table 21.5). As plant nutraceuticals, these compounds are more fully discussed in Chapter 32.

A new class of non-steroidal phyto-oestrogens are the prenylated flavonoids. Many of these compounds are known and this activity has been described for 8-isopentenylnaringenin.

Biflavonoids. The first biflavonoid to be isolated was gingetin, in 1929. Now more than 100 are known with a variety of biological activities being reported. Amentoflavone is of wide distribution, e.g. species of *Ginkgo*, *Hypericum*, *Rhus* and together with robustaflavone has been shown to have activity against influenza A virus, HSV-1 and HSV-2 viruses (Y.-M. Lin *et al.*, *Planta Medica*, 1999, **65**, 120).

Further reading

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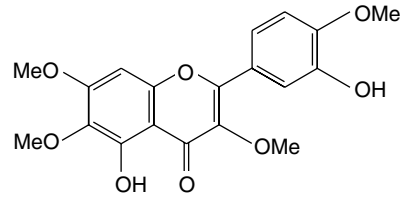
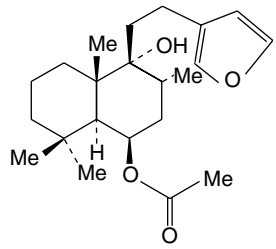
AGNUS CASTUS FRUIT

The *BP*/*EP*, *BHP* drug consists of the whole, ripe, dried fruit of *Vitex agnus castus* L., family Verbenaceae. Synonyms include Chaste tree, Chaste berry and Monk's pepper, alluding to its association with chastity. The plant is a shrub or small tree found in the Mediterranean regions of southern Europe; Morocco and Albania are important commercial suppliers.



Casticin
(Flavonoid)



Rotundifuran
(Labdane-type diterpenoid)

Fig. 21.19
Constituents of agnus castus.

The small dark berries are collected from the wild in autumn and dried. As such, they are blackish-brown in colour with a diameter of up to 5 mm, quadrilocular and four-seeded. A persistent toothed calyx covers up to three-quarters of the fruit. Features of the powder include covering and glandular trichomes from the calyx and numerous diverse fragments from the pericarp and seeds—all detailed in the official monographs.

Extensive studies on the phytochemistry have involved flavonoids (of which the *BP* specifies a minimum content of 0.08% calculated as casticin), also vitexin, penduletin and kaempferol; diterpenes including rotundifuran (Fig. 21.19) and vitexilactone; and various iridoids including aucubin (Fig. 24.1). The latter is used as a reference in the

Table 21.5 Flavonoid types and examples.

Type	Compound	Nature	Occurrence
Flavone	Chrysin	Dihydroxy 5,7	*Prunus, Populus* (heartwood and Balm of Gilead Bud)
Flavone	Butin	Trihydroxy 7,3′,4′	*Butea monosperma* seeds (antifertility activity)
Flavone	Apigenin	Trihydroxy 5,7,4′	Parsley, Roman chamomile flower
Flavone	Luteolin	Tetrahydroxy 5,7,3′,4′	*Reseda luteola* and as glycosides in celery, peppermint, wild carrot etc.
Flavone	Fisetin	Tetrahydroxy 3,7,3′,4′	Yellow cedar wood and the dyestuff sumac
Flavonol	Quercetin	Pentahydroxy 3,5,7,3′,4′	As the rhamnoglucoside rutin and as many other glycosides
Flavonol	Kaempferol	Tetrahydroxy 3,5,7,4′	Senna
Flavonone	Eriodictyol	Tetrahydroxy 5,7,3′,4′	Yerba santa (Hydrophyllaceae)
Flavonone	Liquiritigenin	Dihydroxy 7,4′	Liquorice
Chalcones	Unstable isomers of flavonones		Family Rutaceae and liquorice
Xanthone	Gentisin	1,7-Dihydroxy-3-methoxy	*Gentiana* and *Swertia* spp.
Isoflavone	Formononetin	Dihydroxy 7,4′	Cimicifuga rhizome, red clover flower
	Genistein	Trihydroxy 5,7,4′	Red clover flower, as glucoside in *Genista*
Biflavone	Amentoflavone	Hexahydroxy 2× (5,7,4′)	Ginkgo, hypericum, *Rhus* spp.

TLC test of identity and casticin is assayed by liquid chromatography. Volatile oil (*ca* 0.5%) consists mainly of mono- and sesquiterpenes.

The drug has a long history of use in various menstrual problems and in deficient lactation. In 2001 it was recommended as a therapeutic option for premenstrual syndrome where no cause could be identified (R. Schellenberg *et al*., *Br. Med. J*., 2001, **322**, 134) and further supported in 2006 by research demonstrating its activation of the μ-opiate receptor (D. E. Webster *et al*., *J. Ethnopharmacol*, 2006, **106**, 216).

Allied drug. Vitex negundo fruits are used in Asian medicine and can be distinguished from the *BP* drug by larger fruits.

BIRCH LEAF

Birch leaf has been included in several European pharmacopoeias for many years. The *BP*/*EP* drug consists of the dried whole or broken leaves of *Betula pendula* Roth. and/or *B. pubescens* Ehrh. and hybrids, family Betulaceae, containing not less than 1.5% of flavonoids calculated as hyperoside. *B. pendula* (silver birch) is common throughout Europe and *B. pubescens* (downy or white birch) is Eurasian in origin. Commercial supplies derive largely from Eastern Europe, China and the former USSR.

Leaves from *B. pendula* are 2.5–6.0 cm in length and 2–5 cm wide, glabrous, green on the upper surface, lighter green on the lower; in shape rhomboidal, triangular or ovoid in outline with a broadly tapering or cuneate base attached to a long petiole. The margin is biserrate, the apex long and acuminate. Larger veins are pinnate with an overall reticulation.

B. pubescens has similar, somewhat smaller, more rounded and often slightly pubescent leaves. The apex is neither long nor acuminate and the marginal teeth are smaller and less obviously apically directed than with *B. pendula*.

The microscopical characteristics of both species are similar, the lower epidermi having, in surface view, straight-walled cells and numerous anomocytic stomata with four to eight subsidiary cells. Calcium oxalate crystals occur as clusters in the mesophyll cells and as crystal fibres near the larger veins. Peltate sessile glands situated in shallow depressions are numerous on both surfaces. *B. pubescens* possesses unlignified unicellular thick-walled covering trichomes 80–**100**–**200**–600 μm in length.

The flavonoid compositions of both *B. pendula* and *B. pubescens* are similar with total flavonoids (about 3%) including hyperoside (up to 0.8%), quercitrin (up to 0.14%), myricetin galactoside (up to 0.37%) and other glycosides of quercetin (e.g. the arabinoside avicularin), kaempferol and myricetin. For a detailed analysis of 14 batches of *B. pendula* and three batches of *B. pubescens* see A. Carnat *et al*., *Ann. Pharm. Franc*., 1996, **54**, 231. This group also found the flavonoid level of older leaves of *B. pendula* to be lower than that of young leaves. The small proportion (0.1%) of essential oil contains sesquiterpene oxides. Other constituents of the leaves are (+)-catechin, monoterpene glycosides, triterpene alcohols and esters of the dammarene type. The mineral content (*c*. 4%) is particularly rich in potassium.

The official assay involves an acid hydrolysis of the powdered sample followed by extraction of the flavonoids, and their determination by absorption measurements at 425 nm.

Preparations of the leaf are used as an irrigant of the urinary tract, especially in cases of inflammation and renal gravel. Birch leaf is also an antirheumatic and has been employed to treat gout; as an astringent it is used as a mouthwash.

Birch bark has found traditional use in medicine for the topical treatment of skin diseases; it contains proanthocyanidins and considerable amounts of triterpenoid derivatives of the lupane (betulin, betulinic acid, lupeol) and oleane (oleanolic acid) types. Recently, M. Laszczyk *et al.* (*Planta Medica*, 2006, **72** 1353) have described the isolation and the physical, chemical and pharmacological properties of a gel extract from the outer bark containing triterpenes which can be applied topically to the skin; its cytotoxic properties were demonstrated experimentally. Betulinic acid is a potential agent for the treatment of human melanoma.

CALENDULA FLOWER

Source. Calendula flower derives from the marigold *Calendula officinalis* L., family Compositae, and is not to be confused with 'marigold' referring to *Tagetes* spp. The *EP* and *BP* specify the whole or cut, dried, and fully opened flowers detached from the receptacle and obtained from cultivated varieties. The *BHP* allows also the whole composite flowers which includes the involucre of bracts.

C. officinalis is a native of Central, Eastern and Southern Europe and commercial supplies are obtained largely from Eastern Europe and Egypt.

Characters. Detailed descriptions for the ligulate florets and the composite flower heads will be found in the *BP* and *BHP* respectively. Diagnostic features are the morphology of the ligulate and tubular florets, various biseriate clothing and glandular trichomes (see e.g. Fig. 42.4), pollen grains with a spiny exine, and corolla fragments with yellow oil-droplets, calcium oxalate and fairly large anomocytic stomata.

Constituents. Flavonoids, triterpenoids, essential oil and polysaccharides are the principal constituents of calendula flowers. All groups have been shown to exhibit pharmacological activity and serve to illustrate the difficulty of devising an assay which represents the true therapeutic activity of the drug. The *EP* and *BP* determine the flavonoid content, expressed as hyperoside (not less than 0.4%), utilizing the same method as for Birch Leaf. Other assays based on triterpenoid assessment have been described.

The flavonoid mixture involves quercetin and isorhamnetin derivatives. Triterpenoid saponins (calendulosides A–F, see Table 23.5) are glycosides based on oleanolic acid-3-*O*-β-D-glucuronide and are present in variable proportions (2–10%) depending on time of harvesting and chemotype. The roots are a richer source than the flowers. These saponins have haemolytic and anti-inflammatory activity. Polysaccharides include a rhamnoarabinogalactan (M_r 15 000; rhamnose 24.8%, arabinose 34.2%, galactose 41.0%) and two arabinogalactans with M_r's of 25 000 and 35 000 (J. Varljen *et al*., *Phytochemistry*, 1989, **28**, 2379). Antitumour and phagocytosis stimulation properties have been reported for the polysaccharide fraction. At least 15 compounds have been identified in the essential oil.

Other constituents of the flowers are triterpene alcohols (e.g. α- and β-amyrin, calenduladiol, etc.), sesquiterpenes and carotenoids.

Uses. Calendula is used internally for the alleviation of gastrointestinal disorders and externally, as an ointment or lotion, for the treatment of minor wounds and rashes.

ELDER FLOWER

Elder flower consists of the dried flowers of *Sambucus nigra* L., family Caprifoliaceae. This shrub or small tree is native throughout Europe and Western and Central Asia; commercial supplies of the flowers come principally from Eastern Europe, small quantities are collected in the UK.

Characters. The elder inflorescence consists of small regular flowers arranged in compound umbel-like cymes; calyx superior,

5-toothed; corolla flat, rotate, deeply 5-lobed, creamy white with 5 stamens inserted in the tube; anthers yellow. The flowers have a slightly bitter taste and a sweet, not altogether agreeable odour.

The microscopical characters of the corolla include numerous small oil globules and an upper epidermis with cells having slightly thickened, beaded walls and a striated cuticle. Epidermal cells of the calyx also have striated walls and those at the basal end exhibit unicellular marginal teeth. Calcium oxalate is seen as idioblasts of sandy crystals. There are numerous pollen grains about 30 μm in diameter (as measured in a chloral hydrate mountant) with a faintly pitted exine and three germinal pores and furrows.

Constituents. The drug contains a small proportion (up to *c*. 0.2%) of a semi-solid volatile oil consisting of free acids, principally palmitic acid, and C_{14}–C_{31} *n*-alkanes. By 1985 over 80 components had been identified in the oil.

Flavonoids (up to 3.0%) are predominantly flavonols and their glycosides: rutin predominates with smaller quantities of isoquercetrin, astragalin and hyperoside together with the aglycones quercetin and kaempferol.

Other constituents are triterpenes (α- and β-amyrin principally as esters of fatty acids), triterpene acids (ursolic, oleanolic and 20β-hydroxyursolic acids), various other plant acids (chlorogenic, *p*-coumaric, caffeic and ferulic acids, (Fig. 19.5), and their β-glucosides), sterols, mucilage, tannin and traces of sambunigrin (Table 25.1).

Standards. There are *BP/EP* limits for discoloured, brown flowers (15%) and for fragments of coarse pedicels and other foreign matter (8%). Thin-layer chromatography is employed as a test for identity with further modification to detect adulteration with *Sambucus ebulus*. Flavonoids, calculated as isoquercetrin are determined by absorbance measurements at 425 nm.

Allied species. *Sambucus ebulus* (danewort) is a perennial, foetid glabrous herb with a creeping rhizome and upright little-branched stems. It occurs throughout Europe and apart from habit, is distinguished from *S. nigra* by obvious ovate stipules. *S. canadensis*, American elder, is a somewhat smaller tree than *S. nigra* and is widely spread throughout North America; it is used similarly to *S. nigra*.

Uses. Elder flowers are administered principally as an infusion or herbal tea for the treatment of feverish conditions and the common cold; it acts as a diaphoretic but the mechanism and constituents involved are unclear. The flowers also have diuretic properties.

It may be noted that the sialic acid-binding lectin present in elder stem-bark extracts finds considerable current use in certain biochemical procedures.

HORSETAIL

Equisetum *BHP* 1996, Horsetail *BP/EP* consists of the dried sterile aerial parts of *Equisetum arvense* L. (common horsetail). The plant is found throughout Europe, common in Britain, central China and parts of N. America, preferring the moist sandy or loamy soil of hedgebanks, fields or waste places. Classed within the Pteridophyta (p. 21) it is a flowerless perennial, 20–80 cm in height producing in the spring chlorophyll-free jointed stems each terminating in a sporangia-producing cone. Apparent in the summer are jointed green stems with grooved, toothed sheaths at the nodes together with whorls of many-jointed, spreading branches. It is these sterile structures that constitute the medicinal drug.

Macroscopically, the drug consists of broken green stems and branches, the larger pieces being up to 80 cm in length and 5 cm in diameter. The surface is rough to the touch and the fracture short, exposing a large central cavity. The internodes of the main stem have up to 15 vertical grooves and at the nodes a sheath with as many triangular lanceolate teeth as grooves on the internodes. The branches are solid, again with internodes, the lowest of which on each branch is longer than the sheath with which it is associated.

Diagnostic features of the powder include: characteristic epidermis with paracytic stomata overlapped by the two adjacent subsidiary cells; two-celled non-lignified protuberances on the ridged areas; large-celled parenchyma with many lacunae; non-lignified fibres up to 1 mm long with narrow lumens; small spirally or annularly thickened lignified vessels.

Constituents. Various flavonoids occur in horsetail to the extent of 1.0%, the *BP/EP* requiring a content of at least 0.3% total flavonoids expressed as isoquercitroside. A major component is quercetin 3-glucoside, also luteolin glycosides in some samples (Fig. 21.18). Chemical races of horsetail involve flavonoids, see M. Veit *et al.*, *Planta Medica*, 1989, **55**, 214. Horsetail also contains a naturally high mineral content, with silicic acid and silicates comprising about 8% of the drug; also present are potassium and aluminium chlorides, all contributing to a high ash value for the drug for which, unusually for crude drugs, the pharmacopoeia sets a minimum, as well as higher limit, viz: total ash within 12–27%, acid-insoluble ash within 3–15%. Alkaloids are usually absent or present in small amounts, but see below for poisonous adulterants. Phenolic acids, saponins and phytosterols are among other reported constituents.

Uses. At one time the high siliceous mineral content of horsetail rendered it useful for the abrasive cleaning of copper and bare wooden objects. Traditionally, it has been used medicinally for its diuretic, haemostatic and astringent properties in particular for genitourinary complaints and externally to assist wound healing.

Allied species. A number of *Equisetum* spp. grow in similar damp localities to that of *E. arvense*, including *E. sylvaticum* the wood horsetail, *E. palustre* the marsh horsetail and *E. fluviatile* the water horsetail and might be mistaken or substituted for the genuine drug. As these are known to cause animal poisoning due to the alkaloids, e.g. palustrine and saponins, which have been reported, correct identification of the drug is essential. This is addressed by the pharmacopoeial macroscopic and microscopic descriptions of the drug, briefly covered above, and the TLC test for 'Other *Equisetum* species and hybrids'.

JAVA TEA

Java tea *BP/EP*, *BHP* consists of the dried leaves and tops of stems of *Orthosiphon stamineus* Benth. (*O. aristatus* Miq., *O. spicatus* Bak.), family Labiatae/Laminiaceae.

The plant is a perennial shrub up to 1 m in height with a four-angled stem bearing pointed leaves and lilac-coloured flowers arranged in whorls with four very long blue–violet stamens. It is native to S.E. Asia and Australia. Commercial supplies come from plants cultivated in Indonesia; the leaves and tips are collected shortly before flowering.

Characters. The shortly petiolate leaves are 2–7 cm long with pointed apex, cuneate base, coarsely serrate margin, deep green to yellowish-green upper surface, greenish-grey lower surfaces and, often pigmented pinnate venation.

Microscopical characteristics include wavy-walled, slightly beaded epidermal cells, diacytic stomata more numerous on the lower surface, laminaceous glandular trichomes having in contrast to many other species only four secretory cells, multicellular uniseriate conical clothing trichomes often with reddish contents. The stems afford considerable non-specific vascular tissue.

Constituents. Flavonoids as represented by sinensetin (Fig. 21.18) and various derivatives; diterpenes as illustrated by orthosiphols A–J and other highly oxygenated diterpenes (see S. Awale *et al.*, *Chem. Pharm. Bull.*, 2003, **51**, 268); various benzochromenes, including methylripariochromene A in variable amounts and others; volatile oil up to 0.7% and containing β-caryophyllene and its oxide, α-humulene, β-elemene, etc.; caffeic acid and its derivatives, particularly rosmarinic acid; phytosterols such as β-sitosterol; and inorganic salts, particularly potassium at around 3.0%.

The *BP*/*EP* requires a minimum content of 0.05% sinensetin for the drug determined by liquid chromatography using sinensetin as the reference compound.

Uses. Traditional uses in Europe invoke the diuretic properties of the drug for the treatment of urinary and kidney problems; in S.E. Asia it is used for the treatment of diabetes and hypertension.

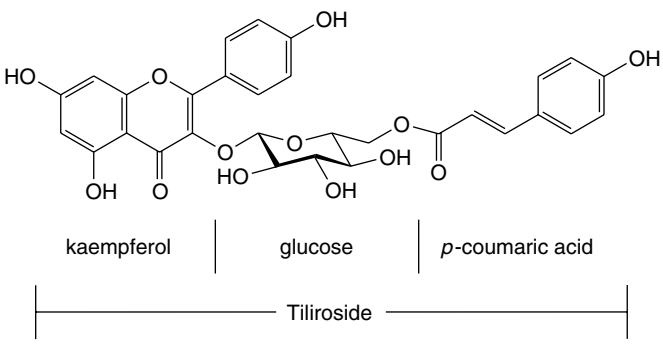
LIME FLOWER

Lime Flower *BP/EP* consists of the dried inflorescences of *Tilia cordata* Miller (small-leaved lime), *T. platyphyllos* Scop. (broad-leaved lime), *T.* × *vulgaris* Heyne or a mixture of these, family Tiliaceae. The trees are native throughout Europe and extensively planted. Commercial supplies of the flowers come from China, the Balkans, Turkey and Hungary, the latter exporting (1997) over 100 tonnes.

The inflorescences consist of pendulous long-peduncled cymes consisting of yellowish-green flowers, the peduncles being adnated to almost glabrous, strap-shaped bracts for about half their lengths. Each flower has five petals, five sepals, numerous stamens forming five groups, and a five-lobed stigma. The odour is faintly aromatic and the taste sweet and mucilaginous.

Features of the microscopy are the mucilaginous cells of the sepals and petals; small clusters of calcium oxalate crystals throughout the parenchymatous tissues; oval to slightly triangular pollen grains, 30–40 µm in diameter and having three germinal pores and a finely granulated exine; other general features of the sepals and petals.

The flavonoid constituents comprise quercetin glycosides (rutin, hyperoside, quercitrin, etc.) and kaempferol glycosides (tiliroside, astragalin). Mucilage, present chiefly in the bracts consists largely of galactomannans, and a small proportion of volatile oil (*c.* 0.02–0.1%) containing farnesol, farnesyl acetate, geraniol and eugenol gives the drug its characteristic faint odour, more pronounced with the fresh flowers. Phenolic acids and proanthocyanidins are also present.



The official upper limit for foreign matter in Lime Flower is 2.0% with observations for the absence of *T. americana* (basswood) and *T. tomentosa* based on flower structure. No assay is given but methods have been published in the German literature.

As with most herbal remedies lime flowers have a multiplicity of applications. In action they are diaphoretic, antispasmodic and expectorant and as such are used, often in conjunction with other herbs, as a nerve tonic, for the treatment of catarrh and indigestion, and for the alleviation of headaches.

MOTHERWORT

The dried aerial parts of *Leonurus cardiaca* L. family Labiatae/Lamiaceae constitute the drug Motherwort *BP*/*EP*, Leonurus *BHP* 1983 (common name lion's tail). The herb is collected during the flowering period.

L. cardiaca is native to Siberia and found generally throughout Europe from Scandinavia to the N. Mediterranean countries; it is rare in Britain, occurring on waste land, in hedges, ditches etc., and has become naturalized in N. America. A perennial herb 60–150 cm in height, it has square stems and alternate leaves. The lower and middle leaves are distinctly divided into five to seven pointed, dentate lobes, whereas the upper leaves are three-lobed, dark green on the upper surface with few trichomes, paler green and felted on the lower surface. Labiate flowers occur in well-separated whorls in the axils of the upper leaves, the general form giving rise to the generic and one of the common names of the plant (see above). The pubescent flower is white or pink, often spotted on the lower lip of the corolla; the angled calyx has five teeth the two lower ones being sharply recurved.

The microscopic features of the green powder are characteristic of the family and include: straight-walled cells of the upper epidermis with striated cuticles, the lower epidermis with anisocytic stomata, glandular trichomes with short unicellular stalks and multicellular heads, numerous covering trichomes occasionally up to 1500 µm in length, vascular tissue of stems and veins, small clusters and single crystals of calcium oxalate.

For the dried drug the *BP*/*EP* limits brown or yellow leaves to 2% and other foreign matter to 2%.

Constituents. Apart from the earlier isolation of stachydrine (formula, see Fig. 26.2), it was in the period 1970–1985 that the major constituents of motherwort were elucidated; these include flavonoids, iridoids, terpenoids and tannins.

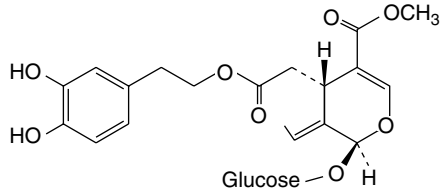
Flavonoids include hyperoside, kaemferol-3-D-glucoside, quercitrin and rutin (for formulae of these see Fig. 21.18 and Table 21.5). Iridoids include leonuride (see Fig. 24.1), ajugol and others. Minor alkaloids in addition to the major alkaloid stachydrine include the stereoisomers of its 4-hydroxy derivative (turicin) and betonicine (see formula under 'Yarrow'). Diterpenes of the labdane type include leocardin and a diterpene similar to marrubiin (q.v.). For the structural determination of three new labdane diterpenes see K. Vijai *et al*., *Planta Medica*, 2008, **74**, 1288.

The *BP*/*EP* requires a minimum flavonoid content for the drug of 0.2% expressed as hyperoside and assayed by absorbance measurements at 425 nm on a hydrolysed extract.

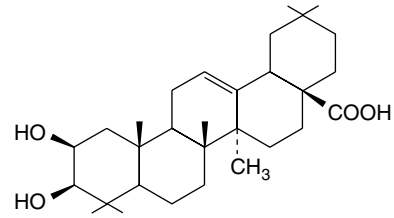
Traditional uses. For nervous tension and menstrual problems. There is some pharmacological support for its use as a uterotonic and for cardiovascular disorders.

OLIVE LEAVES

The olive (*Olea europaea* L., family Oleaceae), best known medicinally for its expressed oil (q.v.), is also used in continental Europe for



Oleuropein



Maslinic acid

Fig. 21.20
Constituents of olive leaves.

the antiseptic, astringent and sedative properties of the leaves. It can be employed both internally and externally.

The dried, leathery leaves, 30–50 mm long and 10–15 mm wide are elliptic, oblong or lanceolate in shape. The apex is mucronate, base shortly petiolate and tapering; margin entire and somewhat recurved. The upper surface is dark green, the lower paler due to a covering of silvery trichomes.

Microscopy of the powder shows thick walled polygonal epidermal cells with small anomocytic stomata on the lower surface together with large peltate trichomes, often broken. Sclereids are apparent.

Flavonols, including rutin (Fig. 21.18) and oleuropein are the principal components. Both compounds are used in the *BP*/*EP* TLC test for identity and there is a minimum requirement of 5.0% for oleuropein determined by liquid chromatography using a standard solution of it as reference. The pentacyclic triterpenoid maslinic acid occurs in the petioles and has various biological activities; it may offer advantages in the resistance to oxidative stress in animals (Montilla M. P. *et al.*, *Planta Med.*, 2003, **69**, 472).

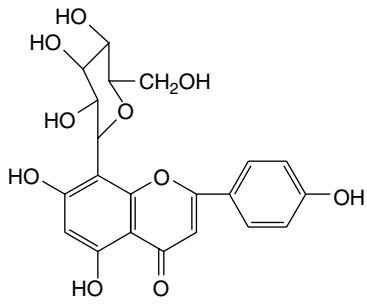
Triterpene acids have been isolated from plant-cell suspension cultures; they include six ursane-type acids and two oleane-type acids (oleanolic and maslinic) with the ursane-type predominant (H. Saimaru *et al.*, *Chem. Pharm. Bull.*, 2007, **55**, 784).

Olive leaves are used as an infusion for their tranquillizing effect in nervous tension and for their antiseptic, astringent and febrifuge properties.

PASSIFLORA

Passiflora (Passion Flower) consists of the dried aerial parts of *Passiflora incarnata* L. collected during the flowering and fruiting period. The drug is described in the *BP*, *EP*, *BHP*, the *British Herbal Compendium*, Vol. 1 and in an *ESCOP* monograph; it is also official in the French, German and Swiss pharmacopoeias. The genus is native to South America and species are widely cultivated as ornamentals. *P. incarnata* is imported from the USA, India and to some extent from the West Indies.

By the nature of its definition, the macroscopical and microscopical characteristics of the drug are numerous and for these the reader should consult one of the sources mentioned above.



Vitexin

Constituents of the genus include flavonoids, mainly *C*-glycosides of apigenin and luteolin such as vitexin, isovitexin, orientin, iso-orientin and their 2″-β-D-glucosides. The *BP* drug is required to contain not less than 1.5% total flavonoids calculated as vitexin (measurement at 401 nm) after treatment of a dry extract with methanol in glacial acetic acid followed by boric acid and oxalic acid in anhydrous formic acid. Other constituents include traces of volatile oil, cyanogenetic glycosides and possibly traces of alkaloids of the harman type. Species other than *D. incarnata* (*P. coerulea*, *P. edulis*) are eliminated by thin-layer chromatography.

Passiflora has sedative actions; it is a popular ingredient of herbal preparations designed to counteract sleeplessness, restlessness and irritability.

For a review update covering all aspects of the drug (over 200 refs). see K. Dhawan *et al.*, *J. Ethnopharmacol*., 2004, **94**, 1–23.

SPINY RESTHARROW ROOT

Spiny restharrow, *Ononis spinosa* L., family Leguminosae/Papilionaceae (syn. Restharrow, Cammock, Stayplough) is widely distributed throughout most of Europe except for the mountainous regions and the extreme north, also western Asia and North Africa; in England it is less common than the related species *O. repens* (Common restharrow). The plant is cultivated in Europe for medicinal purposes and harvested in the autumn. It is principally the roots that are used for medicinal purposes.

The dried drug consists of brown, longitudinally grooved roots, somewhat flattened and twisted and showing in transverse section a distinct radial arrangement of the xylem vascular tissue. The fracture is short and fibrous. Microscopical features include rounded starch grains and vascular tissue having vessels with small bordered pits.

Constituents. Isoflavones include the pterocarpan medicarpin (Fig. 21.18) (also a constituent of lucerne), homopterocarpin-7-*O*-glucoside and trifolirhizin (maackianin-7-*O*-glucoside); possibly the isoflavone formonoetin and its 7-*O*-glucoside. Tannins, lectins and triterpenoids, including α-onocerin (α-onoceradiendiol) have also been recorded. Volatile oil (0.02–0.29%), occurring in the entire plant, gives the drug a somewhat unpleasant odour and contains principally anethole, carvone and menthol; among other constituents are methone, camphor and estragole.

The *BP*/*EP* identification of the drug includes a TLC test.

Uses. The roots are traditionally used for their diuretic, antilithic and anti-inflammatory properties in the treatment of infections of the urinary tract and in removal of kidney and bladder stones.

Hesperidin and rutin

Although flavonoid preparations such as hesperidin and rutin are used in medicine, they do not appear to have justified the high hopes which followed the work of Szent–Györgyi in 1935 on the 'citrin' (sometimes

known as vitamin P) of paprika and lemon peel. Citrus and other fruits have long been included in the human diet and, in addition to ascorbic acid and other compounds, provide flavonoids which decrease capillary fragility and are therefore employed in cases of hypertension and radiation injuries. The substance formerly known as 'citrin' is now known to be a mixture of the rhamnoglucosides of eriodictyol (a tetrahydroxyflavone) and methyl eriodictyol (hesperetin). Among the commercially available products of this type are some produced by the citrus industry containing hesperidin. A similar glycoside, rutin, (Fig. 21.18) the rhamnoglucoside of quercetin, is found in many plants, and commercial supplies are made from tobacco residues, *Sophora* and *Eucalyptus* spp. or buckwheat (*Fagopyrum esculentum*), which yields about 3–4%. Hairy root cultures of *F. esculentum* are reported to give a higher flavonol production than normal root cultures (F. Trotin *et al.*, *Phytochemistry*, 1993, **32**, 929). The flowers of *Sambucus nigra* (elder) have long been used in domestic and veterinary medicine, particularly in the form of ointment. They contain *p*-coumaric acid, rutin and kaempferol.

Rutin occurs as a yellow crystalline powder, soluble in alkali but only slightly soluble in water. Rutin on hydrolysis yields quercetin, rhamnose and glucose, while hesperidin yields hesperetin (or methyl eriodictyol), rhamnose and glucose.

BUCKWHEAT HERB

The drug consists of the dried aerial parts of *Fagopyrum esculentum* Moench, family Polygonaceae, collected when the plant is flowering and prior to fruiting.

Fagopyrum esculentum originated in Central Asia and by the Middle Ages was being cultivated in Europe as a source of grain and green fodder. It has been cultivated in the UK and is now found wild on wasteground as an escape. The fresh plant contains a photosensitizing agent, which, if consumed by animals exposed to sunlight, can cause them damage.

The plant is a little-branched, glabrous herb often with reddish stems and producing a cymose paniculate inflorescence with pink or white flowers. Dark green leaves, paler on the lower surface, are broadly triangular in outline, cordate–saggitate and acuminate. Features of the powder include anomocytic stomata, epidermal papilla-like projections over the veins, numerous calcium oxalate cluster crystals up to 100 µm and small prismatic crystals, vascular tissue of leaf and stem, spherical pollen grains and corolla fragments.

Rutin (see above) is the most important therapeutic constituent of the herb and the *BP*/*EP* requires a minmum content of 4.0% determined by liquid chromatography with absorbance measurements at 350 nm.

Buckwheat is used in the treatment of various circulatory disorders, including varicose veins, chilblains and retinal bleeding.

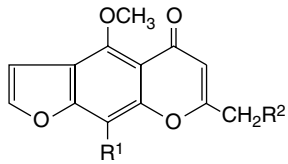
Silybin and silymarin

A number of flavonolignans—for example, silybin and silymarin (a 1,4-dioxan produced by the oxidative combination of taxifolin and coniferyl alcohol)—have antihepatotoxic properties, and extracts of plants containing them—for example, *Silybum marianum* (*Carduus marianus*), Compositae—are widely used in Germany for the treatment of liver ailments. The fruits of *S. marianum* contain silybin, silydianin and silychristin. For further details and structure of silybin see Chapter 29.

Visnaga

The drug consists of the dried ripe fruits of *Ammi visnaga* (Umbelliferae), an annual plant about 1–1.5 m high. It grows in the Middle East and is collected, particularly in Egypt. The greyish-brown mericarps are usually separate but are sometimes attached to the carpophore. Each mericarp is broadly ovoid and about 0.5 mm long. It has five prominent primary ridges and six vittae. Odour, slightly aromatic; taste, very bitter.

Khellin, the most important active constituent, is crystalline and has been synthesized. It is 2-methyl-5,8-dimethoxyfuranochrome. It occurs to the extent of about 1%, the highest concentration being reported in the immature fruits, and is accompanied by two other crystalline compounds, visnagin (about 0.1%) and khellol glucoside (about 0.3%). The fruits contain a minute amount (less than 0.03%) of volatile oil. Contrary to previous ideas that khellin and visnagin are located in the vittae, Franchi *et al*. (*Int. J. Crude Drugs. Res*., 1987, **25**, 137) have shown that, for fruits collected in Southern Tuscany, the furanochromones are present in the large secretory canals of the primary ribs and in the endosperm.

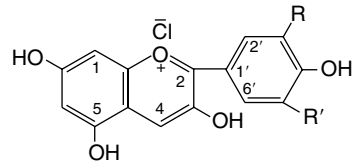


Khellin: $R^1 = OCH_3$, $R^2 = H$
Visnagin: $R^1 = R^2 = H$
Khellol glucoside: $R^1 = H$, $R^2 = O$-Glucoside

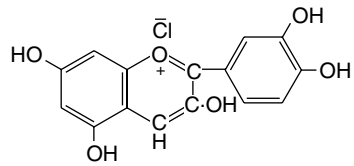
The drug has long been used in Egypt. Khellin, which is now commercially available in tablets and injection, is a potent coronary vasodilator. It has been employed in the treatment of angina pectoris and bronchial asthma, but its use appears to be limited by undesirable side-reactions.

ANTHOCYANIDINS AND GLYCOSIDES

Anthocyanidins are flavonoids structurally related to the flavones. Their glycosides are known as anthocyanins. These names are derived from the Greek *antho*-, flower, and *kyanos*, blue. They are sap pigments and the actual colour of the plant organ is determined by the pH of the sap. For example, the blue colour of the cornflower and the red of roses is due to the same glycosides and both of these plants on hydrolysis with hydrochloric acid yield cyanidin hydrochloride.



Anthocyanidin structure



Cyanidin chloride

Table 21.6 gives a few examples of these numerous and very widely distributed compounds. The most common anthocyanidin, cyanidin, occurs in about 80% of permanently pigmented leaves, 69% of fruits and 50% of flowers. Cyanidin is followed in order of frequency by delphinidin and pelargonidin.

Table 21.6 Common anthocyanidins.

Anthocyanidin	R	R′	Occurrence of glycosides
Pelargonidin	H	H	Flowers of *Pelargonium* (Geraniaceae) and pomegranate
Cyanidin	OH	H	Cornflowers, red poppies, *Rosa* spp., cocoa and cherries
Peonidin	OCH_3	H	Peony (Ranunculaceae)
Delphinidin	OH	OH	*Delphinium* and *Viola* spp.
Petunidin	OH	OCH_3	*Petunia* spp. (Solanaceae)
Malvidin	OCH_3	OCH_3	*Malva* spp., purple grades

Anthocyanidins are precipitated from aqueous solutions as lead salts or as picrates. After hydrolysis with 20% hydrochloric acid, anthocyanidin hydrochlorides, being only slightly soluble, often crystallize out. Chromatographic methods are widely used for the separation and identification of both the aglycones and sugars.

The sugar components are usually attached in the 3- or (more rarely) 5-position. It may be noted that in flavone glycosides the attachment is usually in the 7-position. They may be monosaccharides (glucose, galactose, rhamnose or arabinose); disaccharides (e.g. the rhamnoglucoside of *Antirrhinum* spp.); or trisaccharides (e.g. the 5-glucoside-3-rutinoside of certain Solanaceae such as *Atropa* and *Solanum*). Diglucosides, in which separate glucose molecules are attached in both the 3- and 5-positions, are common (e.g. *Campanula* and *Dahlia* spp.).

Despite their considerable biological importance the anthocyanidins are of little pharmaceutical significance as such, but as previously considered they constitute the monomers of the polymeric condensed tannins (q.v.).

For a review covering the analysis and biological activities of anthocyanins, see J.-M. Kong *et al.*, *Phytochemistry*, 2003, **64**, 923–933.

BILBERRY FRUIT

Bilberry (*Vaccinium myrtillus* L., family Vacciniaceae/Ericaceae) also known as blaeberry, whortleberry and huckleberry, is distributed throughout Europe, N. Asia and N. America including Canada. It grows on the acid soils of mountainous regions, heaths and moorlands and is found in most, particularly northern, regions of the British Isles. The plant is a glabrous, deciduous shrub up to 60 cm tall with creeping rhizomes and numerous erect stems and branches. It bears ovate, bright green leaves, 1–3 cm in length, pitcher-shaped greenish-pink flowers and globose berries about 8 mm in diameter, blue–black when ripe with a glaucous bloom. The quadri- or quinque-locular mesocarp of the fruit contains many ovoid, small, brown seeds. The edible sweet-tasting berries are collected from July to September. Both leaves and fruits were recognized in the Middle Ages for their medicinal value and separate monographs for fresh and dried fruits are now included in the *BP*/*EP*.

Constituents of the fruits. Anthocyanins, particularly glucosides and galactosides of cyanidin, peonidin, delphinidin, petunidin and malvidin (Table 21.6) are responsible for the final colour of the berries. These pigments increase in quantity during ripening whereas that of the polyphenols (−)-epicatechin, (+)-catechin and dimeric proanthocyanidins (Fig. 21.7) decrease. Other constituents include a number of common phenolic acids, vitamin C and volatile compounds. Over 100 volatiles have been identified, the principal ones that afford the characteristic odour of the berries being *trans*-2-hexenal, ethyl 3-methylbutyrate and ethyl 2-methylbutyrate.

For the dried fruits, the *BP*/*EP* specifies a minimum tannin content of 1.0% expressed as pyrogallol and for the fresh fruits a minimum of 0.30% anthocyanins expressed as cyanidin-3-glucoside chloride. The latter is determined by absorption measurements at 528 nm on an acidified aqueous extract of the dried fresh berries. The loss on drying of the fresh berries is 80–90%.

Frozen fresh berries should be stored at or below −18°C. It is important to inspect the dried drug for insect and mouldiness.

Uses and actions. Bilberry has many traditional medicinal uses, a number of which have been supported by fairly extensive pharmacological research. For detailed references, the reader should consult J. Barnes *et al*., *Herbal Medicines*, 2nd edn. 2002, p. 73, The Pharmaceutical Press, London. See also P. Morazzoni *et al*., *Fitoterapia*, 1996, **66**, 3 for an overall review of bilberry.

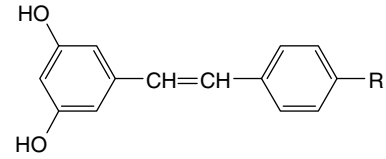
STILBENES

Two stilbenes of pharmacognostical interest are rhaponticin and resveratrol. The former is a glycosidal constituent of rhapontic rhubarb (q.v.) and due to its fluorescence in u.v. light has long been used to detect adulteration of the official rhubarb. Resveratrol is a constituent of species of *Arachis*, *Cassia*, *Eucalyptus*, *Polygonum* and *Veratrum*. Recent interest has centred on its occurrence in grape preparations including red wine, and its therapeutic properties as an antioxidant, anti-inflammatory, anti-PAF and anticancer agent. It may also reduce the risk of coronary heart disease (J. S. Soleas *et al.*, *Clinical Biochemistry*, 1997, **30**, 91). As a constituent of 'darakchasava', it has long featured in Indian medicine (B. Paul *et al.*, *J. Ethnopharmacology*, 1999, **68**, 71).

Pinosylvin, a natural stilbene of the heartwood of *Pinus* spp. is related to, and has similar antibacterial properties to, resveratrol. S. K. Lee *et al*., *Fitoterapia*, 2005, **76**, 258.

Stilbenes are biosynthesized from hydroxycinnamic acids and acetate.

For further discussion, see Chapter 32: The plant nutraceuticals.



Resveratrol, R = OH
Pinosylvin, R = H

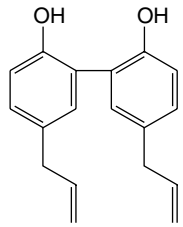
LIGNANS AND LIGNIN

Lignans are dimeric compounds formed essentially by the union of two molecules of a phenylpropene derivative. At one time it was thought that these compounds were early intermediates in the formation of lignin but it is now recognized that they are offshoots of the principal lignin biosynthetic pathway. Unlike lignin they are optically active compounds and probably arise by stereospecific, reductive coupling between the middle carbons of the side-chain of the monomer. Some 300 lignans have been isolated and categorized into a number of groups according to structural features. Important pharmaceutical examples are the lignans of *Podophyllum* spp. (q.v.) which appear to be formed from two molecules of coniferyl alcohol or the corresponding acid

with subsequent modification; apparently, a sinapic acid derivative, as might be expected by the inspection of the podophyllotoxin molecule, is not involved.

Some medicinal plants which contain lignans and which illustrate some of the structural types of this class of compound are given in Table 21.7. The lignans cited are not, however, necessarily the therapeutically active constituents of the plant.

Neolignans are also derived from the same units as lignans but the C_6–C_3 moieties are linked head to tail or head to head and not through the β-β′ carbons. They occur in the heart-woods of trees of the Magnoliaceae, Lauraceae and Piperaceae. *Magnolia officinalis* and *M. obovata* are used in Chinese medicine. Magnolol, a neolignan isolated from the bark, has the following reported activities: CNS depressant and muscle relaxant, antiplatelet, antimicrobial, antitumour, anticancer, insecticidal, etc. (S. D. Sarker, *Fitoterapia*, 1997, **63**, 3).



Magnolol

Lignin is an important polymeric substance, $(C_6–C_3)_n$, laid down in a matrix of cellulose microfibrils to strengthen certain cell walls. It is an essential component of most woody tissues and involves vessels, tracheids, fibres and sclereids.

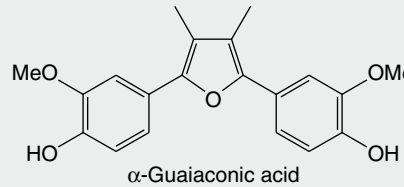
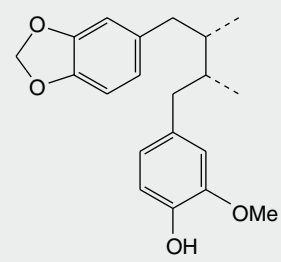
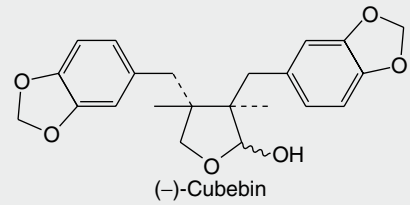
Lignins from different biological sources vary in composition, depending on the particular monomeric units of which they are composed (see Fig. 21.2). Variations in lignin constitution also arise as a result of random condensations of the appropriate alcohols with mesomeric free radicals formed from them by the action of a laccase-type (oxidase) enzyme. As there is no template for this non-enzymic condensation the lignin molecules formed vary in structure and so it is not possible to isolate lignin as a compound of defined composition.

The diagnostic value of lignin in crude drug analysis is covered in Chapter 42.

Further reading

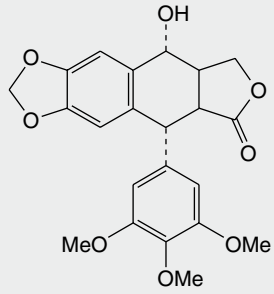
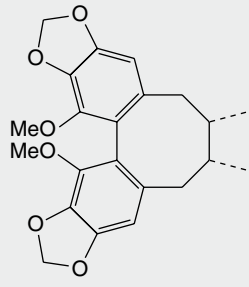
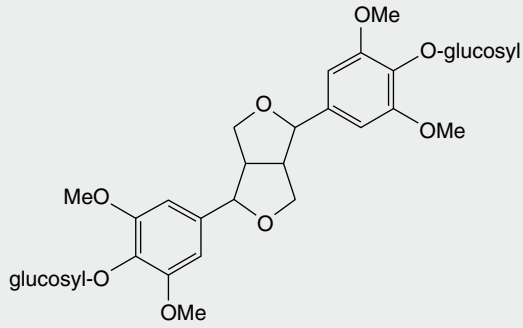
Ward RD 1999 Lignans, neolignans and related compounds. Natural Product Reports 16(1): 75–96

Table 21.7 Occurrence of lignans in medicinal plants.

Species	Lignans	Notes
Guaiacum officinale, *G. sanctum* Source of Guaiacum Resin	 α-Guaiaconic acid	A furano-type lignan. See 'Guaiacum resin' for other details. (+)-Neo-olivil of similar structure is the principal lignan of *Urtica dioica*
Myristica fragrans (Nutmeg)	 Macelignan	A dibenzylbutane-type lignan
Piper cubeba (Tailed pepper)	 (–)-Cubebin	A tetrahydrofuran-type lignan. Fruits contain *c.* 2% of (–)-cubebin together with related compounds

(Continued)

Table 21.7 Occurrence of lignans in medicinal plants. (Cont'd)

Species	Lignans	Notes
Podophyllum spp.	 Podophyllotoxin	This aryltetralin-type lignan and other related compounds are the principal active constituents of *Podophyllum* root and rhizome (see Chapter 27). Also found in linseed, q.v.
Schisandra chinensis	 Wuweizisu C	A dibenzocyclooctadiene-type lignan. This and other lignans of the same class have antihepatotoxic activity—see Chapter 29. Similar compounds are found in Korean Red Ginseng, q.v.
Silybum marianum *Urtica dioica* (Stinging nettle) *Viscum album* (Mistletoe) *Eleutherococcus senticosus* (*Acanthopanax senticosus*)	Silybin and others Neo-olivil derivatives  Eleutheroside E [(–)-syringaresinoldiglucoside]	*Flavonolignans* (see Chapter 29) Tetrahydrofuran-type lignans A tetrahydrofurofuran-type lignan. Occurs in a number of plants together with related compounds including eleutheroside D, the diastereoisomer
Zanthoxylum clava-herculis (Prickly ash bark)	(+)-Asarinin	A tetrahydrofurofuran-type lignan. Occurs in prickly ash bark together with alkaloids, coumarins, amides, resins etc. Plants of this genus used in Western, Indian and Chinese herbal medicine

22

Volatile oils and resins

VOLATILE OILS

Volatile or essential oils, as their name implies, are volatile in steam. They differ entirely in both chemical and physical properties from fixed oils. With the exception of oils such as oil of bitter almonds, which are produced by the hydrolysis of glycosides, these oils are contained largely as such in the plant. They are secreted in oil cells, in secretion ducts or cavities or in glandular hairs (see Chapter 42). They are frequently associated with other substances such as gums and resins and themselves tend to resinify on exposure to air.

Production and uses of volatile oils

Large quantities of volatile oil are produced annually; as examples, for lemon oil, eucalyptus oil, clove leaf oil and peppermint oil world production annually runs into several thousand metric tons each.

Although the production of major oils is highly organized, a number of developing countries have volatile oil-rich flora not fully utilized or cultivated and the United Nations Industrial Development Organisation has taken steps to inform on the setting-up of rural based small-scale essential oil industries (see 'Further reading'). India and China now produce large quantities of oil for export.

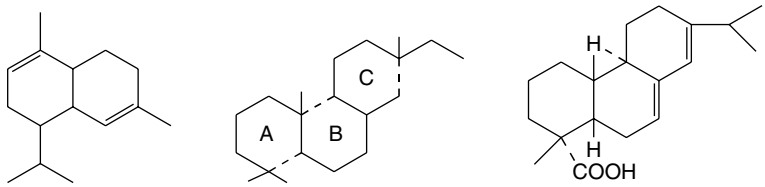
Volatile oils are used for their therapeutic action, for flavouring (e.g. oil of lemon), in perfumery (e.g. oil of rose) or as starting materials for the synthesis of other compounds (e.g. oil of turpentine). For therapeutic purposes they are administered as inhalations (e.g. eucalyptus oil), orally (e.g. peppermint oil), as gargles and mouthwashes (e.g. thymol) and transdermally (many essential oils including those of lavender, rosemary and bergamot are employed in the practice of aromatherapy.

Those oils with a high phenol content, e.g. clove and thyme, have antiseptic properties, whereas others are used as carminatives. Oils showing antispasmodic activity, and much used in popular medicine, are those of *Melissa officinalis, Rosmarinus officinalis, Mentha piperita, Matricaria chamomilla, Foeniculum vulgare, Carum carvi* and *Citrus aurantium*. The antispasmodic activity of some of these oils has also been demonstrated experimentally. The constituents of many volatile oils are stated to interfere with respiration and electron transport in a variety of bacteria, hence accounting for their use in food preservation and in cosmetic preparations.

Composition of volatile oils

With the exception of oils derived from glycosides (e.g. bitter almond oil and mustard oil) volatile oils are generally mixtures of hydrocarbons and oxygenated compounds derived from these hydrocarbons. In some oils (e.g. oil of turpentine) the hydrocarbons predominate and only limited amounts of oxygenated constituents are present; in others (e.g. oil of cloves) the bulk of the oil consists of oxygenated compounds. The odour and taste of volatile oils is mainly determined by these oxygenated constituents, which are to some extent soluble in water (note orange-flower water, rose water, etc.) but more soluble in alcohol (note tinctures or essences of lemon, etc.). Many oils are terpenoid in origin; a smaller number such as those of cinnamon and clove contain principally aromatic (benzene) derivatives mixed with the terpenes. A few compounds (e.g. thymol, carvacrol), although aromatic in structure, are terpenoid in origin.

Evaluation. Various pharmacopoeial procedures are given for the evaluation of volatile oils. Odour and taste are obviously important in the preliminary examination; however oils should not be tasted neat but only after dilution with a sugar solution in ethanol as prescribed in the *BP*. Physical measurements including optical rotation, relative density and refractive index are regularly employed for identification and



α-Cadinene Diterpene skeleton Abietic acid

assessment of purity; similarly thin-layer chromatograms. Capillary gas chromatographic profiles are used to determine the proportions of individual components of certain oils. Advances in gas chromatography have now made possible the ready determination of the chirality of particular components of volatile oils thus detecting adulteration with synthetic material or unwanted other oils; examples of its use are carvone in caraway oil, linalol in coriander oil and linalol and linalyl acetate in neroli oil. The ketone and aldehyde contents of oils such as caraway and lemon respectively are determined by reaction with hydroxylamine hydrochloride (oxime formation) and titration of the liberated acid. Other general tests described in the *BP* include examination for fixed and resinified oils (residue after evaporation), foreign esters (conversion to a crystalline deposit) and presence of water (turbidity of a carbon disulphide solution).

There have been a number of recent problems (1999) with the occurrence of tetrachloromethane in essential oils, particularly spearmint oil. This is probably not due to deliberate adulteration (the adulterant is present at low ppm levels) but a consequence of cleaning the drums before use with offending solvent and inefficient removal of it before filling the drums. Unfortunately on detection it renders the oil useless, as in food tetrachloromethane is prohibited.

The volatile oil content of crude drugs is commonly determined by distillation (Chapter 16).

Biogenesis. The origin of metabolites with phenylpropane and terpenoid structures has been discussed in Chapter 18. In medicinal essential oils the number of the former is limited but for the monoterpenes which arise at the geranyl pyrophosphate (GPP) level of terpenoid synthesis there are numerous examples. Analyses show that these oils commonly contain 40–80 monoterpenoids, many in relatively small proportion. A major constituent of one oil may be a minor one in another.

Three groups of monoterpenoid structures are involved: (1) acyclic or linear; (2) monocyclic; and (3) bicyclic. In the plant they are sequentially derived from limonene in this order as illustrated in Fig. 22.1. Relatively few enzymes, termed cyclases, appear to determine the skeletal class (e.g. menthanes, pinanes, thujanes etc.) and it is possible that these serve as rate-limiting enzymes. However, regulatory factors for the control of synthesis operate not only at the biosynthetic enzyme level *per se* but in a hierarchical manner up to the whole-organism level.

Because of the *trans* geometry of the double bond at the C-2 of GPP, direct cyclization to limonene is not possible and for *Mentha* spp. it has been shown that (−)-limonene synthase located within the oil glands catalyses the isomerization of GPP to enzyme-bound (+)-3*S*-linalyl pyrophosphate with subsequent cyclization to (−)-limonene. However, many enzymatic steps are involved in the subsequent modifications and interconversions of these monoterpenes. Some components of volatile oils are sesquiterpenes ($C_{15}H_{24}$) (q.v.) and they include cadinene, zingiberene (structure, Fig. 18.17) and caryophyllene. The formulae of some of the more common constituents of pharmaceutical volatile oils are given in Fig. 22.2.

It is increasingly evident that some monoterpenes and other components of volatile oils also occur in plants in the glycosidic form. Thus, geraniol, nerol and citronellol occur as glycosides in the petals of *Rosa dilecta*, thymol and carvacrol as glucosides and galactosides in *Thymus vulgaris* and eugenol, benzyl alcohol, β-phenylethyl alcohol, nerol, geraniol and geranic acid as glucosides in *Melissa officinalis*. It is considered that these glucosides of monoterpenols and of 2-phenylethanol are translocated from leaves to flowers as aroma precursors.

Table 22.1 may be used to compare the compositions of important volatile oils. The classification is arbitrary, since an oil may contain a number of compounds all about equally important but belonging to different chemical classes. The substance used for classification is not necessarily the one present in greatest amount. Thus, nutmeg is classified

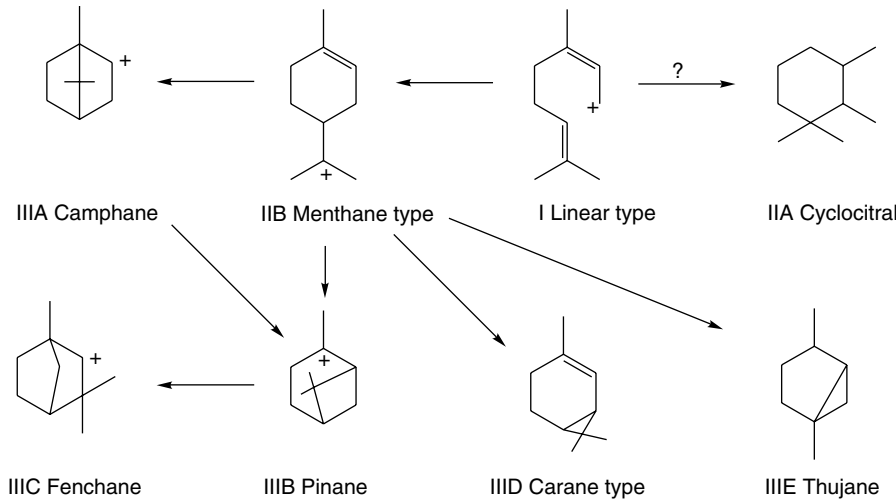


Fig. 22.1
Possible biogenetic relationships between some monoterpene types.

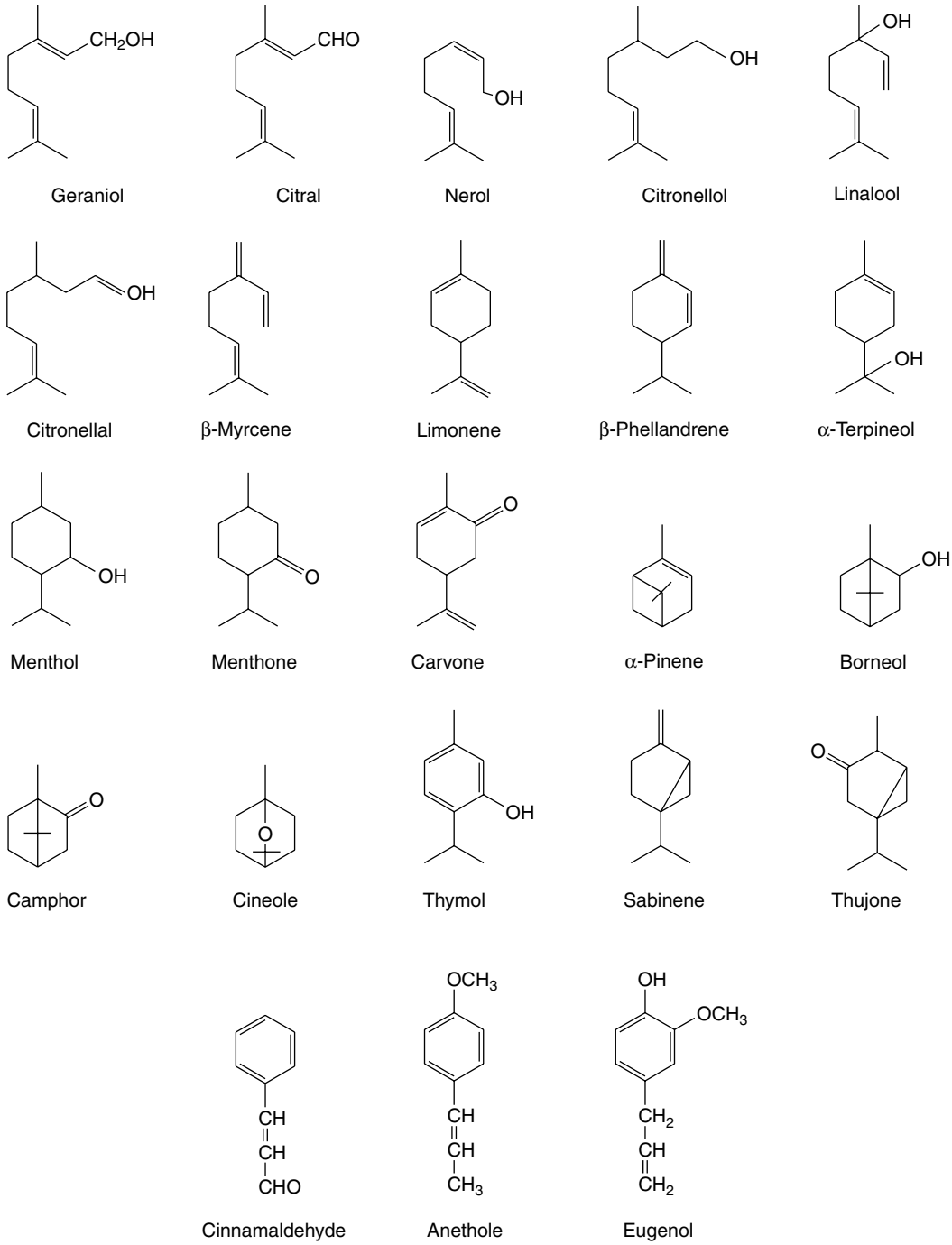


Fig. 22.2
Some components of pharmaceutical oils.

on its myristicin and lemon on citral, although these constituents form only a small percentage of these oils.

C_{20} or diterpenoid compounds include such resin acids as (+)- and (−)-pimaric acid and their isomer, the abietic acid of pine resin. The abietane acids have antimicrobial, antiulcer and cardiovascular properties (for a review covering some 56 acids over the period 1967–92 see A. S. Feliciano *et al*., *Planta Med*., 1993, **59**, 485). Many diterpenoids (e.g. vitamin A and gibberellic acid) do not belong to the volatile oil–resin group. Similarly, only a small proportion of triterpenoid compounds (C_{30}) are found as resin constituents (e.g. in *Euphorbia resinifera*).

Preparation of volatile oils

All the official volatile oils are extracted by distillation with the exception of oil of lemon and oil of cade. The distillation of volatile oils by means of water or steam has long been practised, but modern plants possess many advantages over the older stills, in which charring and undesirable decomposition of the oil often took place. Modern volatile oil stills contain the raw material on perforated trays or in perforated baskets. The still contains water at the base which is heated by steam coils, and free steam under pressure may also be passed in. Tough material such as barks, seeds and roots may be comminuted to

Table 22.1 Composition of volatile oils.

Name	Botanical name	Important constituents
Terpenes or sesquiterpenes		
Tea-tree	*Melaleuca alternifolia*	Cyclic monoterpenes
Turpentine	*Pinus* spp.	Terpenes (pinenes, camphene)
Juniper	*Juniperus communis*	Terpenes (pinene, camphene); sesquiterpene (cadinene); alcohols
Cade (Juniper Tar Oil)	*Juniperus oxycedrus*	Sesquiterpenes (cadinene); phenols (guaiacol, cresol)
Alcohols		
Coriander	*Coriandrum sativum*	Linalol (65–80% alcohols); terpenes
Otto of rose	*Rosa* spp.	Geraniol, citronellol (70–75% alcohols); esters
Geranium	*Pelargonium* spp.	Geraniol; citronellol; esters
Indian or Turkish geranium (Palmarosa)	*Cymbopogon* spp.	Geraniol (85–90%)
Sandalwood	*Santalum album*	Santalols (sesquiterpene alcohols), esters, aldehydes
Esters and alcohols		
Bergamot	*Citrus bergamia*	Linalyl acetate, linalol
Lavender	*Lavandula officinalis*	Linalol; linalyl acetate (much); ethyl-pentyl ketone
Rosemary	*Rosmarinus officinalis*	Borneol and linalol (10–18%); bornyl acetate, etc. (2–5%); terpenes; cineole
Pumilio pine	*Pinus mugo* var. *pumilio*	Bornyl acetate (about 10%); terpenes; sesquiterpenes
Peppermint	*Mentha piperita*	Menthol (about 45%); menthyl acetate (4–9%)
Aldehydes		
Cinnamon bark	*Cinnamomum verum* Presl.	Cinnamaic aldehyde (60–75%); eugenol; terpenes
Cassia	*Cinnamomum cassia*	Cinnamic aldehyde (80%)
Lemon	*Citrus limon*	Citral (over 3.5%); limonene (about 90%)
Lemon grass	*Cymbopogon* spp.	Citral and citronellal (75–85%); terpenes
'Citron-scented' eucalyptus	*Eucalyptus citriodora*	Citronellal (about 70%)
Ketones		
Spearmint	*Mentha spicata* and *M. cardiaca*	Carvone (55–70%); limonene, esters
Caraway	*Carum carvi*	Carvone (60%); limonene, etc.
Dill	*Anethum graveolens*	Carvone (50%); limonene, etc.
Sage	*Salvia officinalis*	Thujone (about 50%); camphor; cineole etc.
Wormwood	*Artemisia absinthium*	Thujone (up to 35%); thujyl alcohol; azulenes
Phenols		
Cinnamon leaf	*Cinnamomum verum* Presl.	Eugenol (up to 80%)
Clove	*Syzygium aromaticum* (L.) Merr & L. M. Perry	Eugenol (85–90%); acetyl eugenol, methylpentyl ketone, vanillin
Thyme	*Thymus vulgaris*	Thymol (20–30%)
Horsemint	*Monarda punctata*	Thymol (about 60%)
Ajowan	*Trachyspermum ammi*	Thymol (4–55%)
Ethers		
Anise and Star-anise	*Pimpinella anisum* and *Illicium verum*	Anethole (80–90%); chavicol methyl ether, etc.
Fennel	*Foeniculum vulgare*	Anethole (60%); fenchone, a ketone (20%)
Eucalyptus	*Eucalyptus globulus*	Cineole (over 70%); terpenes, etc.
Cajuput	*Melaleuca* spp.	Cineole (50–60%); terpenes, alcohols and esters
Camphor	*Cinnamomum camphora*	After removal of the ketone camphor contains safrole; terpenes, etc.
Parsley	*Petroselinum sativum*	Apiole (dimethoxysafrole)
Indian dill	*Peucedanum soja*	Dill-apiole (dimethoxysafrole)
Nutmeg	*Myristica fragrans*	Myristicin (methoxysafrole) up to 4%; terpenes (60–85%); alcohols, phenols
Peroxides		
Chenopodium	*Chenopodium ambrosioides* var. *anthelmintica*	Ascaridole (60–77%), an unsaturated terpene peroxide
Non-terpenoid and derived from glycosides		
Mustard	*Brassica* spp.	Glucosinolates
Wintergreen	*Gaultheria procumbens*	Methyl salicylate
Bitter almond	*Prunus communis* var. *amara*	Benzaldehyde and HCN (from amygdalin)

facilitate extraction but flowers are usually placed in the still without further treatment as soon as possible after collection. Distillation is frequently performed in the field.

The distillate, which consists of a mixture of oil and water, is condensed and collected in a suitable receiver which is usually a Florentine flask or a large glass jar with one outlet near the base and another near the top. The distillate separates into two layers, the oil being withdrawn through the upper outlet and the water from the lower outlet, or vice versa in the case of oils, such as oil of cloves, which are heavier than water. The oil-saturated aqueous layer may be returned to the still or may form an article of commerce, as in the case of rose water and orange-flower water.

Certain oils (e.g. oil of cajuput, oil of caraway, oil of turpentine and oil of Australian sandalwood) are rectified. Rectification usually takes the form of a second distillation in steam, which frees the oil from resinous and other impurities. Light and atmospheric oxygen appear to have an adverse effect on most volatile oils and the official directions with regard to storage should be rigidly followed. The distillation of oil of chenopodium must be done as rapidly as possible, as the chief constituent, ascaridole, gradually decomposes on boiling with water.

Extraction of oils used in perfumery. Certain oils used in perfumery, such as oil of rose, are prepared by steam distillation as described above but many of the flower perfumes require other treatment. An important centre for the extraction of flower oils is Grasse, in the south of France. Here the oils are extracted by *enfleurage*, by digestion in melted fats, by pneumatic methods or by means of solvents. In the *enfleurage* process glass plates are covered with a thin layer of fixed oil or fat upon which the fresh flowers are spread. The volatile oil gradually passes into the fat and the exhausted flowers are removed and replaced by a fresh supply. Formerly the flowers had to be picked off by hand but this is now done mechanically. Only a small percentage of the flowers, which resist the action of the machine, require removal by the fingers or by means of a vacuum cleaner. The pneumatic method, which is similar in principles to the *enfleurage* process, involves the passage of a current of warm air through the flowers. The air, laden with suspended volatile oil, is then passed through a spray of melted fat in which the volatile oil is absorbed. In the digestion process the flowers are gently heated in melted fat until exhausted, when they are strained out and the perfume-containing fat is allowed to cool. It will be seen that in each of the above processes the volatile oil has now been obtained in a fatty base. The volatile oil is obtained from this by three successive extractions with alcohol. The alcoholic solutions may be put on the market as flower perfumes or the oil may be obtained in a pure form by recovery of the alcohol. Solvent extraction is based on the Soxhlet principle (see Chapter 16).

Further reading

De Silva KT 1995 A manual on the essential oil industry. UNIDO, Vienna

Oil of rose

Oil of rose (*Otto* or *Attar of Rose*, *Oleum Rosae*) is a volatile oil obtained by distillation from the fresh flowers of *Rosa damascena*, *R. gallica*, *R. alba* and *R. centifolia* (Rosaceae). It is included in the *USP/NF*, 1995. The chief producing countries are Bulgaria, Turkey and Morocco but smaller quantities are prepared elsewhere.

The oil is prepared in copper alembic stills by the peasants or in large factories under careful scientific control. Some 3000 parts of flowers yield only one part of oil. The oil is very expensive and very liable to adulteration. The 'peasant distilled' oil usually fetches a lower price than that produced in the larger works.

The oil is a pale yellow semisolid. The portion which is solid at ordinary temperatures forms about 15–20% and consists of odourless stearoptene containing principally saturated aliphatic hydrocarbons (C_{14}–C_{23} normal paraffins). The liquid portion forms a clear solution with 70% alcohol. It consists of the alcohols geraniol, citronellol, nerol and 2-phenylethanol with smaller quantities of esters and other odorous principles. Although the alcohols form about 70–75% of the oil, the odour is so modified by the other constituents, such as sulphur containing compounds, that no artificial mixture of the known constituents can be made to reproduce the odour of the natural oil. Phenylalanine has been shown to act as a precursor of the 2-phenylethanol; acetate and mevalonate are incorporated into the terpene alcohols. A citronellyl disaccharide glycoside has been identified as an aroma precursor of citronellol in flowers of *R. damascena* var. *bulgaria* (N. Oka *et al*., *Phytochemistry*, 1998, **47**, 1527). Among a number of lines of callus derived from the leaf-bud of *R. damascena* a few have been shown to produce 2-phenylethanol.

From *Rosa rugosa* var. *plena* growing in central China some 108 compounds have been identified in the flower oil; these include citronellol (60%), geraniol (8.6%), nerol (2.8%), citronellyl acetate (2.7%) and *E*,*E*-farnesol (2.5%). For a review see Y. Hashidoko, *Phytochemistry*, 1996, **43**, 535.

Oil of rose is of great importance in perfumery (for a fuller account of its history and utilization see Widrlechner, *Econ. Bot*., 1981, **35**, 42).

PEPPERMINT LEAF AND PEPPERMINT OIL

Peppermint Leaf as defined in the *BP* and *EP* is the dried leaves of *Mentha* × *piperita* L. (Labiatae). It is required to contain not less than 1.2% of volatile oil. The oil is obtained from the same plant by steam distillation using the flowering tops. The European and American oil appears to be derived to a large extent from the two varieties *M. piperita* var. *vulgaris* Sole ('black mint') and *M. piperita* var. *officinalis* Sole ('white mint').

Mentha × *piperita* is, as implied by the written botanical name, a hybrid species from the two parents, *M. spicata* ($2n = 36$ or 48) and *M. aquatica* ($2n = 96$). *M.* × *piperita* strains commonly have a somatic number of 72, a smaller proportion 66, but other figures have also been reported.

Macroscopical characters. All the mints have square stems and creeping rhizomes. The flowers are arranged in verticillasters and have the floral formula K(5),C(5),A4,G(2). The black mint, which is the one most commonly cultivated in England, has purple stems and dark green petiolate leaves which are tinged with purple. The leaf blades are 3–9 cm long and have a grooved petiole up to 1 cm long. They have a pinnate venation with lateral veins leaving the midrib at about a 45° angle, acuminate apex and sharply dentate margin. Glandular trichomes can be seen as bright yellowish points when the lower surface is examined with a hand lens. The leaves are broader than those of *M. spicata* (spearmint), but narrower than those of *M. aquatica* (water mint). The small, purple flowers appear in late summer.

Microscopical characters. The microscopy of peppermint leaves is typical of the family, showing numerous diacytic stomata on the lower surface (Fig. 42.2G), three- to eight-celled clothing trichomes with a striated cuticle (Fig. 42.4C), and two types of glandular trichome, one with a unicellular base and small single-celled head and the other with a multicellular head characteristic of the family (Fig. 42.5E). Calcium oxalate is absent.

There is a 5% limit of stems over 1 mm in diameter for the official leaves, and as mints are very susceptible to most diseases, there is a 10% limit of leaves infected by *Puccinia menthae*.

Of commercial importance has been the development by mutation breeding at the A. M. Todd Co. of a strain of Mitcham peppermint resistant to the wilt disease *Verticillium albo-duram* var. *menthae*. The strain retains the Mitcham cultivar organoleptic characteristics and gives a good oil production in verticillium-prone soils where cultivation with the ordinary varieties is impossible.

OIL OF PEPPERMINT

The oil of the *BP* (1993) was required to contain 4.5–10% of esters calculated as menthyl acetate, not less than 44% of free alcohols calculated as menthol and 15–32% of ketones calculated as menthone. However, these chemical evaluations are now replaced by a capillary GC profile; limits for individual compounds are limonene 1.0–5.0%, cineole 3.5–14.0%, menthone 14–32%, menthofuran 1.0–9.0%, isomenthone 1.5–10.0%, menthylacetate 2.8–10.0%, menthol 30.0–55.0%, pulegone ⩾4.0%, carvone ⩾1.0%. The ratio of the cineole to limonene contents exceeds 2.

Small quantities of the sesquiterpene viridoflorol form a useful identification characteristic of the oil. A basic fraction of the oil contains a number of pyridine derivatives such as 2-acetyl-4-isopropenyl pyridine which has a powerful grass-like minty odour. High-resolution GC has been used to identify over 85 components of the oil.

As with other cultivated Labiatae, the oil composition of *M.* × *piperita* is greatly influenced by genetic factors and seasonal variations (see relevant chapters). The task of elucidating the nature of the genetic control for the formation of various constituents has been rendered difficult by the hybrid and polyploid nature of the genus. Much progress in this area was achieved by M. J. Murray, a mint breeder with the A. M. Todd Company, Kalamazoo, Michigan. His collection of over 600 accessions of mint species, which has continued to be researched and added to, now forms the basis of the collection of the USDA-ARS-National Clonal Germplasm Repository in Cornvallis, Oregon.

Biogenesis of peppermint monoterpenoids. The biosynthetic isoprenoid origin of monoterpenes was mentioned at the beginning of this chapter. As the essential oils of the Labiatae are synthesized in the cells of the glandular trichomes, techniques such as cell and root culture are of little value as experimental tools. However, new procedures, using gentle abrasion of leaf surfaces with glass beads, have been developed for isolating in high purity and excellent yield, peltate glandular trichomes of peppermint which retain their biosynthetic activity.

Developmental changes in the oil composition of the leaves include the disappearance of limonene, the accumulation of 1,8-cineole, the conversion of menthone to menthol and the acetylation of menthol. All these processes begin at the distal extremity of the leaf and shift progressively to the leaf base (B. Voirin and C. Bayer, *Phytochemistry*, 1996, **43**, 573).

A proposed pathway for the formation of monoterpenes in peppermint is given in Fig. 22.3. A number of enzymes involved in the reactions have been characterized. The hydrolase system involving (−)-limonene-3-hydroxylase in the formation of the alcohol (−)-*trans*-isopiperitenol is cytochrome-P450-dependent and is associated with the oil gland microsomal fraction. The remaining steps are catalysed by operationally soluble enzymes of the oil cells. It will be noted that (+)-pulegone is a branching point for the formation of menthol stereoisomers.

Japanese peppermint oil is derived from *Mentha canadenis* var. *piperascens*; it contains 70–90% menthol, for the extraction of which it is largely used. The commercial dementholized Japanese oil contains approximately the same amount of menthol and its esters as the American oil.

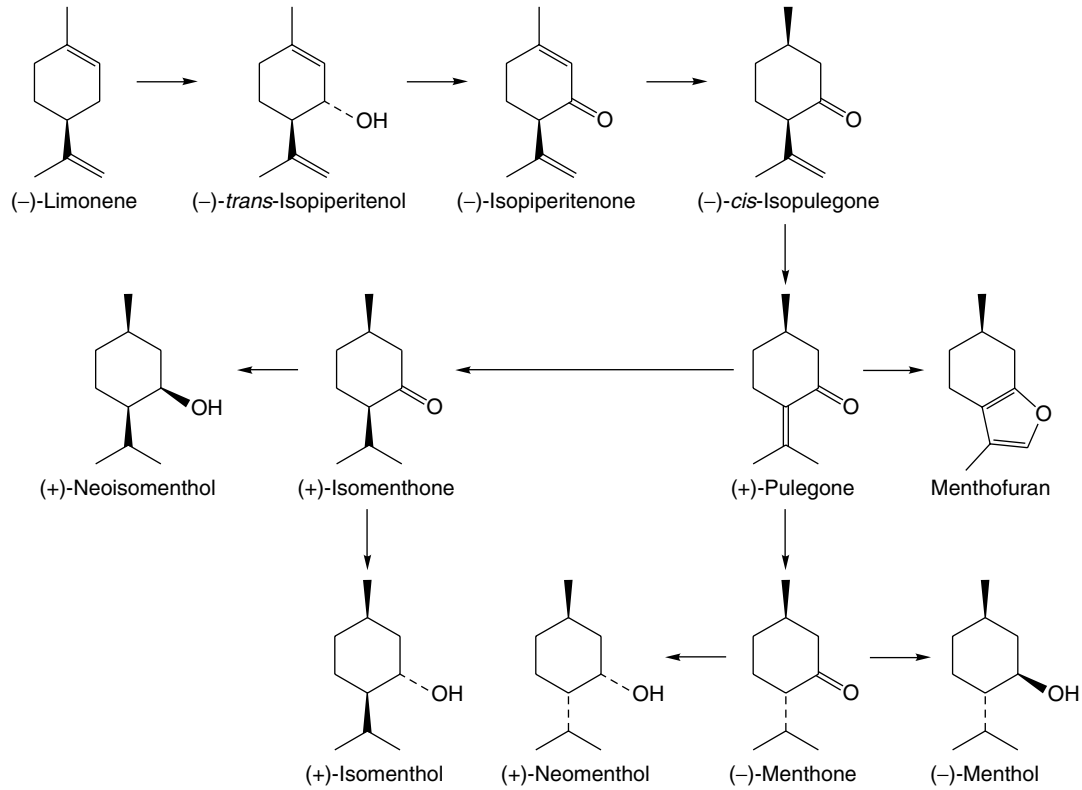


Fig. 22.3
A possible biogenetic scheme for some monoterpenoids of *Mentha* × *piperita*.

DEMENTHOLIZED MINT OIL *BP*

This is cited as the volatile oil from *Mentha arvensis* var. *piperascens* from which the menthol has been partially removed. The two commercial oils, Brazilian and Chinese, differ somewhat in their ranges of ester and alcohol contents; standards are given for each. For both, the cineole:limonene ratio, as determined by GC, is less than unity.

SPEARMINT OIL

Spearmint or ordinary garden mint consists of the dried leaf and flowering top of *Mentha spicata* L. (*M. viridis* Linn.) and *Mentha* × *cardiaca* (Labiatae). The *BP* oil is prepared by steam distillation and should contain not less than 55% of carvone, 2–25% limonene with upper limits for a number of other constituents as determined by gas chromatography. The commercial oil was originally produced in North America but the industry has now largely transferred to India.

Characters. Mint has more or less crumpled, opposite, ovate-lanceolate leaves, 3–7 cm long. The apex is acute or acuminate, and the margin unequally serrate. The leaves differ from those of peppermint in that they are almost sessile and have a bright green colour free from purple.

Constituents. Oil of spearmint contains (–)-carvone, (–)-limonene, phellandrene and esters. As with *M.* × *piperita* limonene is the precursor of the monoterpenoids and in this case the action of a (–)-limonene-6-hydroxylase predominates to give the alcohol (–)-*trans*-carveol which is oxidized to carvone (Fig. 22.4). Dihydrocarvone is formed later in the season and is absent from plantlets produced by shoot-tip culture. Again like peppermint, oil production is influenced by the age of plant, time of collection, chemical varieties and hybridization.

Further reading

Lawrence BM (ed), Hardman R (series ed) 2007 Medicinal and aromatic plants – industrial profiles, Vol 44, Mint: The genus *Mentha*. CRC Press, Boca Raton, FL

SAGE LEAF

The official drug consists of whole or cut leaves of *Salvia officinalis* (Labiatae) containing not less than 1.5% (whole leaf) or 1.0% (cut leaf) of essential oil which is determined by steam distillation. The plant is indigenous to Mediterranean areas but is now cultivated world-wide, principally for its use as a culinary herb.

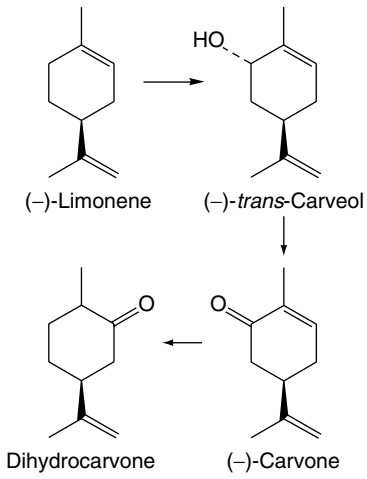


Fig. 22.4
Biosynthetic pathway for major constituents of spearmint oil.

Macroscopical characters. The petiolate oblong-lanceolate leaves are up to 10 cm length and 2 cm in breadth, greenish-grey on the upper surface and tomentose on the lower with a markedly reticulate venation. The leaf apex is rounded, the base rounded or cordate and the margin crenulate. The odour and taste are characteristically pungent.

Microscopical characters. The upper epidermal cells have beaded anticlinal walls, the lower ones are thin-walled and sinuous; both epidermi possess diacytic stomata. Glandular trichomes of the typical labiate type occur on both surfaces with rarer uniseriate glandular trichomes having a double- or single-celled head. Clothing trichomes are numerous, particularly on the lower surface, composed of a short thickened basal cell with articulated and bent terminal cells. A few single-celled warty-walled trichomes are present. The long protective trichomes serve to distinguish *S. officinalis* from *S. sclarea* and *S. pratensis* (M. Then *et al*., *Acta Pharm. Hung*., 1998, **63**, 163).

Constituents. The volatile oil of sage contains about 50% of α- and β-thujone together with cineole, borneol and other constituents (Fig. 22.2). Varieties and other species of sage contain differing amounts of thujone.

Non-volatile components of the leaf include diterpenes, phenolic glycosides based on caffeic and *p*-hydroxybenzoic acids (for recent isolations see M. Wang *et al*., *J. Nat. Prod*., 1999, **62**, 454), and tannins.

Action and uses. Sage as an infusion is used as a mouthwash and gargle for its antiseptic and astringent action. Recent attention has focused on the cholinergic activity of the drug and its possible role in the treatment of Alzheimer's disease and memory loss. It is interesting to note that long before recent advances in the understanding of the neurobiology of Alzheimer's disease, plant materials including sage and balm (*Melissa officinalis*) were recommended in old reference books as possessing memory-improving properties (see E. K. Perry *et al*., *J. Pharm. Pharmacol*., 1999, **51**, 527). The phenolic glycosides of sage together with those of *Melissa officinalis* and *Lavandula angustifolia* possess antioxidant properties (J. Hohmann *et al*., *Planta Medica*, 1999, **65**, 576).

THREE-LOBED SAGE LEAF

Three-lobed sage leaf *BP*/*EP* also known as Greek sage, consists of the whole or cut, dried leaves of *Salvia fructicosa* Mill. (*S. triloba* L. fill) containing not less than 1.8% essential oil in the whole drug and not less than 1.2% in the cut drug. The leaves have a grey–green upper surface and conspicuously downy underside. They are somewhat larger (8–50 mm in length, 4–20 mm in width) than those of common sage and are considerably more pubescent. The clothing trichomes and odour (spicy and similar to that of eucalyptus oil) constitute features of the powder, which otherwise resembles *S. officinalis*.

An alcoholic extract of the drug subjected to TLC is used by the *BP* to detect the presence of cineole and the almost complete absence of thujone in the sample.

SAGE OIL

Sage oils are produced commercially by steam distillation from a number of *Salvia* species but the oil composition is not uniform, as illustrated by the three species considered here. For this reason, the *BP*/*EP* specifies one species, *S. sclarea* L., the clary sage, as the source

of the official oil. The plant is a native of Mediterranean regions and had been introduced into England by the the 14th century.

The pharmacopoeia specifies the acceptable concentration limits for constituents of the oil as follows: α- and β-thujone (less than 0.2%), linalol (6.5–24.0%), linalyl acetate (50–80%); α-terpineol (less than 5.0%), germacrene-D (1.0–12%) and sclareol (0.4–2.6%).

The oil is widely used for flavouring and perfumery purposes.

ROSEMARY LEAF

Rosemary leaf *BP*/*EP*, *BHP* is the whole dried leaf of *Rosmarinus officinalis* L., family Labiatae. The plant is native to Mediterranean regions and is widely cultivated elsewhere in herb gardens and as an aromatic ornamental. Many horticultural varieties varying in habit and flower colour exist. Commercial supplies of the leaf come principally from Spain, Morocco and Tunisia.

R. officinalis is an aromatic evergreen shrub, variable in its form, but mostly with stems reaching a height of over 1 m. The bilobed corollas of the flowers are pale to dark blue and occur clustered in spikes at the ends of the branches; they are larger than those of either lavender or the mints. The leathery, opposite leaves are up to 4 cm long and up to 4 mm wide with entire strongly recurved margins and prominent midribs. The upper surfaces are green, the lower ones grey and somewhat woolly due to numerous branched trichomes. Typical labiate hairs contain the volatile oil, of which the *BP* specifies a minimum content of 1.2% calculated on the anhydrous drug.

Constituents. The compositon of the essential oil is considered under 'Rosemary Oil', below. Hydroxycinnamic acids include caffeic acid and a dimer rosmarinic acid (a characteristic metabolite of the subfamily Saturejoidae to which *Rosmarinus* belongs). For the dried leaf, the *BP* sets a minimum requirement of 3.0% for total hydroxycinnamic acids expressed as rosmarinic acid.

In recent years, a large number of phenolic abietane diterpenoids have been recorded for the leaves including the potent antioxidant carnosic acid together with its degradation products carnosol, rosmanol, epirosmanol and 7-methylepirosmanol. For new recently isolated diterpenes, see A. A. Mahmoud *et al*., *Phytochemistry*, 2005, **66**, 1685; C. L. Cantrell *et al*., *J. Nat. Prod*., 2005, **68**, 98. Triterpenes include the alcohols α- and β-amyrin, ursolic acid and oleanolic acid.

In vitro cell cultures of rosemary have been produced which biosynthesise carnosic acid, carnosol and rosmarinic acid (A. Kuhlmann and C. Rohl, *Pharm. Biol*., 2006, **44**, 401).

Uses. Rosemary leaves have many traditional uses based on their antibacterial, carminative and spasmolytic actions.

Further reading

Petersen M, Simmonds MSJ 2003 Molecules of interest. Rosmarinic acid. Phytochemistry 62: 121–125. *Includes discovery (1958), derivatives, distribution, chemistry, biological activity*

ROSEMARY OIL

Rosemary oil is steam distilled from the flowering aerial parts of *Rosmarinus officinalis* L. The fresh material yields about 1–2% of a colourless to pale yellow volatile oil with a very characteristic odour. It contains 0.8–6% of esters and 8–20% of alcohols. The principal constituents are 1,8-cineole, borneol, camphor, bornyl acetate and monoterpene hydrocarbons, principally α-pinene and camphene. Many minor components have been identified. Chemical races (G. Flamini *et al*., *J. Agric. Food Chem*., 2002, **50**, 1512) and geographical variants concerning the proportions of constituents in the oils are known. The *BP*/*EP* accordingly gives the limits for the percentage content of 12 constituents for oils of the Spanish type and for those of the Moroccan and Tunisian type. These are determined by gas chromatography.

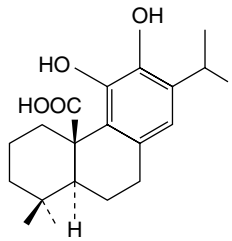
The oil is frequently used in aromatherapy, in the perfumery industry and for the preparation of spirits and liniments for medical use; it has antibacterial and antispasmodic properties.

LEMON BALM

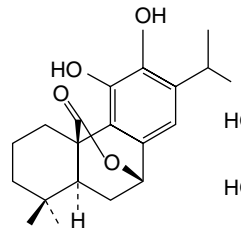
Lemon balm *BP*/*EP* consists of the dried leaf of *Melissa officinalis* L. family Labiatae/Lamiaceae; Balm leaf *BHP* 1990 is from the same source but also includes the flowering tops. The plant is a perennial herb native to southern and eastern Mediterranean regions but now widely grown in gardens for its aroma or for culinary purposes and cultivated commercially in Eastern Europe and Spain.

The leaves are opposite on a hairy quadangular stem; flowering branches arise in the axils of the lower leaves and flowers in clusters at the upper ends of the stems. The two-lipped corollas are initially white, changing later to pale blue or pink; the calyx is toothed with long spreading hairs. Leaf blades are 3–7 cm in length, longly petiolate on the lower parts of stems but shortly so on the upper parts, margins are deeply crenate or serrate, veins are prominent on the lower pale-green surface. Microscopical features are characteristic of the Lamiaceae and include eight-celled glandular trichomes, clothing trichomes and diacytic stomata on the lower epidermis.

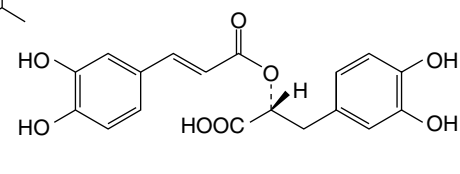
Lemon balm yields only a small quantity of volatile oil (0.06–0.4%), which none the less gives the plant, when crushed, its strong lemon-like odour. Principal components of the oil are the aldehydes citral (composed of the isomers geranial and neral) and citronellal. Other components in smaller proportions are citronellol, nerol and



Carnosic acid



Carnosol



Rosmarinic acid

the sesquiterpene β-caryophyllene; in all, over 70 constituents have been reported. Due to the low yield of oil from the plant, lemon balm oil is subject to adulteration with lemon-grass oil (*Cymbopogon citratus*), lemon-scented verbena oil (*Aloysia triphylla*) or various citrus products.

The *BP*/*EP* drug is assayed on its total content (≮4.0%) of hydroxycinnamic acid derivatives expressed as rosmarinic acid (p. 270); these are mainly structurally related to caffeic acid. Other constituents are flavonoids, principally luteolin glycosides (Table 21.5).

For over 2000 years lemon balm has been used for medicinal and culinary purposes. It is used traditionally for its sedative, spasmolytic and antibacterial properties; more recently it has been investigated by a number of researchers for its topical use in the treatment of Herpes labialis.

THYME

A number of *Thymus* species have been used traditionally for their medicinal and culinary properties. Under the above heading, the *BP*/*EP* recognizes the leaves and flowers separated from the dried aerial parts of *T. vulgaris* L. and *T. zygis* L., family Labiatae, or mixtures of the two. The former is the garden thyme or common thyme, native to Mediterranean regions, and possibly introduced into Britain by the Romans; the latter is also known as Spanish thyme.

Both species have similar morphological and microscopical characteristics and can be difficult to distinguish in the dried state. Stems above 15 mm in length and over 1 mm in diameter are limited to 10% by the pharmacopoeia. The grey–green leaves are slightly hairy on the upper surface and densely so on the lower surface, up to 12 mm long, and 3 mm wide, opposite, sessile and ovate to lanceolate in shape with slightly rolled edges. Under the microscope, both species show on the lower surface volatile oil-containing glandular trichomes typical of the Labiatae and numerous warty-walled clothing trichomes. The characteristic elbow-shaped trichomes of *T. vulgaris* are illustrated in Fig. 42.4. Numerous thick bundles of fibres are apparent in the powder of *T. zygis*.

Constituents. The volatile oil composition of thyme can vary enormously and various chemotypes have been recorded, particularly regarding thymol and carvacrol. The official drug is required to contain not less than 1.2% volatile oil, of which not less than 40% consists of thymol and carvacrol. It is these phenols that are largely responsible for the antiseptic, antitussive and expectorant properties of the drug. Other common variables of the oil are cymene (10–24%) and γ-terpinene (4–18%). Two extreme variations recorded are an almost complete lack of thymol and carvacrol in *T. vulgaris* and an oil containing 74% thymol from *T. zygis*.

A number of monoterpenoid glycosides occur in the leaves, particularly glucosides and galactosides of thymol and terpineol; seven new such constituents have recently been described (J. Katajima *et al*., *Phytochemistry*, 2004, **65**, 3279; *Chem. Pharm. Bull*., 2004, **52**, 1013). Flavones, highly oxygenated flavones, flavanones and dihydroflavonals may be responsible for the spasmolytic effect of the leaves, and the biphenyls reported in 1989 may have deodorant properties. Other constituents include rosmarinic acid (see 'Rosemary') up to 2.6%, various acids, tannins and resins. Rosmarinic acid and 3′-*O*-(8″-Z-caffeoyl) rosmarinic acid have been reported as the most important radical scavengers of the leaves (A. Dapkevicins *et al*., *J. Nat. Prod*., 2002, **65**(6), 892).

THYME OIL

Thyme Oil *BP*/*EP* is obtained by steam distillation from the *fresh* flowering aerial parts of *Thymus vulgaris L.*, *T. zygis* Loefl. *ex* L., or a mixture of both species. The oil therefore resembles that obtained from the official drug described above but reflects any changes that occur during drying and storage. The oil may vary in colour from yellow to dark reddish-brown; it has an aromatic spicy odour suggesting that of thymol.

As with the whole drug, the constituents are subject to variation due to geographic and genetic factors. The *BP* therefore requires a gas chromatographic profile and provides limits for the proportions of major constituents, which are: β-myrcene (1–3%), γ-terpinene (5–10%), *p*-cymene (15–28%), linalol (4.0–6.5%), terpinen-4-ol (0.2–2.5%), thymol (36–55%) and carvacrol (1.0 and 4.0%).

WILD THYME

The *BP*/*EP* drug is defined as the whole or cut, dried, flowering aerial parts of *Thymus serpyllum* L.s.l. It is required to contain a minimum of 0.3% essential oil (dried drug).

The species is an extremely variable aggregate, differing in its forms and chemical constituents both locally and across its wider geographical distribution. It grows on heaths, dry grasslands, dunes and in rocky environments extending from coasts to the lower mountain slopes of central and northern Europe, including the UK.

Wild thyme is used both medicinally and as a flavour in a similar manner to the common thyme, but is less powerful in its actions. The principal constituents are again thymol and carvacrol, which, however, vary appreciably according to source; some chemotypes contain neither of these phenolic compounds. For quality control, the *BP*/*EP* relies on the minimum oil content (as above) and TLC to indicate the presence of thymol and carvacrol and to give an indication of their respective concentrations.

For those essential oils having a low phenol content, major constituents have been variously reported as cineole, β-caryophyllene, nerolidol, myrcene, geranyl acetate and linalyl acetate. Other constituents of the drug (flavonoids, various acids, triterpenes) again resemble those of garden thyme.

The drug has been used traditionally for the treatment of respiratory infections, gastrointestinal problems and skin conditions requiring an antiseptic.

For an elaboration of the chemical constituents, pharmacological actions, therapeutics and research references concerning the thymes, see P. Bradley, 2006, *British Herbal Compendium*, Vol. 2, pp. 369–375; 389–392. British Herbal Medical Association, Bournemouth, UK. For a complete overview of all aspects of the genus, see Stahl-Biskup, E. (ed.), Hardman, R. (series ed.) 2002 Thyme: the genus *Thymus*. Taylor and Francis, New York. 230 pp., *956 references*.

OREGANO

There is a large number of marjorams, and various varieties are cultivated extensively for ornamental and culinary purposes. Two medicinally used species described in the *BP*/*EP* are *Origanum onites* L. (syn. *Majoram onites*) the pot marjoram or Greek oregano, and *O. vulgare* L. subsp. *hirtum* (Link) Ietsw., a subspecies of the wild marjoram, or oregano, family Labiatae. The dried leaves and flowers are separated from the stems; a mixture of both species may be used. Both have a strong, thyme-like odour. Both appear similar in the dried state but the

leaves of *O. onites* are yellowish–green whereas those of *O. vulgare* are more distinctly green. In the powdered form, both show typical laminaceous characteristics.

In view of the diverse nature of the genus, with many varieties of the above and the fact that other plants may be sold commercially under the name 'oregano', the characteristics of the oil are important. The *BP*/*EP* requires a minimum of 2.5% oil in the drug and a minimum 1.5% carvacol and thymol. Other constituents of the oil include caryophyllene, β-bisabolene, cymene, linalool and borneol. Plants grown near the Mediterranean coast are stated to be the most fragrant of all. Tannins, sterols, flavonoids and resin have been reported in the drug. A reddish dye can be obtained from the aerial parts of *O. vulgare*.

Uses. Although in Britain oregano is not used medicinally to any extent, its thymol content gives it strong antiseptic properties. Traditionally its uses include the treatment of digestive disorders, pharnygeal infections and mild fevers.

Further reading

Kintzios SE (ed), Hardman R (series ed) 2002 Oregano: the genus *Origanum* and *Lippia*. Taylor and Francis, New York, 277 pp. *839 references*

LAVENDER FLOWER

The general term 'lavender' applies to a number of species and numerous hybrids and varieties of the genus *Lavandula*, plants used from classical times for their aromatic and medicinal properties. The generic name derives from the Latin *lavare*, referring to the use of lavender by the Romans as a bath perfume. The numerous cultivated varieties vary in their flower colour (blue through purple to white), habit, foliage and, importantly, their oil composition as indicated under 'Lavender Oil' below; many are hybrids and do not breed true.

Lavender flower *BP*/*EP* 2007, *BHP* 1983 consists of the dried flowers of *L. angustifolia* P.Mill. (*L. officinalis* Chaix). It is required (*BP*) to contain a minimum volatile oil content of 1.3% expressed on a dry weight basis.

The flowers, up to 5 mm in length, are packed closely together in verticillasters on a quadrangular stem forming a compact terminal spike. Each verticillaster consists of six to ten shortly stalked flowers. In the fresh condition, oily glandular trichomes can be discerned among the numerous covering trichomes on the surface of the five-lobed calyx. The blue corolla is bilabiate with an upper bifid lip and a lower three-lobed lip.

Microscopical features of the powder include fragments of the calyx and corolla with numerous associated glandular and clothing trichomes; prismatic crystals of calcium oxalate in cells of the calyx; pollen grains up to about 35 µm in diameter with six pores and six pitted lines radiating from the poles; vascular tissue from the pedicel.

Gas chromatographic analysis of the oil isolated in the volatile oil determination above is employed by the *BP*/*EP* to establish the absence in the sample of species and varieties other than *L. angustifolia*. The chromatogram obtained is compared with that of a reference solution containing five compounds expected to be found in the oil of a genuine sample; the peak for camphor should not exceed 1% of the total area of the peaks thus excluding camphoraceous species such as *L. latifolia*.

Uses. Although it is the volatile oil of lavender that is principally used for medicinal purposes the *BHP* 1983 cites flatulent dyspepsia, colic and depressive headache as indications for use of the flowers. It may be used in combination with other drugs such as rosemary, valerian, meadow-sweet and others.

LAVENDER OIL

The botanical source of lavender oil is *Lavandula angustifolia* Miller (*Lavandula officinalis* Chaix), family Labiatae. Originally (*BP* 1980) oil from this species was referred to as 'foreign oil' to distinguish it from that of *L. intermedia* Loisel, which was termed 'English oil'. The latter has a much finer fragrance than the Continental oil and there were separate pharmacopoeial standards for the two oils. Unfortunately the straggly habit of the English lavender does not lend itself to mechanical harvesting and oil produced commercially is now of the Continental type. France, once the principal producer, has been superseded by Bulgaria, with smaller quantities of oil coming from the former USSR, Australia and other countries.

Lavender oil types. The taxonomy of the lavenders is confusing and Continental oils differ among themselves owing to the fact that a number of different species, varieties and hybrids are distilled. The true lavender, *L. officinalis*, yields the best oil when grown at a fairly high altitude, the variety growing under these conditions being known as 'petite lavande'. At a lower altitude the 'lavande moyenne' yields a somewhat less esteemed oil. 'Grande lavande', *L. latifolia* Villers (*L. spica* DC), yields a much coarser oil, which is sold as oil of spike. The above plant readily hybridizes with *L. officinalis* yielding a plant known as 'grosse lavande' or 'lavandin', the oil of which is intermediate in character between that of the parent forms. According to Tucker (*Baileya*, 1981, **21**, 131), of the many names applied to this hybrid species, the correct one is *L.* × *intermedia* Emeric ex Loiseleur. As hybrids the plants do not breed true and are normally propagated vegetatively; a simple efficient method for the *in vitro* shoot regeneration from the leaves has offered possibilities for future breeding (S. Dronne *et al*., *Plant Cell Reports*, 1999, **18**, 429). The lavandin oil market is controlled by the French with Spain the principal producer.

The evergreen plant flowers from July to September and the fresh flowering spikes yield about 0.5% of volatile oil. The amount varies according to variety, season and method of distillation; modern steam stills give a rather larger yield than those in which the flowers are boiled with water. Genuine Continental lavender oil normally contains over 35% of esters. Oil of spike, which is largely used in cheap perfumery, contains little ester but a high proportion of free alcohols (about 23–41% calculated as borneol); 30 components have been identified. The nature of the alcohols also varies from a mixture of linalol and geraniol in the best lavender oil to borneol in oil of spike. Hybrids are of intermediate character (e.g. 'lavandin oil') and contain about 6–9% of esters and about 35% of alcohols. An analysis of the Spanish oil (J. de Pascual Teresa, *Planta Medica*, 1989, **55**, 398) enabled the identification of 50 compounds, the principal ones being 1:8-cineole, linalol and camphor; in contrast to the oil of *L. angustifolia*, linalyl acetate was not present.

A GC profile together with prescribed percentage ranges of 10 components of the pharmaceutical oil is given in the *BP*/*EP*; linalol (20–45%) and linalyl acetate (25–46%) are the principal constituents with a maximum limit for camphor of 1.2%. Chiral chromatography is used to determine the chiral purity of the linalol and linalyl acetate contents.

As with other Labiatae, *Lavandula* cell cultures do not produce essential oil and for *L. vera* rosmarinic acid is the principal phenolic component together with caffeic acid and traces of others. An enol ester of caffeic acid is a blue pigment also found in cell cultures (see E. Kovatcheva *et al*., *Phytochemistry*, 1996, **43**, 1243).

Species of *Lavandula* other than the above are also cultivated. *L. stoechas* has a markedly different odour and of its 51 volatile components, fenchone, pinocaryl acetate, camphor, eucalyptol and myrthenol predominate. Large producers are Spain and France. Oil from wild plants growing in the Algiers region of Algeria contained as significant constituents fenchone (31.6%), camphor (22.4%), *p*-cymene (6.5%) lavandulyl acetate (3.0%) and α-pinene (1.0%). Fifty-four components amounting to *ca* 73% of the oil were identified (T. Dob *et al*., *Pharm Biol*., 2006; **44**, 60).

Uses. Lavender oil is principally used in the toiletry and perfumery industries and occasionally in ointments, etc., to mask disagreeable odours. It is employed pharmaceutically in the antiarthropod preparation Gamma Benzene Hexachloride Application. Lavender flowers are included in the *BHP* and are indicated for the treatment of flatulent dyspepsia and topically, as the oil, for rheumatic pain. The oil is extensively used in aromatherapy (q.v.).

CARAWAY FRUIT

Caraway (*Caraway Fruit*) consists of the dried, ripe fruits of *Carum carvi* (Umbelliferae), a biennial herb about 1 m high. It occurs both wild and cultivated in central and northern Europe (The Netherlands, Denmark, Germany, Russia, Finland, Poland, Hungary and Britain) and in Egypt, Morocco, Australia and China.

History. Caraway fruits were known to the Arabian physicians and probably came into use in Europe in the thirteenth century.

Macroscopical characters. The commercial drug (Fig. 22.5) usually consists of mericarps separated from the pedicels. The fruits are slightly curved, brown and glabrous, about 4–7 mm long, 1–2.3 mm wide and tapered at both ends; they are crowned with a stylopod often with style and stigma attached. Each mericarp shows five almost equal sides, five narrow primary ridges, and, when cut transversely, four dorsal and two commissural vittae. They have a characteristic aromatic odour and taste.

Microscopical characters. A transverse section of a caraway mericarp (Fig. 22.5) shows five primary ridges, in each of which is a vascular strand with associated pitted sclerenchyma and having a single secretory canal at the outer margin of each. The six vittae which appear somewhat flattened and elliptical in transverse section may attain a width of 350 μm; they extend from the base of the fruit to the base of the stylopod. They are lined with small, dark reddish-brown cells and contain a pale yellow or colourless oleoresin (Fig. 22.5B, C). The raphe lies on the inner side of the endosperm, which is non-grooved. Occupying the majority of the transverse section is the endosperm, with thickened cellulose walls (having also deposits of a β-(1,4)-mannan as a reserve polysaccharide) and containing fixed oil and aleurone grains having one or two microrosettes of calcium oxalate. The embryo, which is situated near the apex of the mericarp, will only be seen in sections passing through that region.

More detailed examination shows that the outer epidermis of the pericarp is glabrous (cf. aniseed) and has a striated cuticle (cf. fennel). The mesocarp consists of more or less collapsed parenchyma and lacks the reticulated cells of fennel. The endodermis (or inner epidermis of the pericarp) (Fig. 22.5F) consists of a single layer of elongated cells, arranged more or less parallel to one another and not showing the 'parquetry' arrangement of coriander.

Constituents. Caraway contains 3–7% of volatile oils (*BP* not less than 3.0%), 8–20% of fixed oil, proteins, calcium oxalate, colouring matter and resin.

Uses. Large quantities of caraway fruits are used for culinary purposes. The fruits and oil are used in medicine for flavouring and as carminatives. The carminative and antispasmodic properties have been experimentally verified.

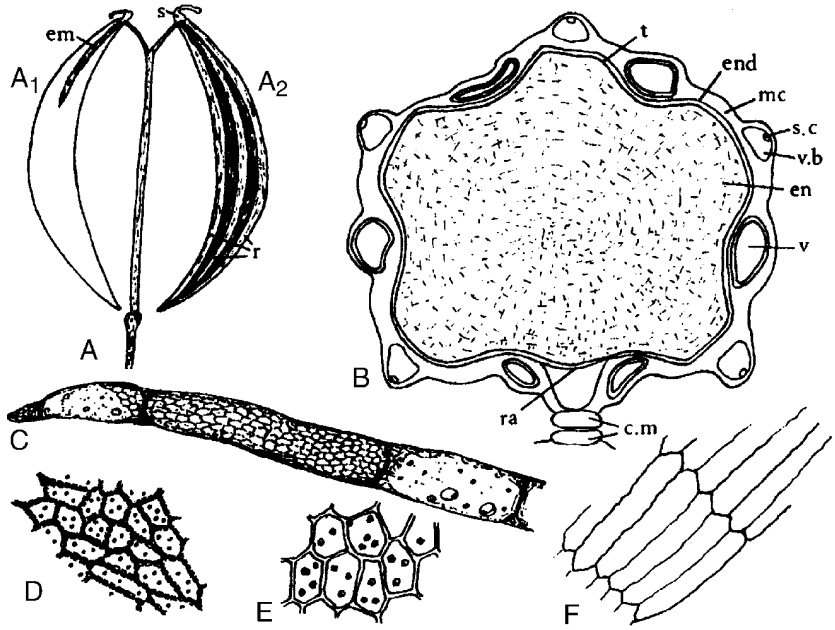


Fig. 22.5
Caraway. A, mericarps showing attachments to carpophore; A_1, mericarp sectioned longitudinally to show position of embryo; A_2, mericarp side view (×8). B, transverse section of mericarp (×50); C, portion of vitta isolated by alkali maceration (×25); D, sclereids of mesocarp; E, endosperm cells with micro-rosette crystals of calcium oxalate; F, endocarp layer in surface view (all ×200). c.m, commissural meristeles; em, embryo; en, endosperm; end, endocarp; mc, mesocarp; r, three of five primary ridges; ra, position of raphe; s, stylopod; s.c, secretory canal; t, testa; v, vitta; v.b, vascular bundle with associated finely pitted sclerenchyma.

Further reading

Nemeth E (ed), Hardman R (series ed) 1998 Medicinal and aromatic plants—industrial profiles, Vol. 7. Caraway: the genus *Carum*. Harwood Academic, Amsterdam. *529 references*

CARAWAY OIL

The volatile oil (Caraway Oil *BP*/*EP*) consists largely of the ketone carvone and the terpene limonene (formulae, Fig. 22.4) with small quantities of dihydrocarvone, carveol and dihydrocarveol. As there is a demand for pure carvone, there is a considerable amount of decarvonized oil available for adulteration.

Official tests include a TLC examination to ascertain the presence of carvone and carveol and the measurement of optical rotation (+65° to +81°), refractive index (1.484–1.490) and acid value (maximum 1.0). The proportions of individual components are required to fall within certain limits as determined by gas chromatography: limonene (30–45%), carvone (50–65%), β-myrcene (0.1–1.0%) with maximum limits for *trans*-dihydrocarvone and *trans*-carveol (both 2.5%). The gas chromatographic chirality assay limits (−)-carvone to 1.0%.

DILL AND DILL OIL

Dill (*Dill Fruit*) consists of the dried, ripe fruits of *Anethum graveolens* (Umbelliferae), a small annual indigenous to southern Europe. It is cultivated in Central and Eastern Europe and Egypt. Dill was known to Dioskurides and was employed in England in Anglo-Saxon times.

Macroscopical characters. The drug usually consists of separate, broadly oval mericarps, about 4 mm long and 2–3 mm broad. The fruits are very much compressed dorsally, the two central ridges being prolonged into membranous wings, while the dorsal ones are inconspicuous. The fruits have an aromatic odour and taste similar to those of caraway.

Microscopical characters. Each mericarp has four vittae on the dorsal surface and two on the commissural. The outer epidermis has a striated cuticle (distinction from fennel), and the mesocarp contains lignified, reticulate parenchyma (distinction from caraway). The endosperm is much flattened but otherwise resembles that of caraway.

Constituents. The volatile oil (Dill Oil *BP*/*EP*) resembles oil of caraway in containing carvone and limonene. The European fruits yield about 3–4% of volatile oil, which should contain from 43 to 63% of carvone; the carvone content is determined by reaction with hydroxylamine hydrochloride (oxime formation) and titration of the liberated acid. Other constituents reported for the oil include *trans*- and *cis*-dihydrocarvone, *trans*- and *cis*-carveol, limonene, D- and L-dihydrocarveol, α- and γ-terpinene, α-phellandrene and others. Chemical types based on the proportion of carvone present, and the presence or absence of dillapiole and myristicin have been distinguished.

Monoterpene glycosides have been isolated from the water-soluble fraction of the fruits.

For further details on constituents, see T. Ishikawa *et al*., *Chem. Pharm. Bull*., 2002, **50**, 501; M. Kosar *et al*., *Pharm. Biol*., 2005, **41**, 491.

Uses. Like caraway, dill is used as a carminative and flavour; it is much used in infant's gripe water.

Allied drug. *Indian Dill*, derived from a variety of *A. graveolens* consists of whole cremocarps which bear pedicels and are narrower and less compressed than the European drug. Indian dill oil contains dillapiole and less carvone.

CORIANDER AND CORIANDER OIL

Coriander (*Coriander Fruit*) of the *BP* is the dried, nearly ripe fruit of *Coriandrum sativum* (Umbelliferae), an annual about 0.7 m high with white or pinkish flowers. It is indigenous to Italy, but is widely cultivated in The Netherlands, Central and Eastern Europe, the Mediterranean (Morocco, Malta, Egypt), China, India and Bangladesh. Coriander is mentioned in the papyrus of Ebers (*c*. 1550 BC), and in the writings of Cato and Pliny. It was well known in England before the Norman Conquest. Ukraine is the major producer of oil and controls the world price on a supply and demand basis; in one large factory continuous distillation has replaced the batch process.

Macroscopical characters. The drug (Fig. 22.6A) usually consists of the whole cremocarps, which, when ripe, are about 2.3–4.3 mm diameter and straw-yellow. Each consists of two hemispherical mericarps united by their margins. Considerable variation exists in coriander. The Indian variety is oval, but the more widely distributed spherical varieties vary in size from the Ukrainian 2.3–3.7 mm to the Moroccan 4.0–4.3 mm. The apex bears two divergent styles. The 10 primary ridges are wavy and inconspicuous; alternating with these are eight more prominent, straight, secondary ridges. The fruits have an aromatic odour and a spicy taste. They are somewhat liable to insect attacks.

Microscopical characters. A transverse section of a fully ripe fruit shows only two mature vittae in each mericarp, both on the commissural surface (Fig. 22.6B). The numerous vittae present in the immature fruit on the dorsal surface of each mericarp gradually join and are eventually compressed into slits. The outer part of the pericarp, which possesses stomata and prisms of calcium oxalate, is more or less completely thrown off. Within the vittae-bearing region of the mesocarp a thick layer of sclerenchyma is formed, which consists of pitted, fusiform cells. These sclerenchymatous fibres tend in the outer layers to be longitudinally directed and in the inner layers to be tangentially directed. In the region of the primary ridges more of the fibres are longitudinally directed; in the secondary ridges nearly all are tangentially directed. Traversing the band of sclerenchyma and corresponding in position to the primary ridges are small vascular strands composed of a small group of spiral vessels. The mesocarp within the sclerenchymatous band is composed of irregular polygonal cells with lignified walls. The inner epidermis of the pericarp is composed of 'parquetry' cells, which in the powder are often seen united to the cells of the inner mesocarp. The testa is composed of brown flattened cells. The endosperm is curved and consists of parenchymatous cells containing fixed oil and aleurone grains. The latter contain rosettes of calcium oxalate 3–10 mm diameter (see Fig. 22.6 C–F).

Constituents. Coriander fruits contain up to 1.8% of volatile oil according to origin (*BP*/*EP* standard not less than 0.2%). The distilled oil (Coriander Oil *BP*/*EP*) contains 65–70% of (+)-linalool (coriandrol), depending on the source, and smaller amounts of α-pinene, γ-terpinene, limonene and *p*-cymene together with various non-linalool alcohols and esters. Some 40 constituents have been identified. The *BP*/*EP* uses GC for the evaluation of the oil with linalool and geraniol as internal standards; there is also a test for chiral purity ((R)-linalool, maximum 14%). Other constituents isolated from the fruits include flavonoids, coumarins, isocoumarins, phthalides and phenolic acids. T. Ishikawa *et al*. (*Chem. Pharm. Bull*., 2003, **51**, 32) obtained 33 compounds from the water-soluble fraction of a methanolic extract of the fruits; new constituents included monoterpenoids, monoterpenoid glycosides, monoterpenoid glucoside sulphates and aromatic compound glycosides. The high content of fats (16–28%) and protein (11–17%) in the fruits make distillation residues suitable for animal feed. The fruits yield 5–7% of ash.

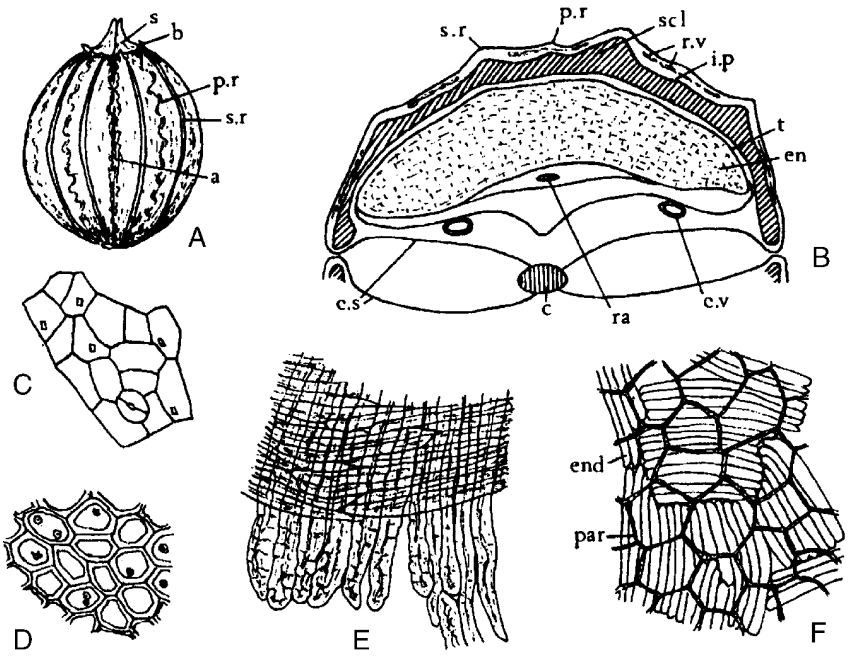


Fig. 22.6
Coriander. A, Whole fruit (×8); B, transverse section of fruit (×16); C, fragment of epicarp in surface view with stoma and small prismatic crystals of calcium oxalate; D, endosperm cells with microrosette crystals of calcium oxalate; E, layers of sclerenchyma from the mesocarp; F, lignified parenchyma of the mesocarp and underlying endodermis showing 'parquetry' arrangement (all ×200). a, line of attachment of mericarps; b, sepal; c, carpophore, c.s, commissural surfaces; c.v, commissural vitta; en, endosperm; end, endodermis; par, lignified parenchyma of mesocarp; p.r, primary ridge; ra, raphe; r.v, remains of dorsal vittae; s, stylopod; scl, sclerenchyma; s.r, secondary ridge; t, testa.

The unripe plant has an unpleasant, mousy odour, which is also present in oil distilled from unripe fruits (mainly aldehydes such as *n*-decanal contained in peripheral vittae). Marked changes occur in volatile oil composition during ontogenesis; the peripheral vittae flatten and lose their oil, all of which is then produced by the commissural vittae. During ordinary storage of the fruits, the oil composition undergoes considerable alteration.

Uses. Very large quantities of the spice are produced in many countries for domestic purposes, such as for use in curries. In the former USSR linalool is isolated from the oil as starting material for other derivatives. Pharmaceutically coriander and its oils are used as a flavouring agent and carminative.

ANISEED AND ANISEED OIL

Aniseed (*Anise Fruit*) of the *BP* and *EP* consists of the dried, ripe fruits of *Pimpinella anisum* (Umbelliferae), an annual plant indigenous to the Levant but widely cultivated both in Europe (Spain, Germany, Italy, Russia, Bulgaria), Egypt and America (Chile, Mexico). Anise is mentioned in the writings of Theophrastus, Dioskurides and Pliny. It was cultivated in Germany in the ninth century. Spain and Egypt are the principal producers of the oil.

Macroscopical characters. The drug (Fig. 22.7A) consists of greyish-brown, pear-shaped, somewhat compressed cremocarps, which are usually attached to pedicels 2–12 mm in length. The cremocarps are

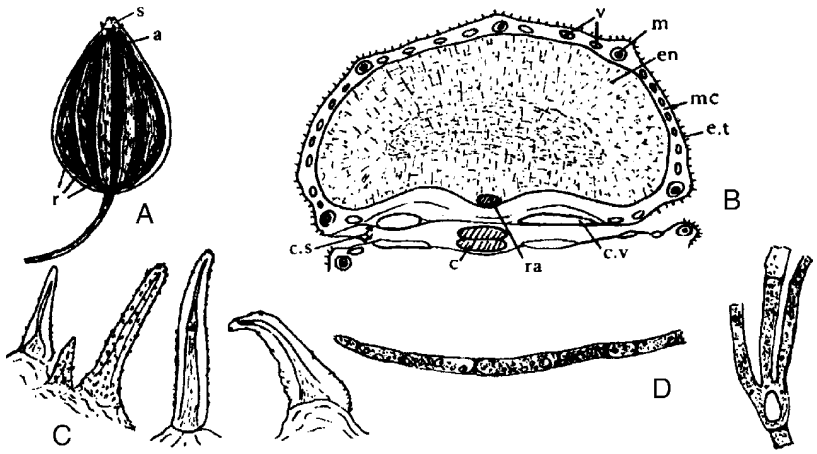


Fig. 22.7
Aniseed. A, Side view of cremocarp showing line of attachment to the two mericarps (×8); B, transverse section of mericarp (×25); C, covering trichomes of epicarp (×200); D, branched and unbranched vittae isolated by alkali maceration (×25). a, Line of attachment of mericarps; c, carpophore; c.s, commissural surfaces; c.v, commissural vitta; en, endosperm; e.t, epicarp bearing trichomes; m, meristele; mc, mesocarp; r, three of five primary ridges of one mericarp; ra, raphe; s, stylopod; v, vittae.

3–6 mm long and 2–3 mm broad. The Spanish (Alicante) and Italian are distinguished by their large size and light colour, while the German and 'Russian' are smaller, more ovoid and darker. Each mericarp has five somewhat wavy ridges and is slightly pubescent on the dorsal surface. They have an aromatic odour and a sweet, aromatic taste.

Microscopical characters. Microscopical examination shows that the epidermis bears numerous papillae and unicellular hairs. On the dorsal surface of each mericarp are from 15 to 45 branched vittae. A small amount of vascular tissue and reticulated parenchyma is present. The endosperm is slightly concave on the commissural surface and contains protein and fixed oil (see Fig. 22.7B–D).

Constituents. Anise fruits yield 2–3% of volatile oil (*BP*/*EP* ≮ 2.0%), which is almost identical with that obtained from star-anise fruits. A number of water-soluble constituents have been isolated from the fruits including various glucosides, see E. Fujimatu *et al*., *Phytochemistry*, 2003, **63** (5).

Aniseed Oil. *BP*/*EP* tests and standards for the oil include: TLC identification of anethole and anisaldehyde; maximum limit of 0.1% for fenchone and 0.01% for foeniculin; *trans*-anethole 87–94%; estragole 0.5–5.0%, anisaldehyde 1.0–1.4% and linalol <1.5% determined by gas chromatography.

STAR ANISE FRUIT AND OIL

The star-anise *Illicium verum* Hook, f., family Illiciaceae, is an evergreen tree about 4–5 m in height, indigenous to the south-west provinces of China. The fruits are collected and the oil distilled locally in China and Vietnam.

The fruits consist of eight (rarely seven or nine) one-seeded follicles. Each follicle is about 12–17 mm long. The pericarp is reddish-brown, woody and only slightly wrinkled. Each carpel has, as a rule, partly dehisced to expose the seed. The latter has a brittle, shining testa and an oily kernel. The beak of each carpel is not turned upwards and the fruit stalk, which is about 3 cm long, is curved (distinction from *I. religiosum*). The oil, which is present in both seed and pericarp, gives the drug an aromatic odour and spicy taste.

The genuine fruits of *I. verum* should yield a minimum of 7.0% volatile oil containing not less than 86.0% of *trans*-anethole. More recently, they have been employed for the extraction of shikimic acid (see Fig. 19.5), which is the starting material for the synthesis of the antiviral drug Tamiflu (Roche). As a consequence, the plant is in some danger of overexploitation and other sources of the acid are being investigated (q.v.).

Bastard star-anise or *shikimi fruits* occur in Eastern commerce and are occasionally exported. They are derived from *I. religiosum* (*I. anisatum*), a species cultivated near the Buddhist temples in Japan and also on the mainland. The carpels are equal in number to those of *I. verum* but are smaller, are much wrinkled and have a curved-up apex. The stalk is shorter than the genuine fruit, and straight. These fruits, which contain shikimic acid, are poisonous, as they contain an amorphous toxic substance sikimitoxin, and a crystalline toxic substance sikimin. New phenylpropanoid glycosides have been recently reported (Z.-H. Jiang *et al*., *Chem. Pharm. Bull*., 1999, **47**, 421). In recent years, Japanese workers have isolated a number of novel sesquiterpene lactones (anisatin-like compounds) from the pericarps of various *Illicium* species including *I. verum*; a number of these compounds are convulsants. T. Nakamura *et al*. (*Chem. Pharm. Bull*., 1996, **44**, 1908) describe the isolation of three sesquiterpenoid compounds (veranistans, A,B,C) which are neurotoxins. For additional isolations, see J.-M. Huang *et al*., *Chem. Pharm. Bull*., 2000, **48**, 657.

Star anise oil

The essential oil should contain 87–94% *trans*-anethole, 0.5–5.0% of estragole and smaller amounts of anisaldehyde (0.1–0.5%) and foeniculin (0.1–3.0%); other minor components include chavicol methyl ether (an isomeride of anethole), *p*-methoxyphenylacetone, safrole and other minor components. The oil is, for all ordinary purposes, indistinguishable from that of *P. anisum* but differences in the gas chromatographic profiles can be seen. The oil is liable to atmospheric oxidation and both anisic aldehyde and anisic acid are normally present. This change is said to diminish the tendency of the oil to solidify, which it normally does on cooling to about 15°C. In the past, the oil was imported in lead containers and some pharmacopoeias give a limit test for heavy metals.

Both aniseed oil and star anise oil are used as flavouring agents and as carminatives. Anethole (a colourless crystalline solid m.p. 21°C) may be prepared from the oil or manufactured synthetically.

BITTER FENNEL AND SWEET FENNEL

Bitter Fennel consists of the dried ripe fruits of *Foeniculum vulgare*, subsp. *vulgare*, var. *vulgare* (Umbelliferae). It is cultivated in many parts of Europe and much is imported from India, China and Egypt. The commercial drug consists partly of whole cremocarps and partly of isolated mericarps. Bitter fennel, now little used in British medicine, is more fully described in the 11th edition of this book. The drug has, however, been re-introduced into the *BP* on account of its *EP* status.

The fruits contain 1–4% of volatile oil with higher yields recorded.

The principal constituents of bitter fennel oil, with *BP*/*EP* prescribed limits, are fenchone (12–25%), *trans*-anethole (55–75%) together with anisaldehyde (maximum 2.0%) and estragole (methyl chavicol) (maximum 5.0%). Minor components include limonene and other monoterpene hydrocarbons.

Anethole is derived via the shikimic acid pathway and fenchone (a bicyclic monoterpene) is formed from fenchol by the action of a dehydrogenase. Other components of the fruits include flavonoids, coumarins and glycosides. The latter, which may have a biogenetic relationship with the volatile oil constituents, have been actively investigated by Japanese workers. Thus M. Ono *et al*. (*Chem. Pharm. Bull*., 1995, **43**, 868; 1996, **44**, 337) describe a number of monoterpene glycosides based on 1,8-cineole and *cis*-miyabenol C which they have termed foeniculosides I–IX. J. Kitajima, T. Ishikawa and co-workers in nine studies on the water-soluble glycosides and sugars of fennel fruit (*Chem. Pharm. Bull*., 1998, **46**, 1587, 1591, 1599, 1603, 1643, 1738; 1999, **47**, 805, 988) have recorded alkyl-, erythro-anethol-, *p*-hydroxyphenylpropylene glycol-, fenchane-, menthane-, aromatic (phenylpropane etc)- and 1,8-cineole-type glycosides. It is of further interest that of the cineole-type glycosides

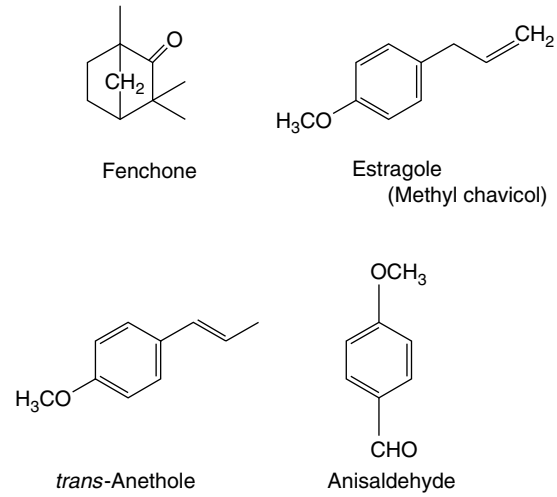


Fig. 22.8
Constituents of fennel oils.

one had previously been isolated from *Cunila spicata* (Lamiaceae), a plant used in Brazilian traditional medicine, one from the peel and flower buds of *Citrus unshiu* and two were biotransformation products from a *Eucalyptus* cell suspension culture following administration of 1,8-cineole. Annual world production of the oil is less than 5 tonnes.

Fennel and its volatile oil are used as an aromatic and carminative.

Sweet Fennel is derived from *F. vulgare*, subsp. *vulgare*, var. *dulce* and is also included in the *BP*/*EP*. The fruits resemble those of the bitter variety but have a sweet taste and lower volatile oil content (not less than 2.0%) of different quantitative composition. Not less than 80% of the oil is required to be anethole, not more than 7.5% fenchone, and not more than 10% estragole.

Further reading

Jodral MM (ed), Hardman R (series ed) 2004 Medicinal and aromatic plants – industrial profiles, Vol 40. Illicium, Pimpinella and Foeniculum. CRC Press, Boca Raton, FL, 256 pp.

Cumin

Cumin consists of the dried ripe fruits of *Cuminum cyminum* (Umbelliferae), a small, annual plant indigenous to Egypt. It is widely cultivated and UK supplies are obtained from Sicily, Malta, Mogadore and India. Spain and Egypt are the major cumin oil producers.

Cumin fruits are about 6 mm long and resemble caraway at first glance. The mericarps, however, are straighter than those of caraway and are densely covered with short, bristly hairs. Whole cremocarps attached to short pedicels occur, as well as isolated mericarps. Each mericarp has four dorsal vittae and two commissural ones. The odour and taste are coarser than those of caraway.

Cumin yields 2.5–4.0% of volatile oil. This contains 25–35% of aldehydes (cuminic aldehyde), pinene and α-terpinol.

As with other umbelliferous fruits the water-soluble constituents of cumin have been recently investigated. Compounds isolated include flavonoid glycosides such as the 7-*O*-β-D-glycopyranosides of apigenin and luteolin, some 16 monoterpenoid glycosides and new sesquiterpenoid glucosides, e.g. cuminosides A and B (T. Ishikawa *et al*., *Chem. Pharm. Bull*., 2002, **50**, 1471; T. Takayanagi *et al*., *Phytochemistry*, 2003, **63**, 479).

Cumin was one of the commonest spices in the Middle Ages. It is employed in Indian medicine (for a study of its activity see S. C. Jain *et al*., *Fitoterapia*, 1992, **63**, 291).

TURPENTINE OIL

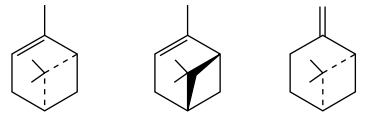
Pharmaceutical turpentine oil is obtained by distillation and rectification from the oleoresin produced by various species of *Pinus*. The unrectified oil is the turpentine of commerce. The resin remaining in the still is the source of colophony (q.v. under 'Resins').

Rectification of the commercial oil consists of treatment with aqueous alkali to remove traces of phenols, cresols, resin acids etc. and possible redistillation.

The genus *Pinus* is widely distributed and many countries have considerable reserves of pine forest. The principal species employed are (1) *Pinus palustris* (longleaf pine) and *P. elliottii* (slash pine) in the S. and S.E. United States; (2) *P. pinaster* (*P. maritima*) in France, Italy, Portugal and Spain; (3) *P. halepensis* in Greece and Spain; (4) *P. roxburghii* (*P. longifolia*) in India and Pakistan; (5) *P. massoniana* and *P. tabuliformis* in China; (6) *P. carribaea* var. *hondurensis* and *P. oocarpa* in Central America and (7) *P. radiata* in New Zealand.

The collection of the oleoresin is very labour-intensive and for this reason output in the USA has declined considerably. Principal world producers are now Portugal and China; other contributors, in addition to the USA, include Spain, Greece, Morocco, France, India, the former USSR, Honduras and Poland. Many other countries produce smaller quantities for their own use. It is considered that about 250 000 trees are required to sustain a small commercial processing plant.

Oil of turpentine is a colourless liquid with a characteristic odour and a pungent taste. It is soluble in alcohol, ether, chloroform and glacial acetic acid. Oil of turpentine is optically active, but the rotation varies not only with the species of pine from which it has been obtained, but also



(+)-α-Pinene (–)-α-Pinene (–)-β-Pinene

in samples taken from the same tree at different periods. Samples taken from the same tree at different times have given rotations varying from −7° 27′ to +18° 18′ in the case of *Pinus palustris*, and −28° 26′ to +1° 23′ in the case of *Pinus heterophylla*. The French oils from *Pinus pinaster* are strongly laevorotatory (−20° to −38°). Over forty components have been identified in French turpentine oil derived from *P. pinaster*.

Oil of turpentine consists chiefly of the terpenes (+)- and (−)-α-pinene, (−)-β-pinene and camphene. These tend to undergo atmospheric oxidation, with the formation of complex resinous substances, the removal of which is accomplished by the process of rectification mentioned above. The varying optical rotations of differing turpentines are mainly due to the varying proportions of the (+)- and (−)-α-pinenes present; (−)-β-pinene is found in almost all *Pinus* spp. in a high state of optical purity and typically occurs with the predominantly (+)-α-pinene. These two isomers have opposite absolute configurations. Other components of the oil which find industrial uses are β-phellandrene, δ-3-carene (a major component of Indian and 'Russian' turpentines), limonene, *p*-cymene, longifoline and estragol.

Oil of turpentine is now rarely given internally. Externally it is used as a counterirritant and rubefacient. For inhalation, terebene is usually preferred. *Terebene* is prepared from oil of turpentine by the action of cold sulphuric acid, which converts the pinene into the optically inactive (±)- limonene (dipentene). Now, most turpentine is processed to give its various constituents which find use in the manufacture of fragrances, flavours, vitamins, insecticides, etc.

PINUS PINASTER TYPE TURPENTINE OIL

This oil, official in the European and British Pharmacopoeias 2007, is obtained by steam distillation of the oleoresin from *Pinus pinaster* Aiton and rectified. The French oils from this species are strongly laevorotatory (limits −40° to −28°), *cf*. Oil of Turpentine *BP*. The principal constituents and official limits are α-pinene (70–85%), β-pinene (11–20%) and limonene (1–7%). Other components in small amounts are camphene, car-3-ene, β-myrcene, longifolene, β-caryophyllene and caryophyllene oxide. Over 40 compounds have been reported in *P. pinaster* oil.

Standards relevant to the quality of turpentine oils are refractive index, relative density, residue on evaporation, optical rotation, acid value and peroxide value. Tests for fixed oils and resinified oil together with solubility in alcohol are also important.

Turpentine oils are used medicinally for their rubifacient acitivity.

Canada turpentine

Canada turpentine, or 'Canada balsam' as it is often incorrectly called, is an oleoresin obtained from the stem of *Abies balsamea* (Pinaceae), the balsam fir. It is collected in eastern Canada and in the State of Maine in the USA. The oleoresin in the bark occurs in schizogenous ducts and large cavities. As the cavities fill with secretion, blister-like swellings develop on the trunk, and it is from these that the oleoresin is collected.

Canada turpentine when fresh is a pale-yellow liquid with a slight, greenish fluorescence and is of honey-like consistency. It has a pleasant, terebinthinate odour and a somewhat bitter and acrid taste. On exposure to air, Canada turpentine becomes more viscous and finally forms a glass-like varnish, a property which rendered it suitable as a microscopic mountant and as a cement for lenses. It contains volatile oil (23–24%) and a number of terpenoid acids.

Pumilio pine oil

A distillation of the fresh leaves of the pumilio pine, *Pinus mugo* var. *pumilio* (Pinaceae) yields the *BP* (1980) oil. It is produced in Eastern Europe.

The oil has an agreeable odour and contains principally terpenes and sesquiterpenes, with up to 10% bornyl acetate (*BP* 1980 limits 4–10% of ester). It may be distinguished from other similar oils by the above ester content and its weight per millilitre value. It is used as a decongestant inhalant, in the preparation of compound thymol glycerin, and as a constituent of zinc undecenoate dusting-powder.

Savin tops

These are the young shoots of *Juniperus sabina* (Cupressaceae), an evergreen shrub about 2–6 m high. It grows wild in the mountains of Austria, Switzerland, Italy, France and Spain. The leaves are imbricated, sessile, more or less adnate to the stem and usually opposite and decussate. The shape and size of the leaves differ very considerably on different parts of the plant. Each leaf has a depression on its dorsal surface, below which is a large oil gland in the mesophyll. This oil gland is oval in young leaves but more elongated in old ones. Savin contains a volatile oil (1–3%) which is a powerful irritant both internally and externally. It contains the terpene alcohol sabinol and its acetate. Other constituents are podophyllotoxin (0.2%), coumarins and savinin. Many diterpenoids with various skeletal structures have been reported among the non-volatile constituents of a hexane fraction of the berries of this plant. (For reports see A. San Feliciano *et al*., *Phytochemistry*, 1991, **30**, 695; *Fitoterapia*, 1991, **62**, 435.)

Oil of cade

Oil of cade is obtained by the destructive distillation of the woody portions of *Juniperus oxycedrus* (Cupressaceae). It is prepared in Portugal, Spain and former Yugoslavia.

The distillate is allowed to stand for at least 15–20 days when a layer constituting oil of cade may be separated.

Oil of cade is a reddish-brown or blackish, oily liquid. Odour, empyreumatic; taste, aromatic, bitter and acrid. The chief constituents are sesquiterpenes (e.g. cadinene) and phenolic compounds (guaiacol, ethyl guaiacol and cresol).

The oil composition of the leaves of *J. oxycedrus* resembles that of *J. communis* (below). The oil is of variable composition; based on geographical location, subspecies and varieties, T. Dob *et al*., (*Pharm. Biol*., 2006, **1**, 1) suggest a classification of the oil based on four chemotypes: α-pinene, limonene, sabinene and *trans*-pinocarveol.

Oil of cade has been used for veterinary purposes for centuries, and has been prescribed for skin diseases.

JUNIPER BERRIES AND OIL

Juniper berries are the dried ripe fruits of *Juniperus communis* (Cupressaceae), an evergreen shrub or small tree. They are collected in former Yugoslavia, Italy, Hungary, Poland, Thuringia, Sweden and other countries. Generally speaking, the berries from the more southern countries contain the most oil.

In Tuscany the collection of the berries is very much a family industry. Bushes are beaten to remove the ripe fruits and the product is roughly cleaned before drying. Importers may further remove extraneous material by a winnowing process involving warm air. Any green berries are removed and the remaining fruits graded.

The female cones consist of scales arranged in whorls of three. The berry-like fruit takes 2 years to ripen, eventually becoming a deep purple colour and having a bluish-grey bloom. On drying, the berries become somewhat darker and shrivel slightly. They are about 3–10 mm in diameter. The apex shows a triradiate mark and depression indicating the sutures of the three fleshy scales. At the base there are usually six, small, pointed bracts arranged in two whorls, but occasionally three or four such whorls are found.

A transverse section of the fruit shows a thin outer skin or epicarp, a yellowish-brown, pulpy mesocarp and three seeds. The seeds lie close together in the centre of the fruit and are hard and woody. Partly embedded in the hard testa of each seed are large oleoresin glands. These usually number from four to eight on the outer side of the seed, and one or two on the inner. The drug has a pleasant, somewhat terebinthinate odour, and a sweetish taste.

The main constituents are volatile oil (about 0.5–1.5%), invert sugar (about 33%) and resin; the *BP*/*EP* specifies a minimum essential oil content of 1.0% with reference to the anhydrous drug.

The aerial parts of this species and its varieties have been examined for water-soluble constituents resulting in the isolation of various phenylpropanoid, neolignan and flavonoid glycosides. Megastigmane glycosides and a new monoterpene glucoside have recently been reported (T. Nakanishi *et al*., *Chem. Pharm. Biol*., 2005, **53**, 783). For a phytochemical and genetic survey of the species, see N. Filipowicz *et al*., *Planta Med*., 2006, **72**, 850.

Juniper berries are used for the preparation of oil of juniper and in making certain varieties of gin. The oil has diuretic and antiseptic properties. It has been reported that commercial oils vary in composition and prolonged intake of some may cause kidney damage. These side-effects are correlated with a high terpene hydrocarbon content and a low proportion of terpinen-4-ol.

Oil of Juniper *BP*/*EP*

This is obtained from the non-fermented berry cones of *Juniperus communis* L. by steam distillation. The oil contains over 60 constituents, although over 100 compounds have been detected in oil from wild berries collected in Greece. Principal components and official limits are α-pinene (20–50%), β-myrcene (1–35%), limonene (2–12%), β-pinene (1–12%), terpinen-4-ol (0.5–10%), sabinene (less than 20%) and β-caryophyllene (less than 7.0%). Other components not quantitatively specified are cadinene, camphene and various alcohols and esters.

The above figures demonstrate the possible variable composition of Juniper oil. For commercial oils in general this variation can be great and, as reported by P. Bradley (*BHPC*, Vol. 2, 2006), such oils are rarely prepared from a uniform source and may involve the distillate from fermented berries after their use in the manufacture of gin or the use of unripe berries, needles and wood of the plant.

Juniper oil is traditionally used for its diuretic, carminative and anti-rheumatic properties. Side-effects of some oils have been attributed to a relatively high proportion of terpene hydrocarbons and a low proportion of terpinen-4-ol.

BITTER ORANGE PEEL

Bitter orange peel is the dried outer part of the pericarp of the ripe or nearly ripe fruit known as the bitter, Seville or Bigarade orange. In botanical characteristics the tree is not unlike the sweet orange and both are regarded as subspecies or varieties of *Citrus aurantium* L. (Rutaceae).

These are named, respectively, *C. aurantium* var. *amara*-L. and *C. aurantium* var. *sinensis* L. (*C. sinensis* (L.) Osbeck.). The bitter orange is not as widely cultivated as the sweet orange and European supplies come from southern Spain (Seville and Malaga), Sicily (Messina and Palermo), Tripoli via Malta and the West Indies. The dried bitter peel is official in the *BP*/*EP*.

History. The bitter orange tree appears to have been introduced from northern India into eastern Africa, Arabia and Syria, whence it was brought to Europe by either the Arabs or Crusaders about AD 1200. The sweet orange was not known in Europe until the fifteenth century and appears to be of Chinese origin.

Collection and preparation. Orange peel may be prepared in the Mediterranean countries or in England. The peel should be removed with as little of white 'zest' as possible. Hand-cut, English dried peel is most esteemed. The peel may be removed in four 'quarters', or in a spiral band. It is also found in thin strips, similar to those found in marmalade, cut by machines. The so-called Maltese is of this type, which is known as 'gelatin-cut'. Fine slicing causes the rupture of a large number of oil glands and some loss in aroma.

Characters. The colour of the dried peel is somewhat variable, but frequently reddish-brown in the spiral form and greenish-brown in the 'quarters'. The other surface is rugged, being somewhat raised over the oil glands, which are clearly seen in sections with the naked eye. The inner surface bears a small amount of white 'zest'. Fragrant odour; aromatic and very bitter taste.

Microscopic examination shows a small-celled epidermis with characteristic stomata; parenchyma containing prismatic crystals of calcium oxalate 20–45 mm long, or sphaerocrystalline masses of hesperidin; small anastomosing vascular bundles; and large oil-filled cavities usually arranged in two irregular rows.

Constituents. Dried bitter orange peel contains not less than 2.0% of volatile oil, vitamin C and the flavonoid glycosides hesperidin and neohesperidin. The latter, present to the extent of 5–14% in the unripe peel, gradually disappears on ripening.

Citrus glycosides and limonoids. *Citrus* fruits contain a large number of flavanone glycosides. The best-known of these, hesperidin (see Fig. 21.18), was first isolated in 1828. It is present in oranges, both bitter and sweet, and in lemons. See also 'Flavone and Related Flavonoid Glycosides' and 'Hesperidin and Rutin'. An isomer of hesperidin, neohesperidin, is present in certain samples of Seville oranges. Naringin, present in some Seville oranges, is the chief flavonoid constituent of the grapefruit. Coniferin (Table 21.1) has been reported in *C. sinensis* and may add to the effects of limonin and naringin.

The bioproduction of neohesperidin and naringin in callus cultures of *C. aurantium* has been demonstrated (J. A. del Río *et al*., *Plant Cell Rep*., 1992, **11**, 592).

Uses. Bitter orange peel is used as a flavouring agent and as a bitter tonic. Hesperidin in the soluble form as in the fresh fruit functions as vitamin P.

Sweet orange peel

The peel of the sweet orange is thinner than that of the bitter, more yellowish in colour and less rough, and the taste, though pungent and aromatic, lacks the extreme bitterness of the Seville peel. As studied in Valencia orange peel, the colour originates from a complex mixture of carbonyl carotenoids, the principal components being violeoxanthin (9-*cis*-violaxanthin), di-*cis*-violaxanthin and all-*trans*-violaxanthin, together with a number of other carotenoids.

ORANGE OILS

The volatile oil from the orange may be extracted by methods other than by distillation (see 'Lemon Oils' for details of methods). That from the bitter orange is known as *Essence de Bigarde* and that from the sweet orange is called *Essence de Portugal*. The latter is official in the *BP*/*EP* and is obtained by mechanical expression of the fresh peel; although chemically almost identical with the bitter orange oil, it does not have the bitter taste or odour of the latter. These oils contain the terpene (+)-limonene and smaller quantities of citral, citronellal, methyl anthranilate, etc. In 1988, 62 components from the steam-distilled oil of Libyan fresh orange peel were identified. Sixteen of the identified compounds had not previously been reported as orange volatiles (A. J. MacLeod *et al.*, *Phytochemistry*, 1988, **27**, 2185). Brazil and the USA are the largest producers of sweet orange oil.

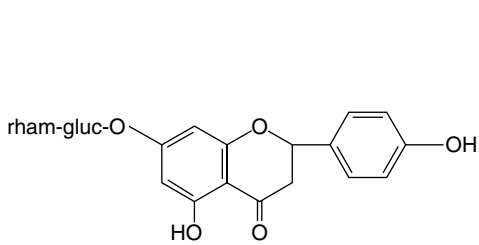
Terpeneless orange oil

By removing about 95% of the terpenes by vacuum distillation a terpeneless oil of orange may be obtained. One part of the terpeneless oil is equivalent to about 15 parts of the sweet orange oil. The *BP*/*EP* oil is required to contain not less than 18% of aldehydes calculated as decanal.

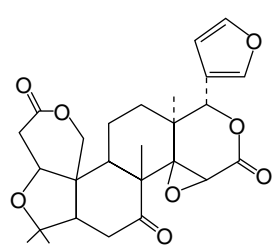
BITTER ORANGE FLOWER OIL

This oil, also known as **Oil of Neroli**, official in the *BP*/*EP* is prepared by steam distillation from fresh flowers of the bitter orange. An alcoholic solution of the oil has a violet-blue fluorescence arising from the small content (0.1–1.0%) of methyl anthranilate which is also responsible for the characteristic odour of the oil. Other constituents, with *BP*/*EP* permitted ranges, are *trans*-nerolidol (1.0–5.0%), geranylacetate (1.0–5.0%), α-terpineol (2.0–5.5%), linalyl acetate (2.0–15.0%), linalol (28.0–44.0%), limonene (9.0–18.0%), β-pinene (7.0–17.0%). There is a TLC test for the absence of bergapten, present should the oil be adulterated with that from the bitter peel.

In Britain the oil was traditionally used for the making of concentrated orange-flower water, syrup of orange flowers and Cologne spirit. It is used in aromatherapy.



Naringin



Limonin

LEMON PEEL

Lemon peel (*Limonis Cortex*) is obtained from the fruit of *Citrus limon* (L.) Burm. f. (Rutaceae), a small tree, 3–5 m high, cultivated in the countries bordering the Mediterranean and elsewhere (see 'Lemon Oils'). The lemon is of Indian origin and appears to have been unknown in Europe until the twelfth century. Numerous varieties and hybrids (particularly with *C. medica* Risso) are cultivated. Dried lemon peel is official in the *BP*/*EP*.

Collection and preparation. Lemons are collected in January, August and November, before their green colour changes to yellow. They are exported in cases containing from 200 to 360 fruits. The smaller fruits, which would not have a ready sale, are used in the preparation of oil of lemon. The peel is removed with a sharp knife in the form of a spiral band.

Characters. Dried lemon peel occurs in spiral bands up to 2 cm wide and 2–3 mm thick. Some pieces bear the apex of the fruit, which has a nipple-like appearance. The outer surface is rough and yellow; the inner surface is pulpy and white. Odour, strong and characteristic; taste, aromatic and bitter. The anatomical structure closely resembles that of orange peel (q.v.).

Constituents. Dried lemon peel contains not less than 2.5% of volatile oil (see below), vitamin C, hesperidin and other flavanone glycosides, mucilage and calcium oxalate. Lemon peel is mainly used for flavouring purposes.

LEMON OILS

Lemons are widely cultivated and the volatile oil is prepared around the Mediterranean, North and South America, in Australia and in parts of Africa. Lemon and other *Citrus* oils are best extracted by means other than distillation. The definition of pharmaceutical lemon oil given in the *British Pharmacopoeia* states that it is obtained by suitable mechanical means, without the aid of heat, from the fresh peel of *C. limon* (L.) Burm. f.

Once the oil has been separated from the peel, it can be distilled without deterioration in quality, and some expressed oil of lemon is fractionally distilled to make terpeneless oil of lemon (q.v.). Distillation direct from the peel is quite different, and, although much oil is prepared from the peel by steam distillation, this is inferior and does not comply with the definition given above. Distilled oil of lemon is cheaper than that prepared by expression and large quantities of it are made and used for non-pharmaceutical purposes.

History. Both expressed and distilled oils of lemon were sold in Paris as early as 1692. The sponge process as used in Sicily was described by Barrett in 1892. Machines were first introduced for oil of lemon production in 1920 and by 1930 about half the Italian oil was produced by their aid. New machines are being frequently introduced, and although some hand-expressed oil is still made (e.g. for eau de cologne, which requires the highest quality), pharmaceutical oil is now machine made.

Preparation. Oil of lemon is only one of several products made from lemons. In addition to dried peel, much lemon peel is candied with sugar. The pulp of the fruit yields on expression lemon juice, which may be canned or used for the preparation of citric acid and citrates. Pulp residues are used for pectin manufacture and as cattle food.

The following processes are used for the production of oil:

Hand methods. As these are no longer applicable to pharmaceutical oils they will not be described here and the reader is referred to earlier editions of this book for accounts of the *spugna* or sponge process, the *scorzetta* process, and the *écuelle à piquer* process.

Machine processes. The quality of machine-produced oil is rather inferior to the best hand-pressed. The machines are designed to set free the oil by puncture, rasping or cutting and by imitating the gentle squeezing action of the sponge method. The superiority of sponge-pressed oil appears to be due to the fact that there is virtually no contact between the oil and the inner white part of the peel (*albedo*). Deterioration in odour results from enzyme action in the finely divided albedo and is likely to be most pronounced when the machines penetrate deeply into the peel and when the resulting finely divided albedo and the water used for spraying are in contact with the oil for any length of time.

For the reasons given above, mincing of the whole fruit or peel followed by expression is unsatisfactory. Machines such as the *pelatric* which abrade the fruit surface are rather better, because they give less admixture of oil and albedo. The *sfumatrice machines* (squeezing machines) as first introduced, imitate more closely the hand method, since they exert a gentle pressing action on the peel passing on a stainless-steel band against stationary protrusions. Spray water is used to remove the oil, which is separated by a centrifuge. The *new sfumatrice* machines are a modification of the above in which fine knives cut into the outer peel (*flavido*), but partly also in the *albedo*. After treatment in these the peel shows 'almost invisible criss-cross cuts'. The newest machines extract the oil more completely than the older ones and therefore give a substantially higher yield.

Distilled oil. Although not official, some lemon oil is produced by distillation, mainly from the residues of the expression processes. It fetches a much lower price than either 'hand-pressed' or 'machine-made' oil.

Constituents. Lemon oil contains terpenes (about 94% mainly (+)-limonene), sesquiterpenes, aldehydes (citral, about 3.4–3.6%, and citronellal) and esters (about 1% geranyl acetate). Limonene (see Fig. 22.2) is a liquid, b.p. 175°C. Citral (see Fig. 22.2) or geranial, a liquid, b.p. 230°C, is the aldehyde corresponding to the alcohol geraniol. Lemon oil shows a marked tendency to resinify and should be protected from the action of air and light as much as possible. It has been shown by the use of GC and TLC that the oil obtained directly from the oil glands of the rind by capillary insertion differs from the fresh and stored commercial oils in composition. Principal reactions which cause these changes are oxidations of monoterpenes, aldehydes and esters, peroxide formation, polymerizations and isomerizations (e.g. limonene $\rightarrow$ α-terpinene).

Adulteration. Oil of lemon was at one time frequently adulterated with oil of turpentine, but analysts now have to contend with more scientific methods of adulteration. These include the addition of terpenes obtained in the preparation of 'terpeneless oil of lemon' and the addition of the cheaper distilled oil of lemon. The value of the oil is judged to some extent on the citral content, but a normal citral content alone is not a sure indication of purity, since citral may be added from a cheaper source such as oil of lemon-grass, which contains 75–85% of this aldehyde. It will be gathered that a careful examination of the oil by both physical and chemical methods is necessary, as exemplified by the standards and tests given in the *BP*.

Uses. Oil of lemon is used for flavouring and in perfumery.

Terpeneless lemon oil

Terpeneless Lemon Oil of the *BP* is prepared by concentrating lemon oil *in vacuo* until most of the terpenes have been removed, or by solvent partition. The concentrate is the terpeneless oil, which has a citral content of 40–50%. Terpeneless lemon oil is equivalent in flavour to about 10–15 times its volume of lemon oil; Lemon Spirit *BP* is a 10% solution in ethanol (96%) and it is also an ingredient of Compound Orange Spirit *BP*.

Buchu leaf

The name 'buchu' is applied to the leaves of several species of *Barosma* (*Agathosma*) (Rutaceae) grown in South Africa. The leaf, official in the *BHP*, is obtained from *Barosma betulina* (Thunb). Bartl. & Wendl. and known in English commerce as 'short' or 'round' buchu. The leaves of *Barosma crenulata* (oval buchu) and *B. serratifolia* (long buchu) are also used.

The leaves of *B. betulina*, *B. crenulata* and *B. serratifolia* are all small, shortly petiolate, green to greenish-yellow in colour, and supplied with numerous oil glands which are readily visible on holding them to the light.

Round or *short buchu* consists of the leaves and a small percentage of the stems, fruits and flowers of *Barosma betulina*. The leaves are 12–20 mm long and 4–25 mm broad. They are rhomboid-obovate in shape, with a blunt and recurved apex. The margin is dentate in the upper two-thirds of the leaf and serrate towards the base. A large oil gland is situated at the base of each marginal indentation and at the apex, while numerous smaller ones are scattered throughout the lamina. The leaves when dry are brittle and coriaceous, but on moistening become cartilaginous or mucilaginous. Odour and taste, strong and characteristic. Reddish-brown fragments of stems, up to about 5 cm, brown fruits with five carpels and flowers with five whitish petals are usually present, but an excessive amount of these must be regarded as an adulteration.

Oval buchu is obtained from *Barosma crenulata* Hooker. The leaves, which are accompanied by a certain amount of stem, are 15–30 mm long and 7–10 mm broad. The shape is more or less oval; the apex is blunt but not recurved and possesses a terminal oil gland; marginal serration very minute. (For a report see E. Wollenweber and E. H. Graven, *Fitoterapia*, 1992, **62**, 86.)

Long buchu is obtained from *Barosma serratifolia* Willd. The leaves are 12–40 mm long and 4–10 mm broad, and linear lanceolate in shape; the apex is truncate and possesses a terminal oil gland; the margin is serrate.

Buchu leaves contain volatile oil, diosmin (see Fig. 42.1B), mucilage, resin and calcium oxalate. In addition to the principal components pulegone, menthone, isomenthone and limonene the oil, in which over 120 components have been identified, contains diosphenol or buchu camphor to which the diuretic activity of the drug has been ascribed. The characteristic odour of the oil has been ascribed to sulphur compounds and *p*-menthane-8-thio-3-one has been characterized; it is present in quantities of up to 0.5% of the oil and is probably derived from (–)-pulegone. Buchu is still occasionally used as a diuretic and urinary antiseptic and is considered effective by herbal practitioners. For a recent review see A. Moola and A. M. Viljoen, *J. Ethnopharm.*, 2008, **119**, 413.

NUTMEG AND NUTMEG OIL

Nutmegs are the dried kernels of the seeds of *Myristica fragrans* (Myristicaceae), an evergreen tree about 10–20 m high, indigenous to the Molucca Islands. The plant is now widely cultivated not only in Indonesia and Malaysia (Molucca Islands, Sumatra, Java and Penang), but also in Ceylon and the West Indies (Grenada). Current world demand for nutmegs stands at about 10 000 tonnes per annum of which about 75% originates from Indonesia and 15% from Grenada.

History. Nutmegs and mace appear to have been first introduced into the Levant by the Arabs in the middle of the twelfth century and by the end of that century were found in northern Europe. The native country of the nutmeg (the Molucca or Spice Islands) was known to Arabian writers of the thirteenth century, and the Banda Islands, a group of the Moluccas where the plant is very abundant, were discovered by the Portuguese in 1512. The Portuguese, after holding the spice trade for about a century, lost it to the Dutch, who maintained a complete monopoly by destroying the trees in neighbouring islands and preventing the export of living seeds. The ordinary drying process destroys the vitality of the seeds, but they were also soaked in milk of lime for many weeks and were seldom sold until they were several years old. The Spice Islands were occupied by the English for a few years (1796–1802), during which period the opportunity was taken to start cultivation in Penang and Sumatra. Until the trees so planted reached maturity the effect of the Dutch restriction was still felt, and in 1806 the import price of mace in London was as high as £10 kg^{-1}.

Cultivation of the spice was subsequently introduced to the West Indies and during the Second World War production of nutmegs in Grenada was expanded enormously. In 1955 a hurricane destroyed 90% of the trees but the industry has now recovered and nutmegs remain the island's main commodity export.

Cultivation, collection and preparation. Nutmeg trees can be grown from fresh seed sown in the shell. The seeds germinate in about 5 weeks, and when the young plants are about 6 months old, they are transplanted to the fields. When the sex can be determined (5–8 years), the male trees are reduced to about 10% of the total. This method leads to irregularly spaced trees in the plantation and now in Grenada vegetative propagation of the female trees is performed by *marcotting* or *air layering*. In this, female shoots are split but not detached, and by the use of hormone powder and suitable packing of the wound, are induced to root. This takes 4–18 months after which the rooted shoots are detached and brought on before planting out. A success rate of over 40% for rooting is now obtainable. Another technique which has been used to increase the number of female trees is the employment of approach grafts. The trees bear fruit from their eighth or ninth year and continue to fruit well for about 20–30 years. The peach-like fruit splits when ripe, exposing the seed with its lobed, red arillus. The plant fruits almost continuously and two or three crops are collected annually. In the East the fruits are collected by hand or by means of a hooked stick, but in Grenada the fruits are allowed to fall to the ground. The orange-yellow pericarp which is about 12.5 mm thick, is usually removed on the spot. Later the arillus is picked off and constitutes, when dried, mace. From mature plantations the annual yield per acre is about 250–500 kg of nutmeg and about 50–100 kg of mace. The nutmegs are dried in the shells, the procedure differing according to local conditions but usually taking about 3–6 weeks. In Malaya sun-drying is used to some extent, but the seeds require adequate cover at night or in wet weather. Large quantities are dried in ovens and in brick buildings. In the latter the seeds are placed on trays over low charcoal fires, being turned and gradually moved nearer to the fires during the process. When drying is completed, the kernel rattles within the brittle testa, which constitutes about one-quarter of the weight of the seed. The testa is cracked by means of a wooden truncheon, mallet or special machine, and the nutmeg extracted. However, machines are liable to cause bruises, and cracking by hand is preferable. The liming of nutmegs to reduce insect attack is now less commonly practised than in the past. After cracking, the nutmegs are now usually graded abroad into sizes represented by numbers per unit weight. Elongated nutmegs, which fetch a lower price, and small or damaged ones are kept separate. Nutmegs are exported in barrels or cases containing about 50 kg.

Macroscopical characters. Nutmegs are broadly oval in outline, 2–3 cm long and about 2 cm broad. If not heavily limed, the surface is of a brown or greyish-brown colour and is reticulately furrowed. At one end is a light-coloured patch with brown lines radiating from the hilum, which is surrounded by a raised ring. From this an ill-defined furrow (imprint of the raphe) runs to the chalaza, at the opposite end of the kernel, where there is a small dark depression. Odour, strong and aromatic; taste, pungent and slightly bitter.

A longitudinal section (Fig. 22.9C) has a lustrous, marbled appearance. The outer tissue, which consists of dark brown perisperm, penetrates the light brown endosperm, the infoldings branching and giving rise to the marbled appearance. The perisperm possesses fibrovascular bundles, the position of which is indicated by the reticulate furrows found on the outer surface.

Microscopical characters. The outer perisperm cells are radially flattened and have brownish contents, insoluble in potassium hydroxide or chloral hydrate. A few of the cells contain prismatic or disc-shaped crystals, thought to consist of potassium acid tartrate. The inner perisperm shows numerous extensive lamellae, corresponding to the furrows on the surface, and penetrating into the endosperm. These lamellae are composed of parenchymatous cells with thin brown walls and of oval oil cells, and show in their outer part vascular strands composed of lignified spiral vessels. The endosperm is composed of parenchymatous cells, with thin brown cell walls, and containing simple or 2–10 compound starch grains (individual grains up to 22 μm in diameter, globular or irregular in shape, with sometimes a slit-like hilum); aleurone grains, the larger of which show a well-defined crystalloid; and feathery crystals of fat. A few tannin cells, containing tannin and starch, occur scattered in the endosperm.

Allied drugs. *Papua nutmegs* are derived from *M. argentea*, a tree grown in New Guinea. They are often taken to Macassar and enter commerce as Macassar, Papua, long or wild nutmegs. They have a uniform, scurfy surface, little odour and a disagreeable taste.

Bombay nutmegs are derived from *M. malabarica*, grown in India. They are very long and narrow and lack the characteristic aroma of the genuine drug.

Mace. Common mace or Banda mace consists of the dried arillus or arillode of *M. fragrans*. This, when fresh, is of a bright red colour and is removed either by the finger or a knife. When removed entire, it forms 'double blade' mace, but if in two pieces, it is known as 'single blade' mace. After flattening by treading under the feet or pressing between boards the mace is slowly dried. The volatile oil of mace resembles that of nutmeg, the major phenolic compounds isolated being dehydrodiisoeugenol and 5′-methoxydehydrodiisoeugenol, both of which have a significant antibacterial action. In recent years a series of lignans and neolignans has been isolated from mace; see Table 21.7 for the formula of macelignan.

Bombay mace is a regular article of commerce, although almost valueless as a spice. It is dark red in colour, is lacking in aroma and yields about 30% of extractive to light petroleum (genuine mace yields about 3.5%). Papua mace is distinguished by its shape, dull brownish surface, lack of aroma and acrid taste.

Constituents. Nutmegs yield 5–15% of volatile oil and also contain 30–40% fat, phytosterin, starch, amylodextrin, colouring matter and a saponin. They yield about 3% of total ash and about 0.2% of acid-insoluble ash. The psychotropic properties of nutmeg are discussed under 'Hallucinogens'.

Uses. Nutmegs, maces, and their oils are largely used for flavouring and as carminatives. In traditional Indian medicine an aqueous extract of nutmeg is used for the treatment of infantile diarrhoea.

NUTMEG OIL

Nutmeg oil is distilled from the kernels imported into Europe and the USA, and is produced in Indonesia (about 120 tons annually), Sri Lanka (30 tons) and India (5 tons). It contains (*BP*/*EP* limits as determined by gas chromatography), α-pinene (15–26%), β-pinene (13–18%), sabinene (14–29%), myristicin (5–12%), limonene (2–7%), γ-terpinene (2–6%), terpinen-4-ol (2–6%), car-3-ene (0.5–2%), safrole (2.5% maximum). Other minor constituents include elemicin and isoelemicin, eugenol, methyleugenol, methoxyeugenol, methylisoeugenol and isoeugenol.

There are differences in optical rotation, refractive index, weight per millilitre and solubility in alcohol between the West Indian and East Indian oils. Myristicin (formula Chapter 39), is 4-allyl-6-methoxy-1,2-methylenedioxybenzene. It is crystalline and, owing to its high boiling point, is mainly found in the last portions of the distillate. Myristicin is toxic to human beings and large doses of nutmeg or its oil may cause convulsions. Workers in Canada and Japan have isolated a considerable

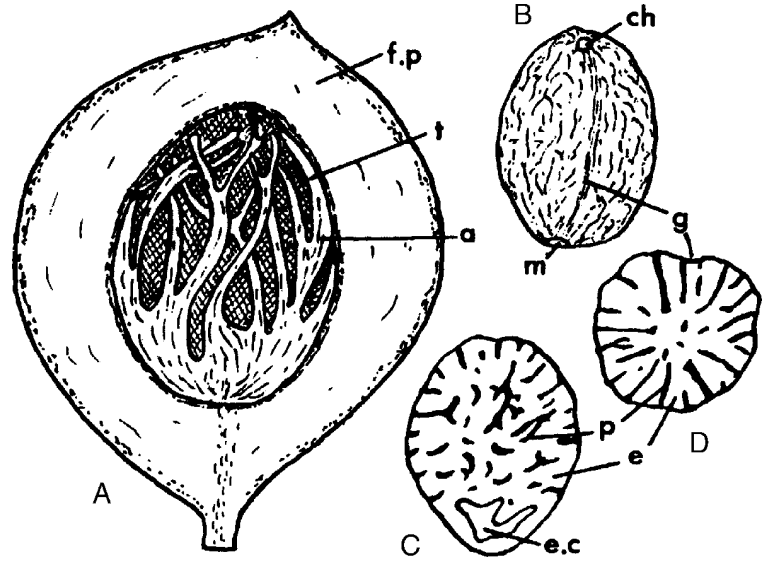


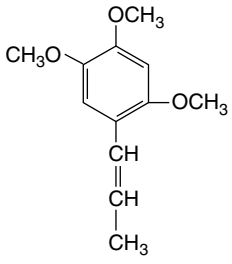
Fig. 22.9
Myristica fragrans. A, Fruit with half of the pericarp removed; B, nutmeg (dried kernel); C, longitudinal section nutmeg; D, transverse section nutmeg (all × 1). a, Aril (mace); ch, chalaza; e, endosperm; e.c, cavity left by embryo; f.p, fleshy pericarp; g, groove marking line of raphe; m, micropyle region; p, perisperm; t, testa.

number of dimeric phenyl-propanoids from the seed; the units include isoeugenol, elemicin and myristicin. Similar dimeric compounds, shown to cause significant changes in hepatic enzyme systems, have been isolated from mace oil (W. S. Woo *et al*., *Phytochemistry*, 1987, **26**, 1542).

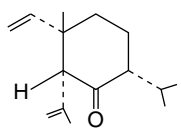
Calamus

Calamus or sweet flag consists of the rhizome of *Acorus calamus* (Araceae), which occurs in commerce both peeled and unpeeled. The perennial plant is common on the banks of streams. Originating in Asia, it is now widely distributed in Asia, Europe and North America. The subcylindrical rhizome is up to 20 cm long and 2 cm diameter; longitudinally furrowed on the upper surface and with conspicuous root scars on the lower surface.

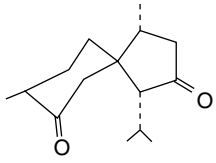
Calamus contains 2–4% of volatile oil containing a number of sesquiterpenes and asarone, a compound related to myristicin (see 'Nutmeg and Nutmeg Oil'). Calamus has been official in many pharmacopoeias, and although still used in some regions, is now mainly used as a source of calamus oil, which is employed in perfumery. The composition of the oil from 2*n*, 3*n* and 4*n* varieties differs and the β-asarone content increases with ploidy. As the phenylpropane derivatives have been shown to be carcinogenic in animal tests, Keller and Stahl (*Planta Med.*, 1983, **47**, 71; 1985, p. 6) recommend the selection of races for pharmaceutical use. The oil from the rhizome of the American 2*n* race contains no β-asarone but consists of shyobunones and acorones, which are also components of the pharmaceutically used oils. GC-MS studies combined with gene sequencing have also been employed for the identification of a 2*n* β-asarone-free race (C. M. Bertea *et al*., *Phytochemistry*, 2005, **66**, 507).



Asarone



2,6-Di-epi-shyobunone
(Main constituent of
diploid calamus oil)



Acorone

Chemotypes of *A. calamus* having differences in essential oil composition have been DNA profiled (N. Sugimoto *et al*., *Biol. Pharm. Bull*., 1999, **22**, 481).

A number of sesquiterpenes based on the cadinane, acorane and eudesmane skeletons have been isolated from *A. calamus* and some of these are strong germination inhibitors of lettuce seeds. Such secondary metabolites are called allelochemicals, well-known examples being the nagilactones of *Podocarpus nagi* (K. Nawamaki and M. Kuroyanagi, *Phytochemistry*, 1996, **43**, 1175).

For details of the main constituents of calamus and the acetylcholinesterase inhibitory activity of the oil, see P. K. Mukhergee *et al*., *Planta Medica*, 2007, **73**, 191.

Further reading

Mukherjee PK, Kumar V, Mal M, Houghton PJ 2007 *Acorus calamus*: scientific validation of Ayurvedic tradition from natural resources. Pharmaceutical Biology 45(8): 651–666. *A review* (*ca 130 refs*) *exploring the various constituents and pharmacological activities of the drug*

Motley TJ 1994 Ethnobotany of sweet flag (*Acorus calamus*). Economic Botany 48(4): 397–412. *A comprehensive review with over 160 references*

CINNAMON AND CINNAMON OIL

The *BP*/*EP* states that 'cinnamon is the dried bark of the shoots grown on cut stock of *Cinnamomum zeylanicum* Blume, freed from the outer cork and underlying parenchyma.' However, Kostermans (see *Bibliographia Lauracearum*, 1964) indicates the plant to be more correctly named *C. verum* Presl. (Lauraceae), of which there are two varieties, better called subspecies, one (var. *subcordata* Nees) with ovate, subcordate leaves, the other (var. *vulgare* Nees, now properly called var. *verum*) with oblong or elliptic leaves pointed at both ends; both produce a good drug. Many other 'varieties' (about 23) have been described and exist wild in Sri Lanka and southern India; most of these, however, on current taxonomic grounds, represent other species. The tree is also cultivated in the Seychelles, Madagascar, Martinique, Cayenne, Jamaica and Brazil. Ceylon cinnamon is the commonest variety on the English market, but good-quality Seychelles drug, which closely resembles the product from Sri Lanka, is also available.

The British and Americans do not give the same meaning to the words 'cinnamon' and 'oil of cinnamon'. In Britain cinnamon and oil of cinnamon are derived as above. In the USA, however, 'Cinnamon NF' is Saigon cinnamon and 'Oil of Cinnamon NF' is the oil which British call Cassia Oil, which is derived from Cassia bark (q.v.).

History. Cassia bark was known to the Chinese in 2700 BC but it is not until the thirteenth century that any reference is found to the collection of cinnamon in Ceylon. Ceylon was occupied by the Portuguese in 1536, the Dutch in 1656, and the English East India Company in 1796. Cinnamon cultivation was started by the Dutch in 1770 and they exercised a strict monopoly comparable with their monopoly of nutmegs. This was continued until the monopoly of the English East India Company was abolished in 1833.

Cultivation, collection and preparation. In Sri Lanka about 26 000 acres are devoted to cinnamon plantations. Most of the plantations are small and are situated in the southern or western provinces. Sri Lanka and the Seychelles both export large quantities of cinnamon leaf oil.

The production of the characteristic compound quills of the inner bark is a multistage process and was fully described and illustrated in earlier editions of this book. Briefly, the cinnamon plants are grown from seed and coppiced almost to the ground when 2 or 3 years old. About five or six shoots are allowed to grow from the stump and are kept vertical by pruning. After about 18 months of growth, and when some 3 m long and 2 cm diameter, shoots are harvested, trimmed and, following a few hours 'fermentation', they have the bark removed with a non-ferrous knife. The peeled bark is then stretched over a suitable stick and the outer cork and cortex scraped off with a curved scraper. Individual pieces of scraped bark are then placed one inside the other and built up to a length of about 42 in (*c*. 106 cm). The compound quills are dried on wooden frames in the open air without exposure to direct sunlight and then finally sorted into grades and made into compact bales of about 45 kg.

The traditional grades of cinnamon are designated: 00000, 0000, 000, 00, 0, 1, 2, 3, 4, quillings, featherings, chips. Most commercial material corresponds to Nos. 1–4 grades. Quillings and featherings

consist of small pieces, the latter often containing some outer bark; they are used for grinding and for oil distillation. Chips consist mainly of outer pieces of bark, and the oil derived from them has a lower specific gravity and a lower aldehyde content than that from the inner bark.

Macroscopical characters. Cinnamon is imported in large bundles about 1 m in length. Retailers generally receive their supplies in shorter lengths known as 'cigar lengths'. The drug consists of single or double compound quills about 6–10 mm diameter and of varying length (Fig. 22.10). In the different grades the thickness of each piece of bark varies considerably, but in good-quality cinnamon it is usually not more than about 0.5 mm, while the number of pieces of bark forming the compound quill varies from about 10 to 40. The external surface of each piece is yellowish-brown and shows longitudinal shining, wavy lines (pericyclic fibres) and occasional scars and holes (indicating the positions of leaves or twigs). The inner surface is somewhat darker and longitudinally striated. The bark breaks with a short, splintery fracture. Odour is fragrant; taste, warm, sweet and aromatic.

Microscopical characters. Transverse sections of cinnamon (Fig. 22.10) show under the microscope a complete absence of epidermis and cork. Shrivelled remains of cortex occur in patches. The outer limit of the bark is marked by a pericycle composed of a continuous ring of three to four layers of sclereids with small groups of pericyclic fibres embedded in it at intervals. The latter produce the lighter-coloured, wavy, longitudinal lines on the outside of the commercial bark. The sclereids (Fig. 22.10) have thickened lignified walls, showing well-defined pit-canals. The thickening on the outer walls is often less pronounced than on the radial and inner tangential walls. The lumen is clearly visible and sometimes contains starch. The pericyclic fibres range from 1000 to 2500 µm long and have strongly thickened lignified walls showing stratification and pit-canals. Primary phloem cannot be distinguished. The secondary phloem is composed of phloem parenchyma containing oil and mucilage cells; phloem fibres; and medullary rays. The sieve-tube tissue, embedded in the phloem parenchyma, is often obliterated. The phloem parenchyma is composed of thin-walled cells, with yellowish-brown walls, and contains starch in compound and simple grains, the latter not exceeding 10 µm diameter (those of *Cinnamomum cassia* often exceed this figure) and numerous acicular crystals of calcium oxalate about 5–8 µm long. Some of the phloem parenchyma cells contain tannin. The secretion cells, containing volatile oil or mucilage, are two or three times the diameter of the phloem fibres, and are axially elongated. The phloem fibres, which occur isolated or in tangential rows, are more abundant towards the inner part of the bark. They are usually less than 30 µm in diameter (those of *C. cassia* measure 30–40 µm in diameter) and have a length of 200–600 µm. The thick lignified walls show stratification. The secondary phloem is divided up by the radial medullary rays, which are uni- or biseriate near the cambium but become broader towards the outside by tangential growth of the cells. The rays are 7–14 cells high. The medullary ray cells are radially elongated, thin-walled with yellow–brown cell contents containing numerous acicular crystals of calcium oxalate. For illustrations of the powdered elements, see Fig. 22.10.

Constituents. Cinnamon contains volatile oil (*BP*/*EP* not less than 1.2%), phlobatannins (which have been little investigated compared with those of cassia) mucilage, calcium oxalate and starch.

CINNAMON OIL

Oil of cinnamon contains about 60–75% w/w of *trans*-cinnamic aldehyde, $C_6H_5CH{=}CHCHO$. Genuine oils also contain 4–10% of phenols (chiefly eugenol), hydrocarbons (pinene, phellandrene and caryophyllene), and small quantities of ketones, alcohols and esters; GLC shows the presence of many compounds and limits for specific constituents are given in the *Pharmacopoeia*. Oil distilled from fresh bark samples collected in Ghana by Angmor *et al*. (*Planta Med*., 1979, **35**, 342) contained a high proportion of cinnamyl acetate, but by a protracted preparation of the drug which simulated the commercial preparation this ester was largely converted into aldehyde. Phenylalanine has been shown to be a precursor of both cinnamic aldehyde and eugenol in the

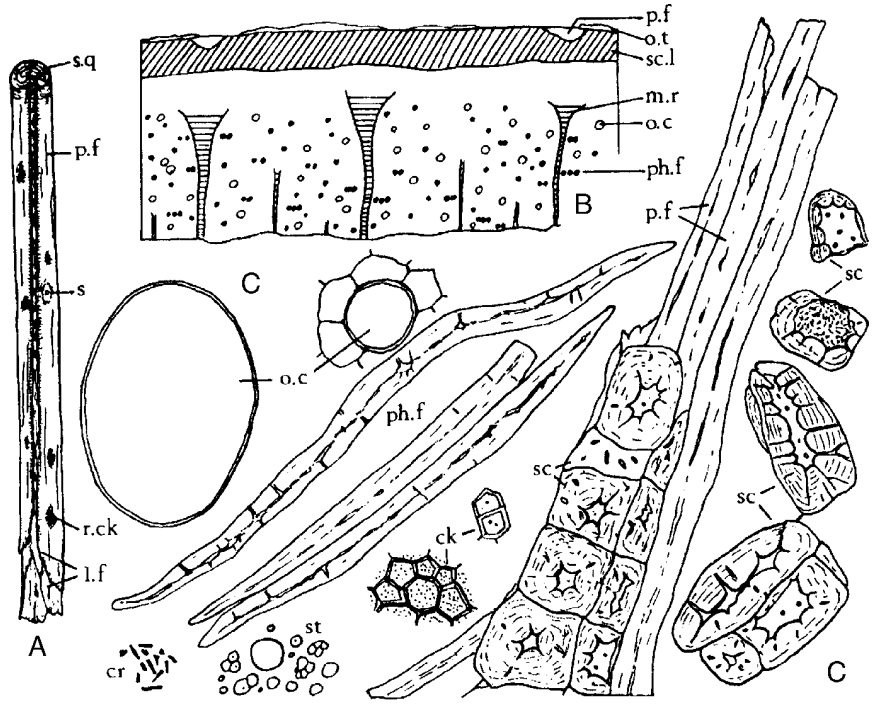


Fig. 22.10
Cinnamon. A, Compound double quill (× 0.5); B, transverse section (×50); C, elements of the powder (×200). ck, Cork cells; cr, acicular crystals of calcium oxalate; l.f, laminated fracture of compound quill; m.r, medullary ray; o.c, oil cells; o.t, remains of outer tissues; p.f, pericyclic fibres; ph.f, phloem fibres; r.ck, residual patches of cork; s, scar of twig; sc, sclereids; sc.l, sclereid layer of pericycle; s.q, transverse surface of compound quill; st, starch granules.

living plant, but the metabolic interrelationships between the aromatic compounds appear complex.

The oil is liable to adulteration with cinnamon leaf oil and with oil of cassia. Oil of cassia contains about 80–95% of aldehydes and a similar test with ferric chloride gives a brown colour. Oil from the root-bark contains much camphor and other monoterpenes but negligible phenylpropanes.

Allied drugs. *Cayenne cinnamon* consists of the bark of cultivated plants of *Cinnamomum zeylanicum* grown in French Guiana, Brazil and some of the islands of the West Indies. It is generally obtained from older branches than the Ceylon drug and appears to be inferior to it in quality. It is not used to any extent in Britain.

C. loureirii is commercially important in Vietnam and grows in the mountainous districts of Annam. The plant, which is closely related to *C. cassia* is also found in China and Japan. It resembles cassia bark more closely than cinnamon, and occurs in quills up to 30 cm long, 4 cm wide and 0.5–7.0 mm thick. The outer surface is greyish-brown, warty and ridged. The odour is coarser than that of Ceylon cinnamon and the taste sweeter.

Uses. Cinnamon is used as a flavouring agent and mild astringent. The oil has carminative properties and is a powerful germicide.

CEYLON CINNAMON LEAF OIL

The oil is obtained by steam distillation of the leaves of *Cinnamomum verum* J. S. Presl. It is a commercial article, and some twenty times the amount of the bark oil is produced. It contains 70–95% of eugenol (*BP*/*EP* limits 70–85%) giving the oil a clove-like odour. An alcoholic solution of the oil gives with ferric chloride solution a blue colour; other components of the oil, which are limited individually within the range 1.0–7% and determined by gas chromatography, are cineole, linalol, β-caryophyllene, safrole, *trans*-cinnamic aldehyde, cinnamyl acetate, eugenol and coumarin. Other standard specifications are relative density, refractive index and optical rotation.

The high eugenol content gives the oil antiseptic and anaesthetic properties; it is mainly employed for the extraction of eugenol and in the cosmetic industry in carnation-type perfumes.

Cassia, Chinese cinnamon or cassia lignea

Various barks have been imported under the name of 'cassia'. That known in the London market as Chinese cassia lignea is derived from *C. cassia* Blume, a tree cultivated in the south-eastern provinces of China (Jiangxi and Guangdong). When about 6 years old, the bark is removed from the older branches, the twigs and leaves being used for distillation. The cork and cortex are partly removed by planing, the bark tied into bundles and exported in boxes, via Guangzhou and Hong Kong.

Cassia bark occurs in channelled pieces or single quills up to 40 cm long, 1–2 cm wide and 1–3 mm thick. The outer surface is darker than that of Ceylon cinnamon and, owing to careless planing, shows patches of grey cork. The odour is coarser than that of cinnamon and the taste more astringent.

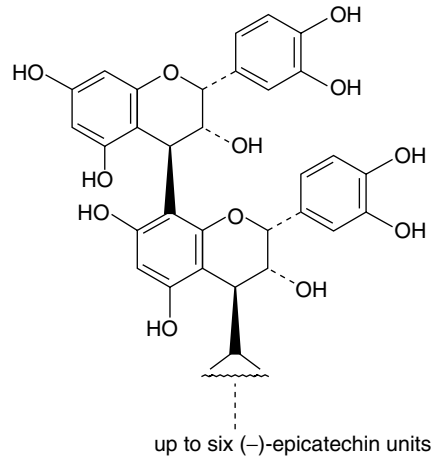
Transverse sections resemble cinnamon as far as the inner part of the bark is concerned, except that the starch grains and phloem fibres are somewhat larger. However, the utility of the fibre size for distinguishing the two barks has been questioned owing to the limited sample numbers used in the original investigation. Outside the sclerenchymatous ring is the cortex and cork layer.

Cassia bark has been reported to contain about 10% mucilage, whereas Ceylon and Seychelles cinnamon samples contained 1.6–2.9%. TLC tests have also been used for distinguishing the barks. Such distinctions could well arise from the fact that the Ceylon cinnamon is an inner bark, whereas with cassia bark outer cortex and cork are present.

Cassia yields 1–2% of volatile oil which, when pure, contains no eugenol but rarely less than 85% of cinnamic aldehyde; other components are cinnamyl acetate, phenylpropyl acetate and numerous trace constituents. 2′-Hydroxycinnamaldehyde has recently been isolated from the stem bark (B.-M. Kwon *et al*., *Planta Medica*, 1996, **62**, 183). The oil is included in the *USP/NF* under the name of Cinnamon Oil and is required to contain not less than 80% by volume of aldehydes. Large quantities of oil are distilled from the leaves and twigs as well as from the bark. Although inferior in flavour to the oil of *C. zeylanicum*, it is cheaper and is described in many pharmacopoeias.

Considerable advances in the chemistry of the non-volatile components of cassia bark have been made by Japanese researchers and have demonstrated the pharmacological activities of these substances. In a number of papers Nohara *et al*. (see *Phytochemistry*, 1985, **24**, 1849 and references therein) have reported the isolation of a series of diterpenes from a fraction of the bark showing antiallergic activity. Aqueous extracts have been shown to have antiulcerogenic activity (T. Akira *et al*., *Planta Med*., 1986, p. 440).

In 1986 Morimonto *et al*. characterized a number of compounds of the tannin complex (*Chem. Pharm. Bull*., **34**, 633, 643) as below. Three flavan-3-ol glucosides were identified as (–epicatechin 3-*O*-, 8-*C*- and 6-*C*-β-D-glucopyranosides respectively. Three oligomeric procyanidins (named cinnamatannins A_2, A_3, A_4) were tetra-, penta- and hexameric compounds respectively, consisting exclusively of (−)-epicatechin units linked linearly through C-4–C-8 bonds (see formula). Free (−)-epicatechin and procyanidins were present, the latter occurring also in dimeric form and as *C*-glucosides.



Cinnamatannins of cassia bark

An arabinoxylan which activates the reticuloendothelial system was described in 1989; this neutral polysaccharide, named cinnaman AX, contains L-arabinose and D-xylose in the respective molar ratio of 4:3. Other pharmacologically similarly-acting polysaccharides have been reported in *Panax* and *Saposhnikovia*.

Callus and suspension cultures of *Cinnamomum cassia* produce large amounts of condensed tannins; the precursors (−)-epicatechin and-procyanidins B2, B4 and C1 have been isolated from callus cultures (see K. Yazaki and T. Okuda, *Phytochemistry*, 1990, **29**, 1559).

Cassia bark is an important drug of Oriental medicine.

Cassia 'buds', perhaps inappropriately named, are the dried immature fruits of *C. cassia*. They yield about 20% of volatile oil having a cinnamaldehyde content of around 80%.

Java or Indonesian cinnamon is derived from *C. burmanii* Blume, and is used in Holland. The tree is found in Sumatra, through Java to Timor. It may be distinguished from ordinary cinnamon when in powder by the presence of tabular crystals of calcium oxalate. The oil contains about 75% of cinnamic aldehyde.

Oliver bark or *black sassafras* is obtained from the so-called Brisbane 'white sassafras' tree, *C. oliveri*, a native of Queensland. It is used locally as a cinnamon substitute. The bark is easily distinguished from the drugs mentioned above. It occurs in flat strips about 20 cm long, 4 cm wide and 1 cm thick. The outer surface is brownish and warty, and bears patches of greyish cork. It yields about 1–2.4% of volatile oil.

Further reading

Ravindran PN *et al* (eds), Hardman R (series ed) 2004 Medicinal and aromatic plants – industrial profiles, Vol. 36 Cinnamon and cassia: the genus *Cinnamonum*. CRC Press, Boca Raton, FL 384 pp.

TEA-TREE OIL

The clear, colourless to pale-yellow oil is obtained by distillation from the leaves and terminal branches of *Melaleuca alternifolia* (Maiden and Betch) Cheel, family Myrtaceae, and other species of *Melaleuca* including *M. linariifolia* Smith and *M. dissitiflora* F. Mueller. These species are closely related to *M. leucadendron* (q.v.) and occur wild in New South Wales, Australia, where they constitute a well-known article of traditional aboriginal medicine.

Cyclic monoterpenes constitute the principal components of the oil, for which the *BP*/*EP* sets specified limits: terpinen-4-ol (30.0% minimum), γ-terpinene (10–28%), *p*-cymene (0.5–12.0%), α-terpinene (5–13%), cineole (less than 15.0%), other components generally present in smaller amounts for which limits are given include α-pinene, sabinene, limonene, terpinolene, aromadendrene and α-terpineol. These compounds are determined by gas chromatography using a reference solution for calculation. Other pharmacopoeial tests are relative density, refractive index, optical rotation and TLC.

In recent years the popularity of tea-tree preparations has increased enormously to include antiseptic creams for skin treatment, inhalations and pastilles for throat infections. A recent report (D. V. Henley *et al*., *New Engl. J. Med.*, 2007, **356**, 479) records that natural lavender and tea-tree oils in moisturisers can cause breast enlargement in prepubertal boys. Laboratory tests on breast cells have shown that the oils activate the female oestrogen receptor and suppress male hormones.

Further reading

Southwell I, Lowe R (eds), Hardman R (series ed) 1999 Medicinal and aromatic plants—industrial profiles, Vol. 9. Tea tree: the genus *Melaleuca*. Harwood Academic, Amsterdam. *589 references*

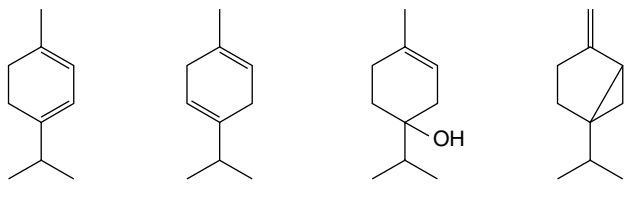


Fig. 22.11
Some monoterpenoids of tea-tree oil; for others see Fig. 22.2.

NATURAL CAMPHOR

Natural camphor is a white, dextrorotatory ketone, $C_{10}H_{16}O$ (see Fig. 18.17), obtained from the wood of *Cinnamomum camphora* (Lauraceae), a tree which is widely grown in Taiwan, Japan and south China; it is also produced commercially in India and Georgia. Synthetic camphor, which is optically inactive, is prepared from turpentine and would probably have completely replaced the natural product had it not been for other important byproducts of the industry. Monographs for both the natural and synthetic camphor are included in the *BP*/*EP*.

Preparation. The best yield of camphor is obtained from old trees. The wood is cut into chips and treated with steam, when a solid sublimate of camphor and liquid volatile oil pass into the receiver. The volatile oil is treated to yield more camphor and much of the residual camphor oil is used as a source of safrole. The impure camphor is treated with lime and charcoal and resublimed into large chambers. It collects in the form of 'flowers of camphor', which can be made into the familiar blocks by hydraulic pressure. Camphor can also be prepared from suitable leaves of the tree and their use is helping to reduce the complete destruction of camphor tree forests.

Synthetic camphor is largely prepared from American turpentine. By the action of hydrogen chloride the pinene is converted into bornyl chloride which, on treatment with sodium acetate, yields isobornyl acetate. Hydrolysis of this is to isoborneol and subsequent oxidation gives camphor.

Tests for identity and purity of natural camphor are important to eliminate synthetic racemic material, excess camphor oil, and camphors from inappropriate natural sources. These tests include melting point (175–179°C), specific optical rotation (+40.0 to +43.0), acidity and limit of halogens (particularly chlorides arising from the synthesis of racemic camphor), gas chromatographic detection of extraneous material arising from the synthesis of camphor and other sources including α- and β-pinene, cineole, fenchone, fenchol and borneol.

Characters. Camphor occurs in small, colourless crystals or in transparent fibrous blocks. It has a characteristic odour and a pungent, aromatic taste, which is followed by a sensation of cold. It volatilizes at ordinary temperatures, forming an encrustation on the walls of the vessel in which it is kept.

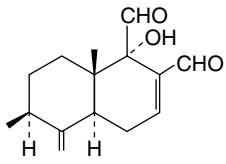
Camphor oil (see above) contains, in addition to camphor, safrole, borneol, heliotropin, vanillin and terpineol, a number of sesquiterpene alcohols. Oil of Pakistan origin has been shown to contain 25 monoterpenoids, and four chemotypes with respect to oil composition have been recorded for Vietnamese material.

Allied drugs. Borneo camphor, obtained from *Dryobalanops aromatica* (Dipterocarpaceae), and Ngai camphor, obtained from *Blumea balsamifera* (Compositae), are used in China and Japan. In California laevorotatory camphor is produced from species of *Artemisia* (Compositae).

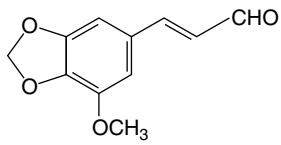
Uses. Camphor is used externally as a rubefacient, and internally as a mild antiseptic and carminative. It finds many non-pharmaceutical uses. Large quantities were once used in the manufacture of celluloid.

Canella bark

Canella bark is the dried rossed bark of *Canella alba* (*C. winterana*) (Canellaceae), a small tree growing in the Bahamas and Florida. It occurs in quills or channelled pieces up to 5 cm long and 5 mm thick. It contains about 1% of volatile oil and as a condiment goes under the names of 'white cinnamon' or 'wild cinnamon'. The oil contains monoterpenes, eugenol and myristicin.



Canellal



3-Methoxy-4,5-methylene-dioxycinnamaldehyde

In 1978 a novel antimicrobial sesquiterpene dialdehyde (canellal) was reported from the bark and more recently 3-methoxy-4,5-methylenedioxy-cinnamaldehyde. Cannellal also has insect antifeedant, antifungal and cytoxic properties. For work on the isolation of drimane sesquiterpenes and other compounds, see D. Kioy *et al*., *J. Nat. Prod*., 1989, **52**, 174; 1990, **53**, 1372 and M. S. Al-Said *et al*., *Phytochemistry*, 1990, **29**, 975.

Oil of Cajuput

Oil of Cajuput is a volatile oil distilled from the fresh leaves of *Melaleuca leucadendron* L. and other species of *Melaleuca* (Myrtaeceae) and rectified by steam distillation. The plants are evergreen shrubs or trees found in the East Indies and Australia. Most of the oil is produced in the islands of Bouru and Banda. It has a pleasant, camphoraceous odour and a bitter aromatic taste. It contains about 50–60% of cineole, terpineol and its acetate, terpenes and sesquiterpenes.

Medicinally, the oil is used both internally and externally as a stimulant and for the treatment of various parasitic conditions. It finds considerable use in India and the Far East.

Pimento or allspice

Pimento (*Jamaica* or *Clove Pepper*) is the dried nearly ripe fruit of *Pimenta dioica* (Myrtaceae), an evergreen tree grown in the West Indies (Jamaica, Cuba, Trinidad etc.) and central America.

The fruits are collected before they are quite ripe, as they otherwise lose much of their aroma and become filled with a sweet pulp; they are normally sun-dried but artificial drying has been recommended.

The pimento flower and fruit closely resemble those of the clove. The biocular ovary, however, develops two seeds, whereas only one is produced in the clove. Pimento fruits are globular and 4–7 mm in diameter. At the apex of the fruit are four small calyx teeth surrounding a short style (cf. clove fruit, Fig. 22.12). The pericarp is reddish-brown, rough and woody, and about 1 mm thick. Sections show numerous oil glands in the pericarp. Each of the two loculi contains a single plano-convex seed. Pimento has a characteristic aromatic odour and taste.

Pimento fruits yield about 3–4.5% of volatile oil which, estimated for eugenol by the method used for oil of cloves, shows a phenol content of 65–80%. The oil also contains cineole, (−)-phellandrene and caryophyllene; in all some 44 compounds have been identified.

CLOVE AND CLOVE OIL

Cloves are the dried flower buds of *Syzygium aromaticum* (*Eugenia caryophyllus*) (Myrtaceae), a tree 10–20 m high which is indigenous to the Molucca or Clove Islands. It is cultivated in Zanzibar and in the neighbouring island of Pemba, which together, for many years, produced more than three-quarters of the world's supply of cloves. However, the industry has deteriorated in these areas and the principal producers are now Madagascar, Indonesia and Brazil. Smaller quantities are grown in Sri Lanka and Tanzania.

History. Cloves were used in China as early as 266 BC and by the fourth century they were known in Europe, although very expensive. The Spice Islands were occupied by the Portuguese at the beginning of the sixteenth century, but they were expelled by the Dutch in 1605. As in the case of nutmegs, the Dutch made every effort to secure a monopoly, destroying all the trees in their native islands (Ternate, Tidor, Mortir, Makiyan and Bachian) and cultivating them only in a group of small islands, of which Amboyna is the largest. In 1770, however, the French succeeded in introducing clove trees into Mauritius, and cultivation was afterwards taken up in Sumatra (1803), Penang, Cayenne, Madagascar, Zanzibar (1818), Pemba and elsewhere.

Collection and preparation. The flower buds are collected when their lower part turns from green to crimson. In Zanzibar and Pemba collection takes place twice yearly, between August and December. The inflorescences are collected from movable platforms. The cloves are dried in the open air on mats and separated from their peduncles, the latter forming a separate article of commerce known as 'clove stalks' (Fig. 22.12D). If left too long on the tree, the buds open and the petals fall, leaving 'blown cloves'; later the fruits (Fig. 22.12C) known as 'mother cloves' are produced. A small proportion of these, usually at a stage intermediate between that of a clove and a fully ripe fruit, are frequently found in the drug. Cloves are imported in bales covered with matting made from strips of coconut leaves.

Macroscopical characters. Cloves are 10–17.5 mm long (cf. A and B in Fig. 22.12). The Penang and Amboyna varieties are the largest and plumpest and are most esteemed, but they are in such demand in the East that relatively small quantities of them reach Europe; they are used principally for the making of pomanders. The Zanzibar variety, however, is of good quality, although smaller and leaner than the Penang and of a blackish-brown rather than a reddish-brown colour.

The 'stalk' of the clove consists of a cylindrical hypanthium or swelling of the torus, above which is a bilocular ovary containing numerous ovules attached to axile placentae. The 'head' consists of four slightly projecting calyx teeth; four membranous, imbricated petals, and numerous incurved stamens around a large style (Fig. 22.12E).

Cloves have a strong, fragrant and spicy odour and a pungent, aromatic taste. When indented with the fingernail, they readily exude oil. Cloves sink in freshly boiled and cooled water (distinction from cloves which have been exhausted of volatile oil).

Microscopical characters. The hypanthium, in the region below the ovary, shows in transverse section (Fig. 22.12) a heavy cuticularized epidermis in which occur stomata, slightly raised above the surface and showing well-defined substomatal spaces. Within this is a zone of roughly radially arranged parenchymatous cells containing numerous schizolysigenous oil glands arranged in two or three more or less intermixed layers. The oil glands are ellipsoidal in shape, with the long axis radial, and show an epithelium composed of two or three layers of flattened cells. The contents of the oil glands are soluble in alcohol and are blackened by treatment with alcoholic ferric chloride or osmic acid. The ground mass of parenchyma also gives the blackening with ferric chloride. Cluster crystals of calcium oxalate (5–25 μm in diameter) occur in many of the parenchymatous cells. Within the oil gland layer is a zone of cells with somewhat thickened walls, embedding a ring of bicollateral vascular bundles. The ground tissue of this zone contains cluster crystals of calcium oxalate. The meristeles are enclosed in an incomplete ring of lignified fibres; the xylem is composed of 3–5 lignified spiral vessels. Within the ring of vascular bundles is a zone of aerenchyma composed of air spaces separated by lamellae one cell thick, which supports the central columella. The ground tissue of the columella is parenchymatous and is particularly rich in calcium oxalate clusters. In the outer region of the columella is a ring of some 17 small vascular bundles.

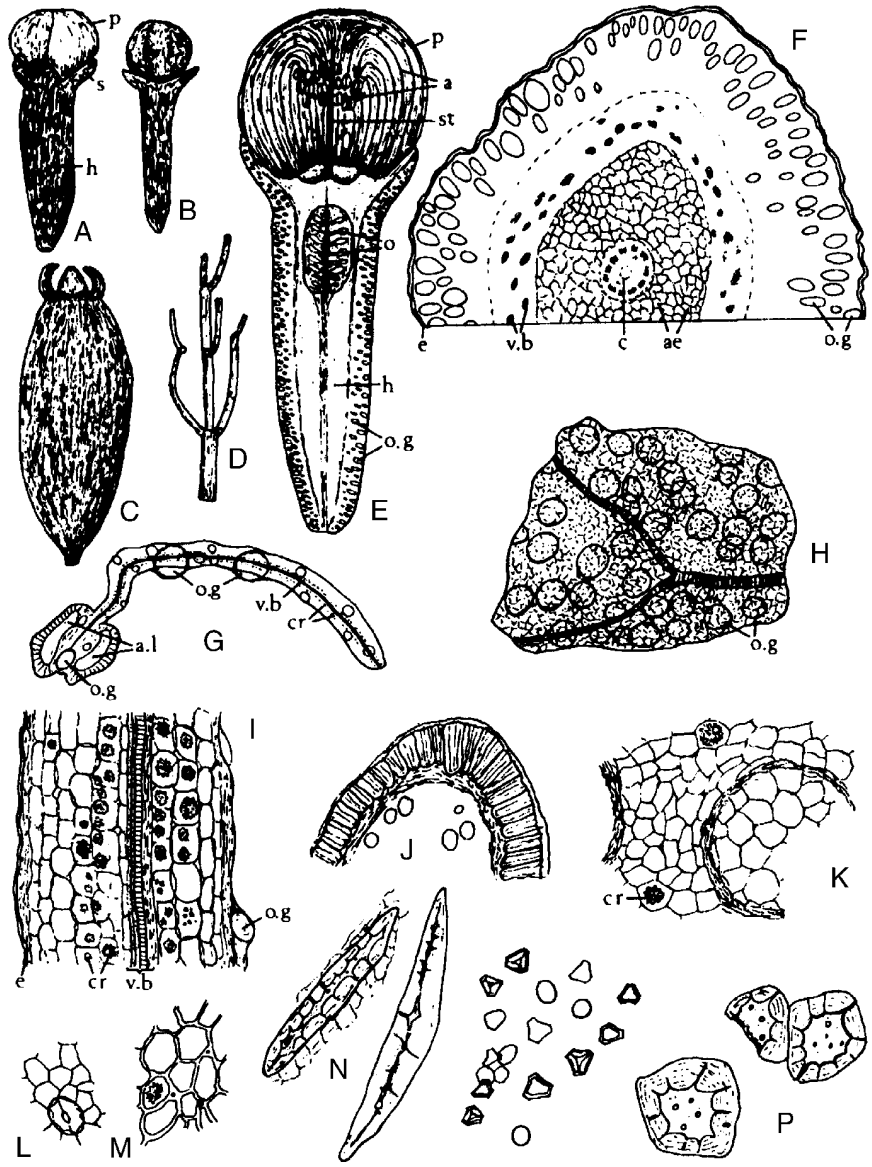


Fig. 22.12

Syzygium aromaticum. A, Penang clove; B, Zanzibar clove; C, fruit (mother clove), (all ×2); D, clove stalk (×1); E, clove cut longitudinally (×5); F, transverse section of hypanthium; G, portion of anther (both ×15); H, surface view of petal (×50); I–P, elements of powdered clove (all ×200); I, portion of anther filament; J, fibrous wall of anther lobe and immature pollen; K, fragment of hypanthium showing portions of oil glands; L, epidermal cells and stoma of hypanthium; M, parenchyma of hypanthium; N, phloem fibres; O, pollen grains; P, sclereids from clove stalk. a, Stamens; ae, aerenchyma; a.l, anther lobes; c, columella; cr, cluster crystal of calcium oxalate; e, epidermis; h, hypanthium; o, ovules; o.g, oil gland; p, imbricated petal; s, sepal; st, style; v.b, vascular bundle.

The hypanthium, in the region of the ovary, shows epidermis, oil gland layer and ring of bicollateral bundles. Within this is a zone of cells with very strongly thickened cellulose walls, limited internally by an inner epidermis forming the wall of the ovary. The dissepiment of the ovary is parenchymatous; the placentae are rich in cluster crystals and contain vascular bundles. If sections of the hypanthium are mounted in a concentrated solution of potassium hydroxide, acicular and radiately aggregate crystals separate, owing to the presence of the phenol eugenol in the oil.

The sepals and petals have a simplified leaf structure. The mesophyll parenchyma contains calcium oxalate and embeds numerous oil glands. The epidermis of the sepals shows stomata. The epidermis of the petals is devoid of stomata and is composed of very irregular cells.

The stamens are composed of filament, connective and anther. The filament shows an epidermis of longitudinal elongated cells, a ground mass of parenchyma embedding numerous oil glands and a single vascular strand enclosed in a sheath of crystal cells. The vascular strand is continuous into the connective, which is terminated by an oil gland. The fibrous layer of the anther wall is composed of cells showing spiral bands of lignified thickening. The pollen grains are triangular in outline and 15–20 µm diameter. The style and stigma yield similar characters to those of the filament. See Fig. 22.12 for the illustrated characters of the powder.

Starch, prisms of calcium oxalate and lignified sclereids are absent from a powder consisting of the flower buds only. Clove stalks contain lignified sclereids (Fig. 22.12P) and reticulately thickened xylem vessels. Clove fruits ('anthophylli', 'mother cloves') contain starch. As there is a permissible pharmacopoeial limit (not more than 6%) for these structures in the drug, a few sclereids and starch grains may therefore be found in the powder. There are also limits for deteriorated cloves (⩾2.0%) and other foreign matter (⩾0.5%).

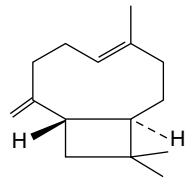
Constituents. Cloves contain about 14–21% of volatile oil (see below), 10–13% of tannin, various triterpene acids and esters, and glycosides of the following: aliphatic and monoterpenoid alcohols, eugenol, isoeugenol, farnesol, nerolidol, sitosterol, stigmasterol and campestrol.

Uses. Cloves are used as a stimulant aromatic, as a spice and for the preparation of the volatile oil. The sesquiterpenes of clove have been cited as potential anticarcinogenic compounds.

Clove oil

Oil distilled in Britain and the US usually requires no purification, but oil distilled abroad (e.g. in Madagascar) is, when imported, usually wet and discoloured by the presence of metallic salts. The latter type of oil is always rectified and may be sold with different eugenol contents. Oil of cloves is a colourless or pale yellow liquid, which is slightly heavier than water (relative density 1.047–1.060). It is soluble in from one to two volumes of alcohol (70%).

Clove oil contains 84–95% of phenols (eugenol with about 3% of acetyleugenol), sesquiterpenes (α- and β-caryophyllenes) and small quantities of esters, ketones and alcohols. Some 28 compounds have been reported in the oil and this rather low number, compared with many other oils, is due to the lack of monoterpene derivatives. The phenols can be estimated by absorption with solution of potassium hydroxide in a graduated flask, as described in the *BP* (1989). The current *BP*/*EP* employs GC using internal standards for the determination of eugenol (limits 75–88%), β-caryophyllene (5–14%), acetyl eugenol (4–15%) and other components. Those oils which have a relatively low phenol content are known in commerce as 'opt' and are the ones mainly used in pharmacy, while the 'strong' oils are used in the manufacture of vanillin.



β-Caryophyllene

Oil of clove, like other essential oils, should be stored in well-filled, airtight containers, protected from light and heat. It is used as a flavouring agent, stimulant, aromatic and antiseptic.

Clove stem oil is produced in Tanzania and in Madagascar; it is used mainly in the flavouring and perfumery industries. *Clove leaf oil* is distilled in Madagascar, Tanzania and in Indonesia, and is used for the isolation of eugenol.

EUCALYPTUS LEAF

Eucalyptus leaf of the *EP* and *BP* consists of whole or cut dried leaves of the older branches of *Eucalyptus globulus* Labill. Eucalyptus trees possess two kinds of leaves, those on young plants being cordate and sessile whereas those on mature trees which constitute the official drug are petiolate and scimitar-shaped. The dried leaves are greyish-brown in colour, coriaceous in texture and have lateral veins which anastomose near the margin. Secretory oil glands are visible in leaves held to the light. Microscopy shows epidermal cells with thick cuticle, anisocytic stomata, together with mesophyll having schizogenous oil glands and prisms and cluster crystals of calcium oxalate.

The leaves are required to contain not less than 2.0% v/w of essential oil and have limits of 3% for dark and brown leaves, 5% for stems and 2% for other foreign matter. Other significant components of the leaves are phloroglucinol-sesquiterpene coupled compounds named macrocarpals, which show antibacterial activity against oral pathogenic microorganisms and inhibition of glycosyltransferase activity. Such substances could have potential in the maintenance of oral hygiene. For further details see K. Osawa and H. Yasuda, *J. Nat. Prod*., 1996, **59**, 823.

EUCALYPTUS OIL

Oil of eucalytus is distilled from the fresh leaves of various species of *Eucalyptus* (Myrtaceae) and rectified. Eucalyptus oils are produced in Portugal, South Africa, Spain, China, Brazil, Australia, India and Paraguay.

Only a certain number of species produce oils suitable for medicinal use. The chief requirements are a high cineole content and the absence of appreciable quantities of phellandrene and aldehydes (for formulae see Fig. 22.2). Suitable oils are derived from *E. polybractea*, *E. smithii*, *E. globulus* and *E. australiana*. In the case of the latter species the oil used in pharmacy is that collected during the first hour of the distillation, that which passes over subsequently being used for mineral separation. 'Citron-scented' eucalyptus oil, which is derived from *E. citriodora*, is used in perfumery and contains a high proportion of the aldehyde citronellal.

Characters. Oil of eucalyptus is a colourless or pale yellow liquid. It has an aromatic and camphoraceous odour; a pungent, camphoraceous taste, which is followed by a sensation of cold. It is required to contain not less than 70.0% of cineole. 1,8-Cineole and *o*-cresol form a solid complex and the crystallizing temperature of this forms the basis for the official assay of the oil. The *Pharmacopoeia* also includes a TLC identification test, and tests which limit the content of aldehydes and phellandrene; these eliminate oils containing citronellal and the so-called industrial eucalyptus oils.

Uses. Eucalyptus oil is much used for alleviating the symptoms of nasopharyngeal infections, for treating coughs and as a decongestant. It is taken internally in the form of mixtures, inhalations, lozenges and pastilles and applied externally as ointments and liniments.

Further reading

Boland DJ, Brophy JJ, House APN (eds) 1991 Eucalyptus leaf oils: use, chemistry, distillation and marketing. ACIAR/CSIRO Inkata Press, Melbourne, Australia

GINGER

Ginger (*Zingiber*) is the scraped or unscraped rhizome of *Zingiber officinale* (Zingiberaceae). The *BP* drug is known in commerce as 'unbleached ginger'. *Z. officinale*, a reed-like plant, is grown in many parts of the world, including Jamaica, China, India and Africa. Jamaica ginger, once the traditional pharmaceutical ginger, has been largely replaced by other sources.

History. Ginger has been cultivated in India from the earliest times; the plant is unknown in the wild state. The spice was used by the Greeks and Romans, and was a common article of European commerce in the Middle Ages. It was well known in England in the eleventh century. Ginger was introduced into Jamaica and other West Indian islands by the Spaniards, and a considerable quantity of the drug was sent from the West Indies to Spain as early as 1547.

Cultivation and preparation. Ginger grows well at subtropical temperatures where the rainfall is at least 1.98 m per annum. As the plant is sterile, it is grown by vegetative means. Selected pieces of rhizome ('seed pieces' or 'setts') each bearing a bud are planted in

holes or trenches during March or April, preferably in a well-drained clayey loam. The procedures resemble potato cultivation. Mulching or manuring is necessary as the plant rapidly exhausts the soil of nutrients. When the stems wither, about December or January, the rhizomes are ready for collection. For the scraped drug, after removal of soil the rhizomes are killed by boiling water. They are then carefully peeled, thoroughly washed and then dried in the sun on mats or barbecues. During drying they are turned from time to time and protected during any damp weather. This first drying usually takes about 5 or 6 days. To obtain a whiter product the ginger is again moistened and dried for a further two days, when it is ready for export. It should not be limed.

With some gingers little or no cork is removed ('coated' or 'unscraped' gingers) and these grades are now also included in the *BP*/*EP*; these are sometimes whitened by dusting with calcium carbonate or lime but are eliminated by the *BP* ash value (not more than 6.0%).

Macroscopical characters. The dried scraped drug (Fig. 22.13) shows little resemblance to the fresh rhizome, owing to loss in weight and shrinkage. It occurs in sympodially branched pieces known as 'hands' or 'races'. These are 7–15 cm long, 1–1.5 cm broad and laterally compressed. The branches arise obliquely from the rhizome, are about 1–3 cm long and terminate in depressed scars or in undeveloped buds. The outer surface is buff-coloured and longitudinally striated or fibrous; it shows no sign of cork. The drug breaks with a short fracture, the fibres of the fibrovascular bundles often projecting from the broken surface. It has an agreeable aromatic odour and a pungent taste.

In transverse section a lens shows the cortex, a dark line (the pericycle and endodermis, the latter without starch) and the stele with numerous scattered fibrovascular bundles. Similar bundles also occur in the cortex. The bundles appear as greyish points, the smaller yellowish points which can also be seen being secretion cells.

The unscraped rhizome resembles the above in structure but is more or less covered by brownish layers of cork with conspicuous ridges; the cork readily exfoliates from the lateral surfaces but persists between the branches.

Microscopical characters. The unpeeled rhizome, in transverse section (Fig. 22.13C), shows a zone of cork tissue, differentiated into an outer zone of irregularly arranged cells produced by suberization of

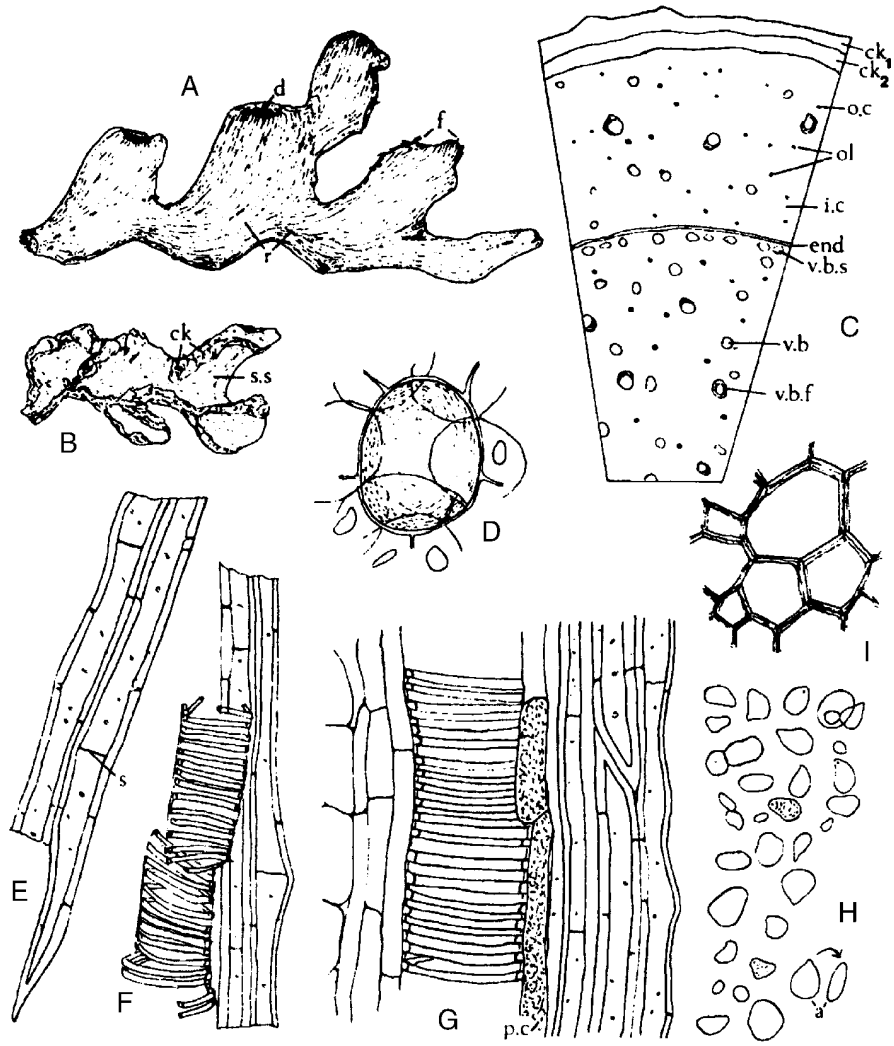


Fig. 22.13
Ginger. A, Peeled Jamaican rhizome; B, partially peeled African root (both ×0.75); C, diagrammatic transverse section of unpeeled rhizome (×15); D, oleoresin cell with adjacent parenchyma; E, portions of septate fibres; F, G, portions of septate fibres with attached vessels; H, starch; I, cork cells in surface view from unpeeled drug (all ×200). a, Starch granule, side- and end-aspects; ck, cork; ck_1, irregularly arranged cells of outer cork; ck_2, radially arranged cork cells; d, depressed scar; end, endodermis; f, projecting fibres; i.c, inner cortex; ol, oleoresin cells; o.c, flattened cells of outer cortex; p.c, pigment cell; r, ridges produced by vascular bundles; s, septum; s.s, scraped surface; v.b, vascular bundle; v.f.b, vascular bundle with fibrous sheath; v.b.s, ring of small vascular bundles.

the cortical cells without division and an inner zone of cells arranged in radial rows and produced by tangential division of the cortical cells. No cork cambium is differentiated. Within the cork is a broad cortex, differentiated into an outer zone of flattened parenchyma and an inner zone of normal parenchyma. The cortical cells contain abundant starch grains. These are almost entirely simple, ovoid or sack-shaped, are 5–15–30–60 μm long and have a markedly eccentric hilum (Fig. 22.13H). Scattered in the cortex are numerous oil cells, with suberized walls enclosing yellow–brown oleoresin. The inner cortical zone usually contains about three rings of collateral, closed vascular bundles. The larger bundles are enclosed in a sheath of septate, non-lignified fibres. Each vascular bundle contains phloem, showing well-marked sieve-tubes and a xylem composed of 1–14 vessels with annular, spiral or reticulate thickening. These vessels do not give a marked lignin reaction with phloroglucinol and hydrochloric acid. Axially elongated secretion cells with dark contents occasionally accompany the vessels. The inner limit of the cortex is marked by a single-layered endodermis free from starch. The outermost layer of the stele is marked by a single-layered pericycle. The vascular bundles of the stele resemble those of the cortex, and are, except for a ring of small bundles immediately within the pericycle, scattered as is typical of monocotyledonous stems. The ground mass of the stele is composed of parenchyma resembling the cortical parenchyma and containing much starch, and numerous oil cells. Cork cells are absent from the scraped drug. (For illustrations of the above features see Fig. 22.13D–H.)

Constituents. Ginger contains about 1–2% of volatile oil (*BP*/*EP* ≮1.5%); for the assay, liquid paraffin (10 drops) or other antifoaming agent may be added to the distillation flask. The rhizomes also contain 5–8% of resinous matter, starch and mucilage. Oil of ginger, to which the drug mainly owes its aroma, contains a mixture of over 50 constituents, consisting of monoterpenes (β-phellandrene, (+)-camphene, cineole, citral and borneol), sesquiterpene hydrocarbons (zingiberene, β-bisabolene, (*E*,*E*)-α-farnesene, β-sesquiphellandrene and *ar*-curcumene) and the sesquiterpene alcohol zingiberol. Over 50 volatile constituents of fresh organically grown ginger (fresh Chinese white and Japanese yellow varieties) have been recorded (S. D. Jolad *et al*., *Phytochemistry*, 2004, **65**, 1937).

The pungency of ginger is due to gingerol, an oily liquid consisting of homologous phenols. The principal one of these is [6]-gingerol (i.e. where $n = 4$). It is formed in the plant from phenylalanine, malonate and hexanoate (see Denniff *et al*., *J. Chem. Soc. Perkin*, *I*, 1980, 2637).

Smaller amounts of gingerols with other chain-lengths are also present. Similarly, [6]-gingerdiol is accompanied by four analogues which were isolated as minor components of the rhizome by Kikuzaki *et al*. from the less-polar fractions of the dichloromethane extracts (*Phytochemistry*, 1992, **31**, 1783). The same group also characterized (*Phytochemistry*, 1991, **30**, 3647; 1996, **43**, 273) a number of diaryl-heptanoids and a further seven have since been reported from Chinese *Z. officinalis* (J. Ma *et al*., *Phytochemistry*, 2004, **65**, 1137). These diarylheptanoids are similar to the curcuminoids present in greater quantity in turmeric (q.v.). A number of diarylheptanones – gingerenones A, B, C and isogingerenone B have been investigated by Endo and colleagues (*Phytochemistry*, 1990, **29**, 797). Other minor components are methylgingediol, gingediacetates, methylgingediacetates and a C_{20}-dialdehyde.

The pungency of gingerol is destroyed by boiling with 2% potassium hydroxide. Boiling with baryta water decomposes it with formation of a phenolic ketone called zingerone and aliphatic aldehydes (mainly normal heptaldehyde). Zingerone also occurs in the rhizome and, like gingerol, is pungent but possesses in addition a sweet odour. Its pungency is destroyed by prolonged contact with 5% sodium hydroxide. Shogaols, components of the oil, represent compounds formed by loss of water from the gingerols and were not thought to be present in the fresh rhizome. However, T.-S. Wu *et al*. (*Phytochemistry*, 1998, **48**, 1889) have now isolated three new dehydroshogaols from fresh roots purchased from a market in Taiwan. For formulae of ginger constituents see Figure 22.14.

Varieties. The plant which yields the official ginger is grown in many tropical countries, including India (Cochin, Calicut and Bengal), Africa (Nigeria, Sierra Leone), China, the East Indies, Cochin China, Australia and Florida. The chief varieties in English commerce are the Chinese, Nigerian, Cochin and African.

A number of commercial varieties of root, oleoresin and essential oil are available, seemingly derived from *Z. officinale*; whether these arise from different chemical races, from differences in cultivation and harvesting techniques or from different climatic conditions is not clear. They vary considerably in sensory characters. Australian oils are characterized by a 'lemon, citrus-like' odour. Oil from Fiji has a high citral content and a relatively high content of 1,8-cineole similar to Japanese oil.

Nigerian ginger. The best Nigerian closely resembles the Jamaica drug, but can be distinguished from it in the whole condition by its somewhat darker colour, its smaller size and that it is rather less deeply scraped. Nigerian ginger has a more pungent taste and rather less aroma than Jamaican. It yields less volatile oil (about 0.7–1%).

Cochin ginger. This is grown in southern India and is imported via Bombay or Madras. It occurs in both coated and scraped forms. The coated variety bears on the upper and lower surfaces a wrinkled reddish-grey cork which readily exfoliates. The lateral surfaces are without cork but are decidedly darker than the surface of the Jamaican drug. Pieces may be found of almost exactly the same size and shape as the Jamaican, but on the whole the pieces are smaller and the branches somewhat thicker. Cochin ginger is more starchy and breaks with a shorter fracture than the official; it is equally pungent but less agreeably aromatic. *Calicut ginger* closely resembles the Cochin, but the latter is usually regarded as the better grade.

Chinese ginger. This is produced in large quantity as various grades; it is sliced as opposed to split and the peeled drug is reported to be of Jamaican quality. It is often the principal variety available in the UK.

African ginger. This is typically smaller and darker than the Cochin. It is 'coated', a brown cork extending over a greater area than in the Cochin. The relatively small exposed portions of cortex on the lateral sides are grey to blackish in colour. It lacks the fine aroma of the Jamaica drug, although exceeding it in pungency. *Bombay ginger* resembles the African.

Allied drugs. *Japanese ginger* is derived from *Z. mioga*. The volatile oil which it contains differs in physical properties from that of the official species and gives the drug a bergamot-like odour. The taste is less pungent than that of *Z. officinale* and the starch grains are compound and less eccentric.

Preserved ginger consists of young undried rhizomes which are preserved by boiling in syrup. The West Indian variety is made from the official plant, but that from China is said to be obtained from the greater galangal, *Alpinia galanga* (Zingiberaceae).

Galangal rhizome, now little used in England although employed on the Continent, is derived from the lesser galangal, *A. officinarum*.

Adulteration. Most of the likely vegetable adulterants can be detected by a routine microscopical examination. Powdered ginger may have been prepared from 'wormy' drug, and so attention should be paid to the absence of insect fragments.

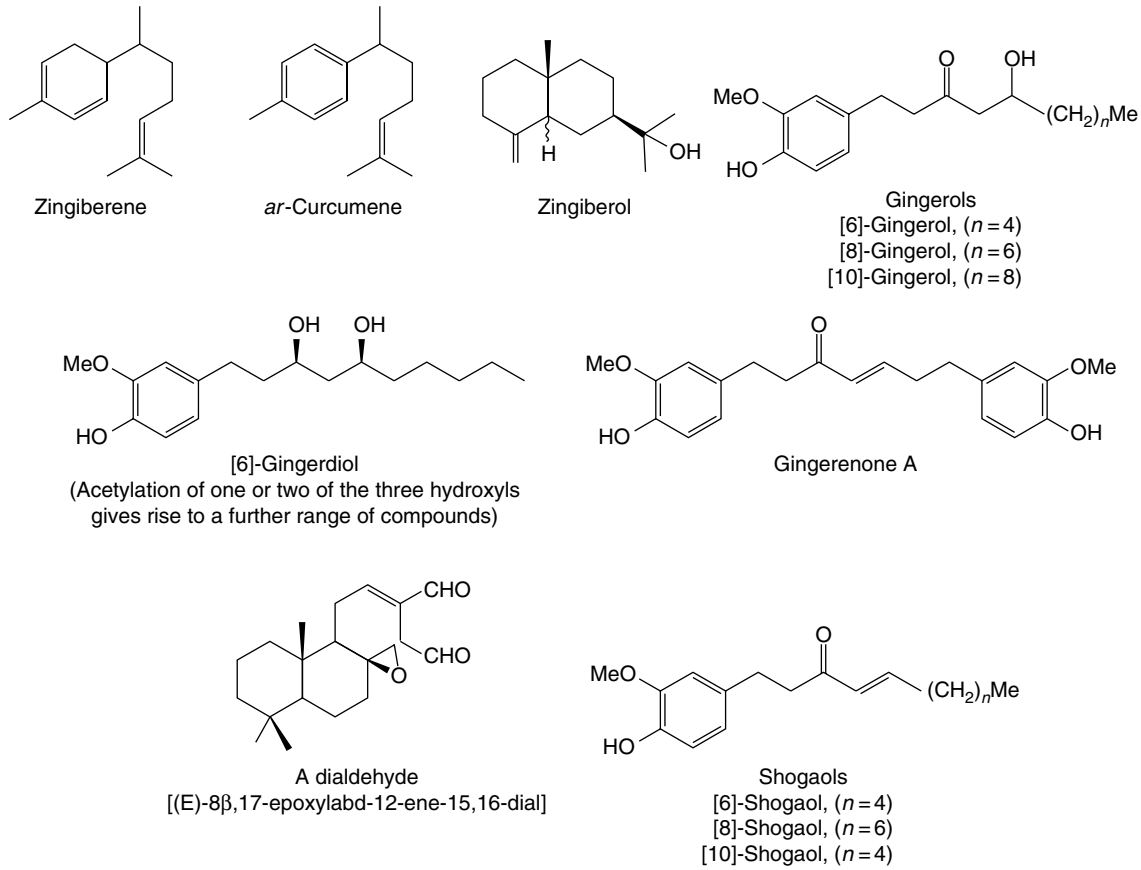


Fig. 22.14
Constituents of ginger.

Adulteration may also take the form of the addition of 'spent ginger' which has been exhausted in the preparation of essence. This may be detected by the official standards for alcohol-soluble extractive, water-soluble extractive, total ash and water-soluble ash.

Exhausted ginger and, more particularly, ginger galenicals may have their pungency increased by the addition of capsicum or grains of paradise. The suspected liquid, or a tincture prepared from the suspected powder, is heated in a water-bath with caustic alkali. The liquid is then evaporated, and the residue acidified with hydrochloric acid and shaken with ether. Some of the ethereal solution evaporated on a watch-glass gives a residue which is not markedly pungent to taste. This test depends on the fact that gingerol is more readily decomposed by alkalis than are capsaicin or paradol.

Uses and actions. Ginger is used as a carminative and stimulant. A US study by Mowrey and Clayson (*Lancet*, 1982, **1**, 655) indicated that powdered ginger may be a more effective antiemetic than dimenhydrinate (Dramamine). The authors suggested that it may ameliorate the effects of motion sickness in the gastrointestinal tract itself, in contrast to antihistamines, which act centrally. Other reports claim that ginger is effective in the control of excessive and uncontrolled vomiting occurring in the first trimester of pregnancy and that it might provide a cheap antiemetic adjunct to cancer therapy (S. S. Sharma *et al*., *J. Ethnopharmacology*, 1997, **57**, 93).

A considerable number of pharmacological studies involving the digestive, central nervous and cardiovascular systems have been reported for the isolated constituents of ginger. These activities include the potent inhibitory actions of the gingerols against prostaglandin synthetase which correspond with the anti-inflammatory and anti-platelet aggregation properties of the drug. These compounds, together with [6]-shogaol, also produce enhanced gastrointestinal activity with effects on bile secretion. The C_{20}-dial mentioned previously has a cholesterol-biosynthesis inhibitory activity in animal preparations and is assumed to be a HMG-CoA reductase inhibitor (M. Tanabe *et al*., *Chem. Pharm. Bull*., 1993, **41**, 710). The sesquiterpene hydrocarbons have also been associated with the antiulcer activity of the drug. A strong antibacterial and antifungal action has been demonstrated for a number of the rhizome constituents.

Further reading

Ravindran PN *et al* (eds), Hardman R (series ed) 2005 Medicinal and aromatic plants – industrial profiles, Vol 41. Ginger: the genus *Zingiber*. CRC Press, Boca Raton, FL. 520 pp.

Turmeric

Turmeric (*Curcuma*) is the dried rhizome of *Curcuma longa* (Zingiberaceae), cultivated in India, West Pakistan, China and Malaya. It contains constituents similar to those of ginger and is described in Chapter 29 under 'Antihepatotoxic Drugs'.

For a review of the principal pharmacological activities of turmeric (anti-inflammatory, hepatoprotective, antimicrobial, wound healing, anticancer, antitumour, antiviral) see R. C. Srimal, *Fitoterapia*, 1997, **68**, 483.

A radioprotective effect ascribed to free radical scavenging and electron/hydrogen donation has been demonstrated in mice (D. Choudhary *et al*., *J. Ethnopharmacology*, 1999, **64**, 1) and an assay-guided fractionation of an ethanolic extract has furnished three DPPH free-radical scavenging diaryheptanoids: curcumin, demethoxycurcumin and bisdemethoxycurcumin (DPPH = 1,1-diphenyl-2-picrylhydrazyl). See E. K. Song *et al.*, *Planta Med.*, 2001, **67**, 876.

Further reading

Ravindran PN *et al* (eds) Hardman R (series ed) 2007 Medicinal and aromatic plants – industrial profiles, Vol 45, Turmeric: the genus *Curcuma*. CRC Press, Taylor and Francis Group, Boca Raton, FL

CARDAMOM FRUIT AND CARDAMOM OIL

Cardamom consists of the dried, nearly ripe fruits of *Elettaria cardamomum* Maton var. *minuscula* Burkill (Zingiberaceae). The seeds, the part used medicinally and as a spice, are directed to be kept in the fruits until required for use. This prevents loss of volatile oil and helps one to distinguish the fruits from those of *E. cardamomum* var. *major* (unofficial long wild native cardamoms) and from the fruits of other genera of the same family. However, to cut costs on transport much seed is now imported in sealed tins. Cardamom is expensive, its price among other common spices being exceeded only by those of saffron and vanilla.

Principal producers are Sri Lanka, southern India and Guatemala.

History. Cardamoms are mentioned in the early Sanskrit writings of Susruta, but it is difficult to say with any certainty when they first appeared in Europe. Immense quantities are still used in Hindu festivals. Both *Amomum* and *Cardamomum* appear in a list of Indian spices liable to duty at Alexandria, about AD 176–180. The Portuguese navigator Barbosa (1514) appears to have been the first to mention the source of our official drug as the Malabar coast.

Production, collection and preparation. Although wild plants are found in India and Sri Lanka, cardamoms are mainly obtained from cultivated plants. Propagation is by seedlings or vegetatively but the latter gives problems owing to possible infection by mosaic or katte virus. The plant is reed-like, 4 m or more high, and bears long leaves arising from the rhizome. As the capsular fruits on the same raceme ripen at different times and it is important to collect them when nearly ripe and before they split to shed their seeds, it is best to cut off each fruit at the correct stage with a pair of short-bladed scissors. Pickers can, by this method, collect about 5 kg of fruit per day, although collecting all the fruits on one raceme together is naturally quicker. In Sri Lanka and India flowering and fruiting continues for practically the whole year but most of the crop is collected from October to December.

The fruits are dried slowly, either outdoors or in a curing house. Too-rapid drying is to be avoided, as it causes the fruits to split and shed their seeds. Sometimes the capsules are remoistened and further exposed to the sun but this sun-bleaching, although improving the appearance, also increases the number of split fruits. Bleaching may also be done by placing trays of the fruit over burning sulphur. Bleached fruits appear to have become less common and there is now an increased proportion of the unbleached Alleppy and Ceylon greens. The green curing procedure is also used in Guatemala and it has been claimed that enhanced colour retention is obtained by soaking the fruits for 10 min in 2% sodium carbonate solution before drying.

The capsules have the remains of the calyx at the apex and a stalk at the base. These may be removed either by hand-clipping or by machines. The fruits are then graded by means of sieves into 'longs', 'mediums', 'shorts' and 'tiny'. If they have been sulphur-bleached, they are aired in the open before being packed for export.

Macroscopical characters. The cardamom fruit is an inferior, ovoid or oblong capsule, about 1–2 cm long. The size, shape and surface vary in the different commercial varieties and grades (see below). The apex is shortly beaked and may show floral remains, while the base is rounded and shows the remains of the stalk. Internally the capsule is three-celled, a double row of seeds attached to axial placentas occurring in each cell. In good samples the seeds form about 70% of the total weight. The seeds in each loculus are tightly pressed together and usually separate in a single mass.

Each seed is about 4 mm long and 3 mm broad and somewhat angular. The colour varies from a dark reddish-brown in fully ripe seeds to a much paler colour in the unripe ones. The testa is transversely wrinkled and is covered by a membranous aril. A groove on one side of the seed indicates the position of the raphe and a depression at one end of the hilum. Cardamom seeds have a strongly aromatic odour and a pleasantly aromatic, although somewhat pungent, taste.

Seeds cut longitudinally and transversely and stained with iodine show the aril, testa, perisperm (containing starch) and the endosperm and embryo (both free from starch), as illustrated in Fig. 22.15.

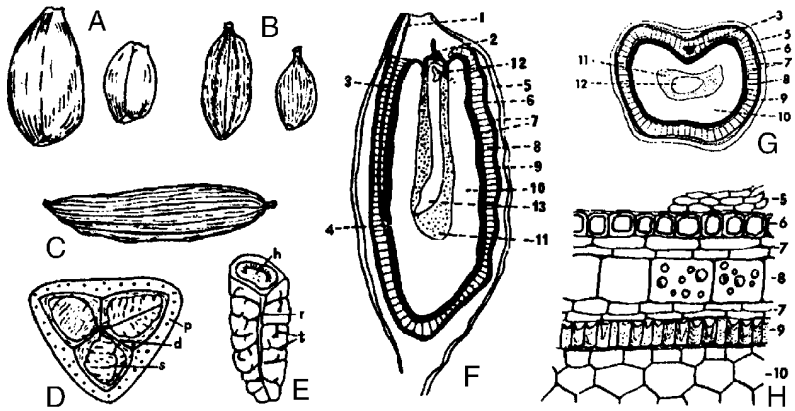


Fig. 22.15
Cardamom fruits and seeds. A, Mysore; B, Alleppy green; C, long, wild native (all × 1); D, transverse section of fruit (×1.5); E, whole seed (about × 4); F, longitudinal section of seed; G, transverse section of seed; H, arrangement of cells in transverse section of seed coat. d, Dissepiment of fruit; p, pericarp; r, raphe; s, seed; t, wrinkled testa; 1, funicle; 2, operculum or embryonic cap; 3, raphe; 4, chalaza; 5, arillus; 6, epidermis of testa; 7, parenchyma layers of the testa; 8, oil cell layer; 9, sclerenchymatous layer of testa; 10, perisperm; 11, endosperm; 12, embryo; 13, haustorium.

Varieties. *Mysore* fruits have a cream or pale buff colour and a nearly smooth surface. *Malabar* are usually smaller and have a rather darker and less smooth pericarp. *Mangalore* resemble the Malabar but are usually more globular and have a rougher pericarp; they occur both bleached and semi-bleached. *Alleppy* fruits are narrower than the above varieties, have a markedly striated pericarp and vary in colour from greenish-buff to green. *Ceylon greens* resemble Alleppy, but are generally greener and more elongated. The seeds of the above varieties are almost indistinguishable from one another, and also from the seeds of the *long wild native* cardamom (see below under 'Allied drugs'.)

Microscopical characters. Sections of the seed (Fig. 22.15) show a very thin membranous arillus, enveloping the seed and composed of several layers of collapsed cells, yellow in colour and containing oil. The brownish testa is composed of the following layers. (1) An outer epidermis consisting of a single layer of cells rectangular in transverse section, longitudinally elongated and with prosenchymatous end walls in surface view; light yellow in colour and having slightly thick end walls. (2) A single or double layer of parenchymatous cells, elongated at right angles to the long axis of the overlying epidermal cells (see Fig. 22.15H). (3) A single layer of large parenchymatous cells containing volatile oil; in the region of the raphe there are two layers of oil cells separated by the raphe meristele. (4) Several layers of small flattened parenchymatous cells, their structure often partially obliterated. (5) An inner epidermis of sclerenchymatous cells, radially elongated, with anticlinal and inner walls very strongly thickened and reddish-brown in colour. Lumen bowl-shaped and containing a module of silica (see Fig. 22.15H). The operculum or embryonic cap is composed of two or three layers of these sclerenchymatous cells, continuous with those of the inner epidermis. The micropyle is a narrow canal passing through the operculum. Within the testa is a well-developed perisperm composed of parenchymatous cells packed with minute globular starch grains, 4 μm diameter and containing in the centre of each cell a small prismatic crystal of calcium oxalate. The perisperm encloses the endosperm and embryo, both composed of thin-walled cells rich in protein.

Cardamom pericarps or husks which have been used for the adulteration of powdered drugs may be identified in the form of powder by the pitted fibres and spiral vessels of the fibrovascular bundles and by the abundant, empty parenchymatous cells.

Constituents. Samples of cardamom seed yield 2.8–6.2% (*BP* not less than 4.0%) of volatile oil and also contain abundant starch (up to 50.0%), fixed oil (1–10%) and calcium oxalate.

Cardamom oil. The oil is distilled in relatively limited quantities in Sri Lanka, India and Guatemala with an estimated global production of some 4 tonnes in 1984. The oil contains a high proportion of terpinyl acetate and cineole and smaller quantities of other monoterpenes, including alcohols and esters. Over 40 compounds have been identified in the oils of *Elettaria* species. The *BP* requires an ester value of 90–156 and an optical rotation of +20° to +40°. The loss of oil from seeds kept in the pericarp is small but a loss of 30% in 8 months takes place when the seeds are separated from the fruits. Gas chromatography has shown oils from different varieties of cardamom to have qualitatively the same composition, but variations in the proportions of individual components are evident.

Allied drugs. *The long wild native cardamoms* of Sri Lanka (Fig. 22.15C) are derived from *E. cardamomum* var. *major* Thwaites. They are much more elongated than the official variety, sometimes attaining a length of about 4 cm. The pericarps are dark brown and coarsely striated. The oil distilled from them is used in liqueurs.

'*Amomum*', of the *Indian Pharmaceutical Codex*, consists of the ripe or nearly ripe seeds of *Amomum aromaticum* or *A. subulatum*. The former, obtained from Bengal and Assam, is known as Bengal Cardamom; the latter, obtained from Nepal, Bengal, Sikkim and Assam, as Nepal or Greater Cardamom.

No other similar drugs, unless we include grains of paradise (see below), are imported in any quantity or with any regularity; the following is a list of allied species the seeds of which somewhat resemble those of the true cardamom: *A. cardamomum*, the round or cluster cardamom of Siam and Java; *A. xanthioides*, the bastard or wild Siamese cardamom; *A. maximum*, a Javanese plant; *Aframomum korarima*, the Korarima or Abyssinian cardamom; *A. mala*, the East African cardamom; *A. hanburii* and *A. daniellii*, Cameroon cardamoms; *A. angustifolium*, Madagascar cardamom; *Costus speciosus*, Chinese cardamom. The antiplasmodial activity of *A. zambesiacum* seeds has been investigated and five new labdane diterpenoids and five known ones isolated (M. Kenmogne *et al.*, *Phytochemistry*, 2006, **67**, 433).

Uses. The principal uses of cardamom are as a flavouring agent in curries and cake. Large quantities are used in Scandinavia and Germany and, with a large proportion of Asiatics in the population, consumption has increased in Britain. Some is used in the manufacture of liqueurs and a relatively small amount in pharmacy, chiefly in the form of Compound Tincture of Cardamom.

Biological activities demonstrated for cardamom include antimicrobial, anti-inflammatory, analgesic, antispasmodic and, recently, gastroprotective (A. Jamal *et al.*, *J. Ethnopharmacol.*, 2006, **103**, 149).

Grains of paradise

This spice, also known as Guinea grains or melegueta pepper, has been an article of commerce from very early times. It consists of the seeds of the West African reed-like herb *Aframomum melegueta* (Zingiberaceae), which has many of the characters of cardamom (q.v.).

The seeds are hard, reddish-brown, about 3 mm long and of a flattened pyramidal shape. The testa is papillose. Internally the structure resembles that of a cardamom seed. They have an aromatic odour and a pungent taste. The aroma is due to about 0.5% of volatile oil which contains principally β-caryophyllene, α-humulene and their epoxides. The pungency arises from paradol, a substance related to gingerol, and from small quantities of shogaol and gingerol. For the detection of paradol in ginger galenicals, see 'Ginger'. The essential oils from the seeds of *A. melegueta* and other *Aframomum* spp. from the Cameroon have been analysed by GC-MS (C. Menut *et al.*, *Flavour Fragrance J.*, 1991, **6**, 183). The seeds are used in alcoholic liquors and to some extent in veterinary medicine. Excessive consumption of the seeds can lead to ocular toxicity (S. A. Igwe *et al.*, *J. Ethnopharmacology*, 1999, **65**, 203).

CHAMOMILE FLOWERS

Roman Chamomile Flowers are the expanded flower-heads of *Chamaemelum nobile* (L.) All (*Anthemis nobilis* L.) (Compositae), collected from cultivated plants and dried. Chamomiles are cultivated in the south of England and in Belgium, France, Germany, Hungary, Poland, former Yugoslavia, Bulgaria, Egypt and Argentina. As a result of long cultivation most of the tubular florets present in the wild plant have become ligulate, and it is these 'double' or 'semi-double' flower-heads which form the commercial drug. They are included in the *BP*/*EP*.

History. Owing to the large number of similar composite plants, it has proved impossible to trace the drug in classical writings. The double variety was certainly known in the eighteenth century.

Collection. The flowers are collected in dry weather and carefully dried. The crop is often damaged by wet weather and the discoloured flowers then obtained fetch a much lower price than those having a good colour.

Characters. Each dried flower-head (Fig. 22.16A) is hemispherical and about 12–20 mm in diameter (the *BP* imposes a 3% limit on small or blemished heads). The florets are of a white to pale buff colour, the outer ones hiding the involucre of bracts. A few hermaphrodite, tubular florets are usually found near the apex of the solid receptacle (see Fig. 22.16B). A transition between the typical tubular florets and typical ligulate ones is often seen. The ligulate florets show three teeth (or occasionally two), the centre one being most developed. There are four principal veins. The corolla is contracted near its base into a tube from which a bifid style projects. The ovary is inferior and devoid of pappus. Each floret arises in the axil of a thin membranous bract or palea which has a blunt apex. At the base of the receptacle is an involucre consisting of two or three rows of oblong bracts which have membranous margins.

Chamomiles have a strong, aromatic odour and a bitter taste. The *BP*/*EP* includes a TLC test for identity and requires the drug to contain not less than 0.7% of volatile oil and not more than 10.0% water.

Constituents. Chamomiles contain 0.4–1.0% of volatile oil which is blue when freshly distilled owing to the presence of azulene. Other components of the oil are *n*-butyl angelate (principal), isoamyl angelate, 3-phenylpropyl isobutyrate, tridecanal, pentadecanal and terpenes. Chamomiles also contain sesquiterpene lactones of the germacranolide type, hydroperoxides, dihydroxycinnamic acid and apigenin (a trihydroxy flavone) and luteolin both free and as glucosides. (For the isolation of other constituents see A. Carnat *et al.*, *Fitoterapia*, 2004, **75**, 32.

Uses. Considerable quantities of chamomiles are used in domestic medicine in the form of an infusion (for dyspepsia, etc.) or poultice or in shampoo powders. For the production of volatile oil, the entire aerial parts are usually used.

Further reading

Franke R and Schilcher H (eds), Hardman R (series ed) 2005 Medicinal and aromatic plants Vol 42. Chamomile: industrial profiles. CRC Press, Boca Raton, FL. 288 pp.

MATRICARIA FLOWERS

Matricaria flowers (German or Hungarian chamomile flowers) are the dried flower-heads of *Matricaria recutita* L. (*Chamomilla recutita* (L.) Rausch.) (Compositae). The plant is a native to and is cultivated in southern and eastern Europe; Argentina and Egypt are also producers. It is official in the *EP* and described in the *BP*.

The capitulum when spread out, is 10–17 mm in diameter and consists of a receptacle, an involucre, 12–20 marginal ligulate florets and numerous central tubular florets. Unlike chamomile flowers, matricaria possesses a hollow receptacle which is devoid of paleae (see Fig. 22.16C–E). Broken flowers are limited to 25%. The drug has a pleasant aromatic odour.

Constituents. The flower-heads are required to contain not less than 0.4% of a blue volatile oil; this consists mainly of the sesquiterpenes α-bisabolol, chamazulene and farnesene. Chamazulene itself does not occur in the plant but is formed from a sesquiterpene lactone (matricin) during steam distillation.

Flavones and coumarins (e.g. herniarin) are present and the dried ligulate florets contain 7–9% of apigenin glucosides (the 7-glucoside and a mixture of acetates as determined by ^{13}C-NMR analysis) and 0.3–0.5% free apigenin, which may arise by post-harvest hydrolysis of the glucosides. In this respect, V. Švehlíková *et al.* (*Phytochemistry*, 2004, **65**, 2323) have studied the isolation, identification and stability of apigenin-7-*O*-glucoside in the white florets.

A number of chemotypes depending on the proportions of bisabolol, bisabolol oxides and farnesene in the oil have been described. Most Turkish varieties of *M. chamomilla* yield yellow oils containing no chamazulene; 2*n* and 4*n* races have been studied for their respective coumarin variations (A. Pastirová *et al.*, *Pharm. Biol.*, 2005, **43**, 205). For an article listing the many known constituents of matricaria flowers see A. Ahmad and L. N. Nisra, *Int. J. Pharmacognosy*, 1997, **35**, 121.

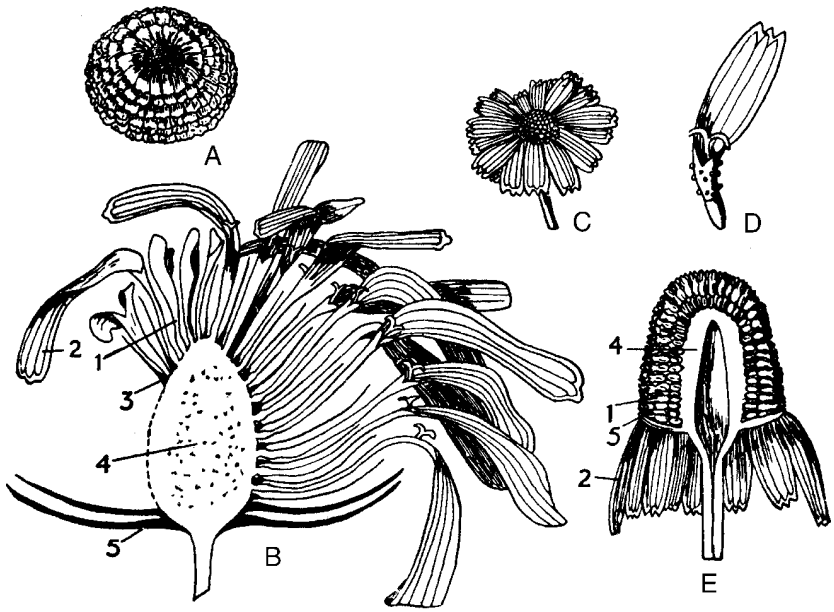


Fig. 22.16
A, Cultivated Roman chamomile; B, the same cut longitudinally; C, German chamomile; D, a ligulate floret of same; E, German chamomile cut longitudinally. 1, Tubular floret; 2, ligulate floret; 3, palea; 4, receptacle; 5, bract of involucre. (B after Greenish, remainder after Gilg.)

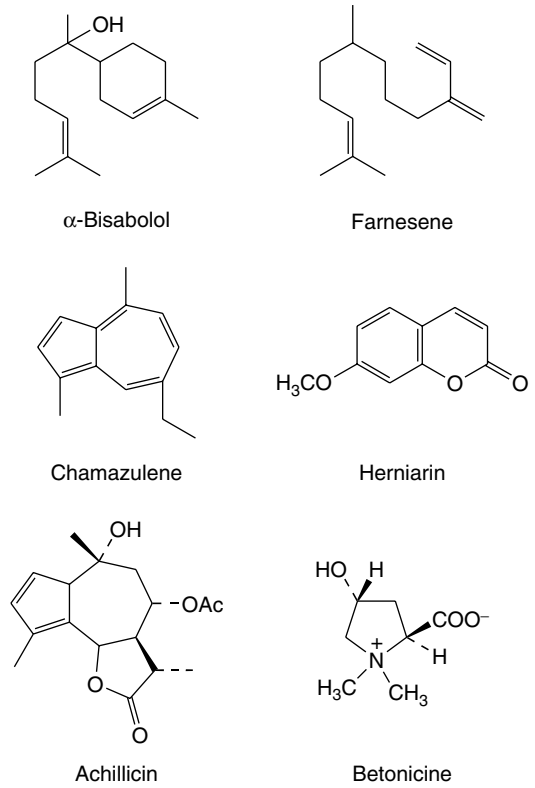


Fig. 22.17
Constituents of matricaria flowers.

Uses. Matricaria flowers are mainly used on the Continent of Europe and in the USA for their anti-inflammatory and spasmolytic properties. The ulcer-protective properties of German chamomile have been ascribed to bisabolol-type constituents, on which considerable pharmacological work has been reported. Four optically active isomers of bisabolol are possible; extracts for pharmaceutical use should be prepared only from clearly defined types containing the active constituents.

Allied drug. *Tanacetum parthenium* (L.) Schultz-Bip.; *Chrysanthemum parthenium* (L.) Bernh., or *feverfew flowers* may be single or double. The receptacle is flatter than that of the Roman chamomile and may or may not bear paleae. If the latter are present, they are acute and less membranous than those of the chamomile. The whole flowering tops are usually sold. Feverfew herb yields 0.07–0.4% of volatile oil. It is used in herbal medicine; for details concerning its content of sesquiterpene lactones see Chapter 24.

MATRICARIA OIL

Matricaria oil is that steam-distilled from the fresh or dried flower heads or flowering tops of *Matricaria recutita* L. Resulting from the chemotypes mentioned above two types of oil are described in the pharmacopoeia—one rich in bisabolol oxides and the other rich in (−)-α-bisabolol. These compounds, together with chamazulene, are determined by gas chromatography.

These oils are blue in colour and have a characteristic odour.

YARROW

Yarrow (millefolium, milfoil) is described in the *BP*/*EP*, *BHP* and a number of continental pharmacopoeias. It is also the subject of German Commission E and ESCOP monographs. The drug consists of the dried flowering tops of *Achillea millefolium* L. (Compositae), an extremely diverse aggregate species with varying chromosome numbers and differences in oil composition. The *British Herbal Compendium*, Vol. 1, 1992 points out that work reported under '*Achilleum millefolium*' may refer to *A. millefolium* sensu stricto or any number of other species which have been more recently and narrowly defined.

Yarrow is native to Europe and Western Asia but is now widespread in most temperate regions including N. America; commercial supplies come largely from south-eastern Europe, although it is also collected in other European countries including the UK.

Characters. The flowers occur in characteristically dense terminal corymbs about 3–5 cm in diameter and composed of capitula 3–5 cm in diameter. Each capitulum possesses an involucre of bracts, usually with five white to reddish ligulate ray florets, and 3–20 tubular disk florets. The fruits are achenes. The powdered material contains numerous elements, not only from the flower but also from stems and leaves. These include the typical Compositae pollen grains, leaf epidermis with anomocytic stomata and glandular and clothing trichomes again typical of the Compositae. Fuller details are given in the *BP* and *EP*.

Constituents. The pharmacopoeia requires an essential oil content of not less than 0.2% and not less than 0.002% of proazulenes calculated as chamazulene (Fig. 22.17). The tetraploid form of the plant (*A. millifolium* L., ssp. *collina* Becker) appears the most suitable as it produces considerable chamazulene as a component of the oil whereas the widespread hexaploid species (*A. millefolium* L., ssp. *millefolium*) lacks this guaianolide sesquiterpene. Germacranolide- and eudesmanolide-type sesquiterpenes are also constituents of the oil together with caryophyllene, sabinene, α- and β-pinene, borneol, bornyl acetate, camphor and small quantities of thujone. The proazulenes are determined by measurement of the absorbance (608 nm) of the oil (in xylene) obtained by distillation from the herb.

Other isolates from yarrow include sesquiterpene lactones (achillin, achillicin, etc.), flavonoids (apigenin, luteolin, quercetin and their 7-*O*-glycosides), alkaloids (betonicine, stachydrine, trigonelline) and various acetylenes, coumarins, triterpenes, sterols and plant acids.

V. K. Agnihotri *et al.* (*Planta Med.*, 2005, **71**, 280) studied plants from two different high-altitude (1600 m) populations propagated under uniform environmental conditions at a lower altitude (300 m); the populations represented two ecotypes, a 1:8-cineole type and a borneol type, which differed in oil content and in composition of mono- and sesqui-terpenes.

Uses. Yarrow is used, as is chamomile and matricaria, to treat various skin conditions and digestive disorders. Its pharmacological actions can arise from various groups of compounds—anti-inflammatory (chamazulene and prochamazulenes, apigenin, salicyclic acid), haemostatic (betonicine), spasmolytic (flavonoids).

Further reading

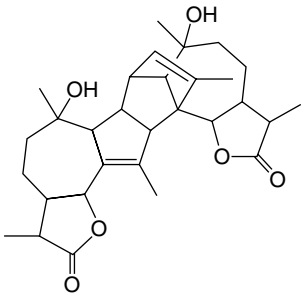
Chandler RF, Hooper SN, Harvey HJ 1982 Ethnobotany and phytochemistry of yarrow, *Achillea millefolium*, Compositae. Economic Botany 36: 203–223

Hofmann L, Fritz D, Nitz S, Kollmannberger H, Drawert F 1992 Essential oil composition of three polyploids in the *Achillea millefolium* 'complex'. Phytochemistry 31: 537–542

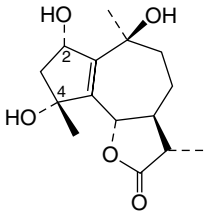
WORMWOOD

Wormwood is essentially the dried leaves and flowering tops of *Artemisia absinthium* L., Compositae, widely distributed in Europe

and the New World and recorded as a household remedy from biblical times. It is now included in the *EP*, *BP*, *BHP* 1983 and a number of European pharmacopoeias. There are official requirements for its volatile oil content and bitterness. The principal producers are the former USSR, Bulgaria, former Yugoslavia, Hungary and Poland; it is also cultivated in the USA and elsewhere.



Absinthin



Artabsinolide C
Artabsinolide A = 2-ketone
Artabsinolide B = 4-epimer, 2-ketone

The plant is a subshrub with deeply dissected leaves. The insignificant globose flowers form loose panicles and consist mainly of tubular florets and a few yellow ray florets. The leaves and grooved stems are covered with silky hairs.

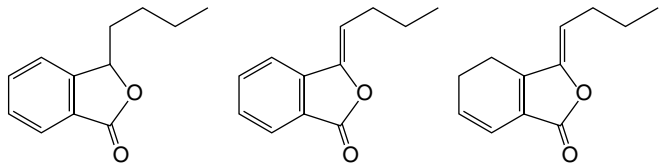
Characteristic features of the microscopy are the T-shaped trichomes (see Fig. 42.3I) on both leaf epidermi; these have uniseriate stalks of up to three cells and long tapering unicellular heads. There are numerous unicellular long, twisted trichomes and secretory trichomes with biseriate two-celled stalks and heads of two to four cells. The stomata are of the anomyocytic type. Numerous spherical pollen grains, 30 µm in diameter with three pores and a spiny exine, are seen in the powdered drug.

The drug has an aromatic odour and is intensely bitter. The active constituents are the bitter substances and essential oil. Bitter substances (0.15–0.4%) consist of sesquiterpene lactones, principally the dimeric guaianolide absinthin (0.20–0.28%), artabsin, artabsinolides A, B, and C and others. They are evaluated in the *BP* by the organoleptic test for 'bitterness value' using a quinine hydrochloride solution for comparison. The essential oil (*BP* requirement not less than 0.2%) is variable in composition according to geographical source and chemotype with any one of *p*-thujone, *trans*-sabinyl acetate, *cis*-epoxyocimene and chrysanthenyl acetate forming over 40% of the mixture; also present are other sesquiterpenes and monoterpenes.

Over the years many medicinal properties have been ascribed to wormwood. It is considered of value for promoting the appetite, for its strengthening effect in the treatment of colds and influenza, for gall bladder and menstrual problems and for the expulsion of round worms. Thujone is toxic, making the cultivation of low-thujune chemotypes desirable. The herb is also used in the making of liqueurs.

LOVAGE

Lovage is the whole or cut dried rhizome and root of *Levisticum officinale* (*Ligusticum levisticum*), family Umbelliferae. The official drug should contain not less than 0.4% essential oil for the whole drug and not less than 0.3% for that in the cut condition calculated with reference to the anhydrous drug.



3-Butylphthalide Ligusticum lactone Ligustilide

The plant is native to southern Europe, western Asia and the Orient but has for a long time been cultivated elsewhere; it is produced commercially in the Balkans, Germany, Holland, Poland and the USA.

In habit, lovage is a tall, aromatic perennial herb with bipinnate, cauline leaves coarsely toothed at the apex and greenish-yellow flowers. The rhizomes and roots are obtained from plants 2 to 3 years old and when split, cut and dried are in pieces up to 5 cm in diameter for the rhizomes and up to 25 cm in length for the roots. Externally the drug is greyish-brown in colour and longitudinally furrowed; a transverse section of the roots shows a thick yellowish-white bark separated from a brownish-yellow radiate wood by a dark line. Oil-containing structures are visible in the outer regions of the transverse section. Microscopic characters of the powdered drug include polygonal or rounded cork cells as seen in surface view, considerable parenchyma, reticulately thickened vessels, fragments of secretory cells and single and compound starch granules.

The drug contains up to 1.0% of volatile oil, the characteristic odoriferous components being alkyl phthalides of which 3-butylphthalide (*c.* 32%), ligustilide (*c.* 24%) and ligusticum lactone are principal components. Terpenes include α- and β-pinene, α- and β-phellandrene, α- and β-terpinene, camphene, myrcene, etc. Other constituents are various coumarins and plant acids. The *BP* includes a TLC examination as a test for identity and for the absence of angelica root.

Lovage has been used for centuries as a herbal remedy. It has carminative, diuretic and antimicrobial properties making it useful for the treatment of dyspepsia, cystitis and as a mouthwash for tonsillitis. Herbalists usually prescribe it in admixture with other drugs.

Tansy

Tansy (*Tanacetum vulgare* (L.); *Chrysanthemum vulgare* (L.) Bernh.) (Compositae) is used as an anthelminthic in herbal medicine but its poisonous properties are well appreciated. The herb contains about 0.2–0.6% volatile oil containing around 70% of thujone. Many sesquiterpene lactones have been isolated from the flowers and herb together with flavones. Numerous chemical races, originating from different geographical areas, are known and involve both the oil constituents and the sesquiterpenes. (For a series of reports involving three other species of *Tanacetum* see O. O. Thomas, *Fitoterapia*, 1989, **60**, 138, 231, 329 and references cited therein. With regard to the anti-inflammatory properties of the herb, C. A. Williams *et al*. (*Phytochemistry*, 1999, **51**, 417) have compared the flavonoids of *T. vulgare* and feverfew, and revised some flavonoid formulae.)

Sandalwood oil

Sandalwood oil is obtained from the heartwood of *Santalum album* (Santalaceae), an evergreen tree 8–12 m in height which is widely distributed in India and the Malay Archipelago.

Supplies are mainly derived from Indonesia and southern India where the trees are systematically cultivated and the cutting is controlled. The volatile oil is contained in all the elements of the wood, medullary ray cells, vessels, wood fibres and wood parenchyma. The oil contains about 90–97% of sesquiterpene alcohols, distinguished for purpose of analysis as 'santalol'. This consists of α-santalol (b.p. 300–301°C) and β-santalol (b.p. 170–171°C). The hydrocarbon fraction contains about nine components.

C. G. Jones *et al.* (*Phytochemistry*, 2006, **67**, 2463) have discussed the biosynthesis of sandalwood oil sesquiterpenes chemotaxonomically with respect to the co-occurrence patterns of the four types studied: (1) α- and β-santalenes and bergamotene, (2) γ- and β-curcumene, (3) β-bisabolene and α-bisabol, and (4) four unidentified sesquiterpenes.

Recent reports suggest that the oil is being adulterated with polyethylene glycols. The oil is now mainly used in perfumery; a possible chemoprotective action on liver carcinogenesis in mice has been demonstrated (S. Banerjee *et al*., *Cancer Lett*., 1993, **68**, 105). Bioassay-guided fractionations coupled with NMR structural determinations have shown that of eleven sesquiterpenes, (*Z*)-α-santalol and (*Z*)-β-santalol have strong anti-*Helicobacter pylori* activities against a clarithromycin resistant strain. (T. Ochi *et al.*, *J. Nat. Prod.*, 2005, **68**, 819).

Australian sandalwood oil is prepared by distillation and rectification from the wood of *Eucarya spicata*, a small tree growing in Western Australia. It contains sesquiterpene alcohols.

RESINS, GUM-RESINS AND SIMILAR SUBSTANCES

The term 'resin' is applied to more or less solid, amorphous products of complex chemical nature. On heating they soften and finally melt. They are insoluble in water and usually insoluble in petroleum spirit but dissolve more or less completely in alcohol, chloroform and ether. Chemically, resins are complex mixtures of resin acids, resin alcohols (resinols), resin phenols (resinotannols), esters and chemically inert compounds known as resenes. The chemical structures of many of these compounds have now been elucidated.

Resins, as described above, are often associated with volatile oils (oleoresins), with gums (gum-resins) or with oil and gum (oleo-gum-resins). However, no hard and fast distinction can be made between these groups, as products such as mastic and ammoniacum, which are usually considered as a resin and a gum-resin, respectively, both contain volatile oil. Resins may also be combined in a glycosidal manner with sugars, as in the Convolvulaceae.

The term 'balsam' is often wrongly applied to oleoresins such as Canada turpentine and copaiba, and should be reserved for such substances as balsam of Peru, balsam of Tolu and storax, which contain a high proportion of aromatic balsamic acids (see Chapter 19). These balsams, if containing free acids, are partially soluble in hot water, owing to the solubility of benzoic and cinnamic acids, while the aromatic esters and resins are insoluble. Benzoin is perhaps best described as a balsamic resin.

The above products are usually contained in schizogenous or schizolysigenous ducts or cavities. They are often preformed in the plant (i.e. they are normally physiological products), but the yield is usually increased by injury (e.g. in the case of *Pinus*). Many products (e.g. benzoin and balsam of Tolu) are not formed by the plant until it has been injured: that is, they are of pathological origin. The gums which are often associated with resins and volatile oils usually resemble acacia gum in chemical nature and in the fact that they are often accompanied by oxidase enzymes. While resins are usually produced in ducts or cavities, they may be found in other positions—for example, in the resin cells of bloodroot, in the elements of the heartwood of guaiacum, in the external glands of Indian hemp, in the internal glands of male fern or in the glands on the surface of the lac insect.

MYRRH

Myrrh (*Arabian* or *Somali Myrrh*) is an oleo-gum resin, obtained from the stem of various species of *Commiphora* (Burseraceae), growing in north-east Africa and Arabia. British texts have traditionally given the principal source as *C. molmol* but Tucker (*Econ. Bot*., 1986, **40**, 425) states that the chief source today is *C. myrrha*. The *EP* and *BP* definition cites *Commiphora molmol* Engler and/or other species of *Commiphora*. Two other species, *C. abyssinica* and *C. schimperi*, both of which may attain a height of 10 m, grow in Arabia and Abyssinia. The drug is chiefly collected in Somaliland and Ethiopia.

History. Products of the myrrh type were well known to the ancients under the names of *bola*, *bal* or *bol*. The drug is still known to the Indian traders as 'heerabol', while the Somalis call it 'mulmul' or 'ogo'. The name 'myrrh' is probably derived from the Arabic and Hebrew word *mur*, which means bitter. Many references occur in the Old Testament, but the product was apparently that derived from *C. erthyaea* var. *glabrescens*, which is known to the Somalis as 'habbak hadi', and commercially as perfumed bdellium or bissabol.

Guban myrrh, which is produced from the trees of the Somali coast area known as the Guban, is rather oily and is regarded as inferior to the more powdery 'ogo' produced further inland.

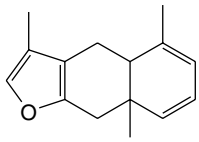
Collection. Almost all members of the Burseraceae possess in the phloem oleoresin canals, which are formed schizogenously and may afterwards unite with one another to form schizolysigenous cavities. This occurs in the species *Commiphora*. Much of the secretion is obtained by spontaneous exudation from the cracks and fissures which commonly form in the bark, and some is obtained from incisions made by the Somalis. The yellowish-white, viscous fluid soon hardens in the great heat to reddish-brown masses, which are collected by the Somalis. As bdelliums and gums are collected at the same time, these frequently find their way into the drug and have subsequently to be picked out.

Characters. Myrrh occurs in somewhat irregular tears or masses weighing up to about 250 g. The surface is reddish-brown or reddish-yellow in colour and powdery. The drug fractures and powders readily, the freshly exposed surface being of a rich brown colour and oily. Whitish marks are sometimes seen and thin splinters are translucent. Myrrh has an aromatic odour and an aromatic, bitter and acrid taste.

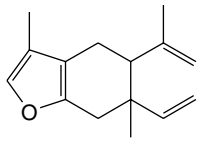
Myrrh forms a yellowish emulsion when triturated with water. When extracted with alcohol (90%), as in the preparation of Tincture of Myrrh, a whitish mass of gum and impurities remains. The *BP* alcohol-insoluble matter should not exceed 70%. Lump myrrh usually yields not more than 5% of ash, but the commercial powdered drug frequently yields more. It may be distinguished from perfumed bdellium and similar products by allowing an ethereal extract of the drug to evaporate to dryness and passing the vapour of bromine over the resinous film produced. A violet colour is given by genuine myrrh but not by bdellium. TLC and visualization with ultraviolet light at 365 nm is used by the *BP* as an identification test and also to establish the absence of *C. mukul*, an inferior bdellium product.

Constituents. Myrrh contains 7–17% of volatile oil, 25–40% of resin, 57–61% of 'gum' and some 3–4% of impurities.

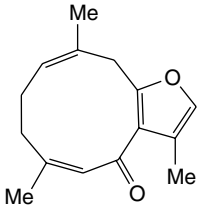
The volatile oil contains terpenes, sesquiterpenes, esters, cuminic aldehyde and eugenol. The sesquiterpene fraction (Fig. 22.18) contains furanosesquiterpenes including furanogermacranes, furanoguaianes and furanoeudesmanes (N. Zhu *et al.*, *J. Nat. Prod.*, 2001, **64**, 1460). Furaneudesma-1,3-diene and curzarene have morphine-like properties and act on the CNS opioid receptors; furanodiene-6-one and methoxy furanoguaia-9-ene-8-one show antibacterial and antifungal activity against standard strains of pathogenic species (P. Dolara *et al*., *Nature*, 1996, **379**, 29; *Planta Medica*, 2000, **66**, 356). The oil, which is distilled outside the countries of origin, readily resinifies and then gives a violet colour with bromine.



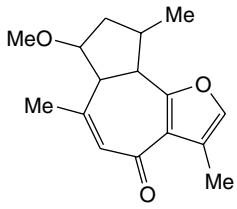
Furaneudesma-1,3-diene



Curzarene



Furanodiene-6-one



Methoxy furanoguaia-9-ene-8-one

Fig. 22.18
Constituents of myrrh.

The chemistry of the resins is complex and not fully elucidated. The larger ether-soluble portion contains α-, β- and γ-commiphoric acids, the esters of another resin acid and two phenolic resins. The smaller ether-insoluble fraction contains α- and β-heerabomyrrholic acids. The crude alcohol-insoluble matter ('gum') contains about 18% of protein and 64% of carbohydrate containing galactose, arabinose and glucuronic acid. This gum is associated with an oxidase enzyme.

Allied drugs. Four different varieties of 'bdellium' were recognized by Holmes. Of these, *perfumed* or *scented bdellium* or *bissabol* is probably derived from *C. erythaea* var. *glabrescens*. It resembles soft myrrh in appearance but is easily distinguished from it by the more aromatic odour and by the fact that it does not give a violet colour with the bromine test. *Hotai bdellium* or *gum hotai* is opaque and odourless; it contains a saponin and is used for washing the hair. The resin of *C. confusa* collected in Kenya contains dammarane triterpenoids, as does *C. kua* (L. O. A. Manguro *et al.*, *Chem. Pharm. Bull.*, 2003, **51**, 479, 483).

Uses. Myrrh is used in incense and perfumes. Like many other resins, it has local stimulant and antiseptic properties. It is chiefly employed in medicine in the form of a mouth-wash or gargle for its astringent effect on mucous membranes. A number of its traditional and historic uses have received experimental support.

Olibanum

Olibanum (*Frankincense*) is an oleo-gum-resin obtained by incision from the bark of *Boswellia carterii*, *B. frereana* and other species of *Boswellia* (Burseraceae), small trees indigenous to north-eastern Africa and Arabia. The drug occurs in more or less ovoid tears, 5–25 mm long, which are sometimes stuck together. The surface is dusty and of a yellowish, bluish or greenish tint. Fracture, brittle; inner surface, waxy and semitranslucent. Odour is characteristic, especially when burned; taste, slightly bitter. The drug contains 3–8% of volatile oil consisting of numerous terpenes (e.g. *p*-cymene) and sesquiterpenes, about 60–70% of resin, and 27–35% of gum. In 1956 the gum was found to contain two polysaccharides; one consisting of units of galactose and arabinose and the other of galactose and galacturonic acid. Modern methods of analysis have allowed the taxonomic identity of diverse frankincense products to be determined; examples are incense mixtures, traditional medicines and archaeological specimens (S. Hamm *et al.*, *Phytochemistry*, 2005, **66**, 1399).

Olibanum is used in incense and fumigating preparations. Formerly, it was considered a stimulant and has been used in China for the treatment of leprosy. With animal models, Duwiejua *et al*. (*Planta Medica*, 1993, **59**, 12) have reported a positive anti-inflammatory activity for the drug.

Asafoetida

Asafoetida is an oleo-gum-resin obtained by incision from the living rhizome and root of *Ferula foetida* Regel, *F. rubricaulis* Boiss., and other species of *Ferula* (Umbelliferae), plants about 3 m in height. The drug is collected in Iran, Pakistan and Afghanistan.

Collection and preparation. The collection of asafoetida involves removal of the stem and the cutting of successive slices from the vertical rootstock. After each slice is removed, oleo-gum-resin exudes and, when sufficiently hardened, is collected. The product is packed in tin-lined cases for export.

Characters. Asafoetida occurs in two principal forms.

Tears. These are rounded or flattened and about 5–30 mm diameter. They are greyish-white, dull yellow or reddish-brown in colour, some specimens acquiring the latter colour with age, while others remain greyish or yellowish.

Mass. This consists of similar tears to those described above agglutinated into masses and usually mixed with fruits, fragments of root, earth and other impurities. Mass asafoetida is the commonest commercial form.

Asafoetida has a strong, alliaceous odour and a bitter, acrid and alliaceous taste. It should yield not more than 50% of matter insoluble in alcohol (90%) and not more than 15% of ash.

Constituents. Asafoetida consists of volatile oil, resin, gum and impurities. The oil has a particularly evil smell and contains sulphur compounds of the formulae $C_7H_{14}S_2$, $C_{16}H_{20}S_2$, $C_8H_{16}S_2$, $C_{10}H_{18}S_2$, $C_7H_{14}S_3$, and $C_8H_{16}S_3$; some of these show pesticidal activity. The flavour is largely due to *R*-2-butyl-1-propenyl disulphide (a mixture of *E* and *Z* isomers), 1-(1-methylthiopropenyl)-1-propenyl disulphide and 2-butyl-3-methylthioallyl disulphide (both as mixtures of diastereoisomers). The drug also contains a complex mixture of sesquiterpene umbelliferyl ethers mostly with a monocyclic or bicyclic terpenoid moiety; more recently (G. Appendino *et al*., *Phytochemistry*, 1994, **35**, 183) three new sesquiterpene coumarin ethers have been isolated. Also present are asaresinol ferulate and free ferulic acid. For selected formulae see Fig. 22.19. The drug contains no free umbelliferone (distinction from galbanum). However, on boiling it with hydrochloric acid and filtering into ammonia, a blue fluorescence is produced owing to the formation of umbelliferone. Ferulic acid is closely related to umbellic acid and umbelliferone (both of which occur in galbanum).

Allied drugs. Galbanum and ammoniacum are oleo-gum-resins obtained, respectively, from *Ferula galbaniflua* and *Dorema ammoniacum*. Galbanum contains, besides umbelliferone, a number of umbelliferone ethers; also gum and up to 30% of volatile oil containing numerous mono- and sesquiterpenes, azulenes and sulphur-containing esters. Ammoniacum, listed in the *BHP*, contains free salicylic acid but no umbelliferone. The major phenolic constituent is ammoresinol; Appendino *et al*. (*Helv. Chim. Acta*, 1991, **74**, 495) isolated an epimeric mixture of prenylated chromandiones termed ammodoremin. The volatile oil (*c*. 0.5%) contains various terpenoids

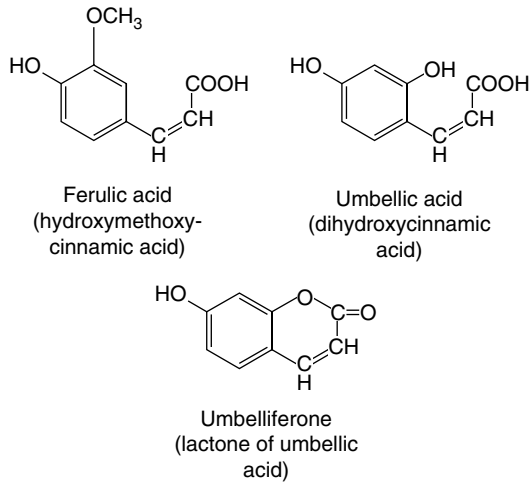


Fig. 22.19
Constituents of asafoetida.

with ferulene as the major component. The demonstration of the broad spectrum and antimicrobial activity of ammoniacum has supported its traditional use for chest infections (M. Rajani *et al.*, *Pharm. Biol.*, 2002, **40**, 534).

Uses. Asafoetida is included in the *BHP* (Vol. 1, 1990) and is employed for the carminative and expectorant properties of the volatile oil fraction. It is an ingredient of certain sauces.

Damiana

Damiana consists of the dried leaves of *Turnera diffusa* var. *aphrodisiaca* (Turneraceae), and probably other species of *Turnera*. The drug is collected in Bolivia and Mexico. The leaves are yellowish-green to green in colour, broadly lanceolate, shortly petiolate, and 10–25 cm long; margin with 3–6 teeth on each side; veins pinnate and prominent on the lower surface. The drug usually contains some of the reddish-brown, cylindrical twigs, flowers and spherical fruits. Damiana has an aromatic odour and taste. It contains 0.5–1.0% of volatile oil, from which thymol, α-copaene, δ-cadinene and calamenene have been isolated; in addition, a brown amorphous substance, damianin, resins and gum.

It would appear that in Mexico the wild populations of the plant are threatened by over-collection, and cultivation is recommended using micropropagation, the latter having now been shown to be a commercial feasibility (L. Alcaraz-Meléndez *et al.*, *Plant Cell Rep.*, 1994, **13**, 679.)

Damiana is traditionally used in Mexico and Southern USA to revive libido where subconscious causative factors are involved. Elixir of Damiana and Saw Palmetto or other admixtures are used as an aphrodisiac for men.

For a review of the genus *Turnera* (*93 references*), see S. Kumar *et al.*, *Pharm. Biol.*, 2005, **43**, 383.

Copaiba

Copaiba is an oleoresin obtained from the trunks of various species of *Copaifera* (Leguminosae) and contains at least 24 sesquiterpene hydrocarbons and a number of diterpenes. It was formerly used as a urinary antiseptic but has now been almost completely replaced by antibiotics and other drugs.

Eriodictyon leaf

Eriodictyon or Yerba Santa consists of the dried leaf of *Eriodictyon californicum* (Hydrophyllaceae), a low evergreen shrub of the hills and mountains of California and northern Mexico.

The leaves usually occur in fragments; when entire, they are lanceolate, 5–15 cm long and 1–3 cm wide. The apex is acute; the base slightly tapering into a short petiole. The margin is irregularly serrate or crenate-dentate. The upper surface is yellowish-brown to greenish-brown and covered with a glistening resin. The lower surface is greenish-grey to yellowish-grey, conspicuously reticulate, with greenish-yellow or brown veins, and minutely tomentose (cottony) between the reticulations. The leaves are thick and brittle. They have an aromatic odour and a balsamic bitter taste, which becomes sweetish and slightly acrid.

Eriodictyon contains volatile oil, resin, eriodictyol (see 'Hesperidin' and 'Eriodictyol'), homoeriodictyol, chrysoeriodictyol, xanthoeriodictyol, eriodonol, eriodictyonic acid and ericolin.

Yerba Santa is employed in the USA for the preparation of a fluid extract and Aromatic Eriodictyon Syrup, which is used to mask the taste of bitter and otherwise disagreeable medicines, particularly quinine. American Indians smoked or chewed the leaves as a cure for asthma. Some herbalists consider it an excellent expectorant. Externally it can be used for the treatment of bruises, insect bites, etc.

Gamboge

Gamboge is a gum-resin obtained from *Garcinia hanburii* (Guttiferae), a tree indigenous to South-East Asia.

Gamboge is a typical gum-resin, and when triturated with water, it forms a yellow emulsion. Good gamboge contains 70–80% of resin (gambogic acid) and 15–20% of water-soluble gum with which is associated an oxidase enzyme. Gamboge acts as a purgative but is now little used in human medicine. It is used as a pigment. For a report on its bioactivity, see A. Panthong *et al.*, *J. Ethnopharmacol.*, 2007, **111**, 335.

MASTIC

Mastic is a resin or, more correctly, an oleoresin containing little oil, obtained from various cultivated varieties of *Pistacia lentiscus* L. (Anacardiaceae); the *BP*/*EP* specifies var. *latifolius* Coss; another quoted in the literature is var. *chia* from the Greek island of Chios, which is the principal exporter.

The plant is an evergreen shrub and tapping is limited by law to the period 15 July–15 October. The base of the shrub is cleared of weeds, flattened and covered with a special white soil to receive some of the flow. The stem and larger branches are then wounded by means of a gouge-like instrument which makes an incision about 2 cm long and 3 mm deep. Each plant is tapped repeatedly for about 5 or 6 weeks, receiving in all about 200–300 wounds. A special tool is used for removing the tears which harden on the plant and the flat plates of mastic which collect on the ground. These are graded by the collector and regraded, washed and dried in a central depot before being exported in wooden boxes. Chios exports about 250 000 kg annually.

Mastic occurs in yellow or greenish-yellow rounded or pear-shaped tears about 3 mm diameter. The shape of the tears is sufficient to distinguish them from those of sandarac. The tears are brittle but become plastic when chewed. Odour, slightly balsamic; taste, mildly terebinthinate.

The resin component of mastic is a complex mixture. It contains tri-, tetra- and penta-cyclic triterpene acids and alcohols (for a report see F.-J. Marner *et al.*, *Phytochemistry*, 1991, **30**, 3790). About 2% of volatile oil is also present, the pharmacopoeial minimum being 1%; over 60 compounds have been reported from mastic and up to 250 recorded in plant oils. The principal components appear to be the monoterpene hydrocarbons α-pinene, β-myrcene and camphene. Four neutral novel triterpenoids and ten triterpenoid acids have now been characterized, see V. P. Papageorgiou *et al.*, *J. Chromat. A*, 1997,

769, 263. The acid value of about 50 (*BP*, 1980, not more than 70) distinguishes it from East Indian or Bombay mastic, which has an acid value of more than 100.

Mastic is used in the preparation of Compound Mastic Paint and as a microscopical mountant. In Greece and the Middle East mastic has been used for centuries as a protective agent for the stomach, and investigations at the University of Nottingham Medical School indicated success in the treatment of gastric ulcers. Research has shown that mastic will kill *Helicobacter pylori* at concentrations of 0.06 mg/ml (see F. U. Huwez *et al*., *New Engl. J. Med*., 1998, 339, 1946). Further studies were planned with patient volunteers infected with *H. pylori* (*Pharm. J*., 2000, **264**, 459).

Sandarac

Sandarac is a resin obtained from the stem of *Tetraclinis articulata* (Cupressaceae), a tree 6–12 m high, which is found in North and North-west Africa and in Spain.

Sandarac occurs in small tears about 0.5–1.5 cm in length. These usually have an elongated, stalactic or cylindrical shape, globular or pear-shaped tears being relatively rare. The surface is covered with a yellowish dust, but the interior is more or less transparent, and if the tears are held up to the light, small insects can frequently be seen embedded in them. The drug is easily powdered and when chewed remains gritty, showing no tendency to form a plastic mass (distinction from mastic). The drug has a faint, terebinthinate odour, and a somewhat bitter taste.

Sandarac resin consists of sandarocopimaric acid (inactive pimaric acid), sandaracinic acid, sandaracinolic acid and sandaracoresene. The drug also contains a bitter principle and 0.26–1.3% of volatile oil.

Grindelia herb

Grindelia or gum plant consists of the dried leaves and flowering tops of *Grindelia camporum (G. robusta)*, *G. humilis* and *G. squarrosa* (Compositae), collected in south-western USA. The plants are herbaceous with cylindrical stems, sessile or amplexicaul leaves, and resinous flower-heads each surrounded by an involucre of linear-lanceolate bracts. Odour, balsamic; taste, aromatic and bitter.

In the wild 2*n* and 4*n* forms occur and selection of the latter for cultivation should produce higher yields of resin (J. L. McLaughlin *et al.*, *Econ. Bot*., 1986, **40**, 155).

Grindelia contains about 20% of resin, which contains a large number of labdane diterpene acids termed grindelanes and methyl esters (see B. A. Timmermann *et al*., *Phytochemistry*, 1985, **24**, 1031; M. Adinolfi *et al.*, *ibid*., 1988, **27**, 1878). The plants yield about 0.2% of a volatile oil containing over 100 components. Oil composition from the different species varies quantitatively with bornyl acetate and α-pinene the major components of the monoterpenoid fraction (see G. Kaltenbach *et al.*, *Planta Medica*, 1991, **57**, (Suppl. 2), A82). For a report on the oil content, and its antioxidant activity, of plants raised experimentally in Central Italy, see D. Fraternale *et al.*, *Fitoterapia*, 2007, **78**, 443.

The herb has been used for the treatment of bronchitis and asthma, but is now mainly employed in the form of a lotion for dermatitis produced by the poison ivy, *Rhus toxicodendron* (Anacardiaceae). Some grindelanes have been shown to have antifeeding deterrent activity towards aphids.

Guaiacum resin

Guaiacum resin is obtained from the heartwood of *Guaiacum officinale* and *G. sanctum* (Zygophyllaceae), small evergreen trees found in the dry coastal regions of tropical America. *Guaiacum officinale* is found on the coast of Venezuela and Colombia and in the West Indies, while *G. sanctum* occurs in Cuba, Haiti, the Bahamas and Florida. Little is now found in commerce.

Guaiacum resin occurs in large blocks or rounded tears about 2–3 cm diameter. The freshly fractured surface is brown and glassy. The powder is greyish but becomes green on exposure. Taste, somewhat acrid; odour, when warmed, aromatic. When free from woody debris, guaiacum is soluble in alcohol, chloroform and solutions of alkalis. An alcoholic solution gives a deep blue colour (guaiac-blue) on the addition of oxidizing agents such as ferric chloride. This colour is destroyed by reducing agents. Colophony, the most likely adulterant, may be detected by the cupric acetate test.

Some of the main resinous constituents are lignans. These are phenolic compounds having a C_{18} structure formed from two C_6–C_3 units (Table 21.7). Guaiaretic acid, which forms about 10% of guaiacum resin, is a diaryl butane.

The flowers, fruit and bark of the tree contain triterpenoid and nortriterpenoid saponins.

For use as a reagent the resin as extracted from the wood by means of chloroform is said to be the most sensitive. An alcoholic solution is used for the detection of blood stains, cyanogenetic glycosides, oxidase and peroxidase enzymes.

Guaiacum resin, included in the *BHP* (Vol. 1, 1990) is indicated for the treatment of chronic rheumatic conditions. It is a permitted food additive in the USA and in Europe.

COLOPHONY

Colophony (rosin) is the resin remaining in the still after removal of the volatile turpentine oil from the oleoresin of species of *Pinus* (see Turpentine Oil). Generally, the resin obtained from trees during their first year of tapping is of a lighter colour than that obtained subsequently. Traditionally, some 17 grades of rosin have been recognized, extending from the almost black wood rosin (B&F grades) through paler colours to the window glass (WG), water white (WW) and extra white (X) grades. For a detailed account of the oleoresin collection (cup and gutter method) and preparation of the rosin, see the 15th edition of this book.

Characters. The colophony described in the *BP*/*EP* occurs in translucent glassy masses of a pale yellow or amber colour. It is brittle and easily powdered. It fuses gradually at about 100°C, and at a higher temperature burns with a smoky flame, leaving not more than about 0.1% of ash. Colophony is insoluble in water but soluble in alcohol, ether, benzene and carbon disulphide.

Chemical tests

1. Dissolve about 0.1 g of powdered resin in 10 ml of acetic anhydride. Add one drop of sulphuric acid on a glass rod. Care should be taken to see that the apparatus used is dry, that the solution is cold and that concentrated sulphuric acid is used. On adding the acid a purple colour, rapidly changing to violet, is produced.
2. Shake a little powdered colophony with light petroleum and filter. Old samples of resin are usually much less soluble in this solvent than fresh ones. Shake the solution with about twice its volume of dilute solution of copper acetate. The petroleum layer becomes emerald-green in colour, a change which is due to the formation of the copper salt of abietic acid.

Constituents. Colophony contains resin acids (about 90%), neutral inert substances formerly known as resenes and esters of fatty acids.

The exact composition varies with biological source, preparation, age and method of storage.

The resin acids are isomeric diterpene acids. It will be noted that colophony has a high acid value of 150–180.

Before distillation the resin contains large amounts of (+)- and (−)-pimaric acids. During distillation the (+)-pimaric acid is stable but the (−)-pimaric acid undergoes isomeric change into abietic acid, the major constituent of the commercial resin (see formula, p. 264). On heating at 300°C abietic acid undergoes further molecular rearrangement to produce some neo-abietic acid. The commercial 'abietic acid' is prepared by digesting colophony with weak alcohol.

The abietane acids have been considerably investigated over recent years; they are obtained chiefly from colophony but are also present in other conifers of the Araucariaceae, Cupressaceae, Pinaceae and Podocarpaceae. Their activities are mainly antimicrobial, antiulcer and cardiovascular; some have filmogenic, surfactant and antifeedant properties.

Uses. The amount of colophony used in pharmacy for the preparation of zinc oxide and other adhesive plasters, ointments, etc., is relatively small. Much rosin is artificially modified by hydrogenation or polymerization; products involving its use include paper size, adhesives, printing inks, rubber, linoleum, thermoplastic floor tiles and surface coatings.

Further reading

Keeling CI, Bohlman J 2006 Diterpene resin acids in conifers. Phytochemistry 67(22): 2415–2423. *A review focusing on recent discoveries in the chemistry, biosynthesis, molecular biology, regulation and biology. Many refs*

Ipomoea

Ipomoea (*Orizaba Jalap*, *Mexican Scammony Root*) is the dried root of *Ipomoea orizabensis* (Convolvulaceae), a convolvulaceous twining plant with a fusiform root about 60 cm long. The drug is collected in the Mexican State of Orizaba and is exported from Vera Cruz.

Orizaba was originally imported as a substitute or adulterant of jalap or its resin ('jalapin'). However, the resin is more soluble in ether than is jalap resin and more closely resembles that obtained from the root of *Convolvulus scammonia*, which was the original source of scammony resin.

Whole roots of ipomoea are rarely imported, and the drug usually consists of transverse or oblique slices about 3–10 cm wide and 2–4 cm thick.

The outer surface is covered with a greyish-brown, wrinkled cork. The transverse surface is greyish or brownish and shows about 3–6 concentric rings of fibrovascular bundles. The parenchymatous tissue of both bark and stele resembles that of jalap in containing starch and calcium oxalate. Like jalap, the section shows numerous scattered secretion cells with resinous contents. Odour, slight; taste, faintly acrid.

Ipomoea, when extracted with alcohol (90%), yields about 10–20% of a complex resinous mixture, of which about 65% is soluble in ether. The chief constituents of ipomoea resin are the methyl pentosides and other glycosides of jalapinolic acid and its methyl ester; these are the orizabins. Also isolated are the scammonins, the structures of which are indicated below. For details of these resin glycosides see B. Hernández-Carlos *et al*., *J. Nat. Prod*., 1999, **62**, 1096. Also present are sitosterol and other phytosterol glycosides.

Ipomoea is mainly used for the preparation of ipomoea resin. It resembles jalap in medicinal properties.

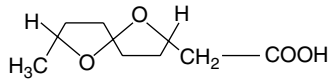
Jalap

Jalap consists of the dried tubercles or tuberous roots of *Ipomoea purga*, a large, twining plant indigenous to Mexico. Most of the drug is imported from eastern Mexico under the name of 'Mexican' or 'Vera Cruz' jalap. Convolvulaceous tubers with purgative properties were brought to Spain about 1565.

The traditional system of production in Central Vera Cruz has been described by A. Linajes *et al*. (*Econ. Bot*., 1994, **48**, 84). Scarification of seeds prior to sowing is the secret of obtaining a 95% germination rate in 8 days. The productive period extends from July to February and the harvested tubers are smoke-dried in small wooden sheds using unseasoned *Liquidamber macrophylla* wood for fuel. During this process there is a weight loss of 50–75%. This method gives a product more resistant to fungal and insect attack than does simple drying. The yield is 2.4–3.0 tons of fresh root/hectare which can be increased, as in India, to 4.8 tons/hectare by the use of cow manure.

Jalap tubercles are fusiform, napiform or irregularly oblong in shape, and 3–5 cm long. They are extremely hard and heavy. The surface is covered with a dark brown, wrinkled cork, which is marked with lighter-coloured, transverse lenticels. The larger pieces may bear gashes, which have been made to facilitate drying. The tubercles may be softened for cutting by prolonged soaking in water. Cut transversely they show a greyish interior, a complete cambium ring fairly close to the outside and within it numerous irregular dark lines. The drug has a slight, smoky odour; the taste is at first sweetish, afterwards acrid. A description of the microscopy of jalap was given in the 11th edition.

Jalap contains 9–18% of resin contained in secretion cells and giving a yellow stain with iodine water. It may be extracted from the powdered drug with boiling alcohol (90%). On pouring a concentrated tincture into water, the resin is precipitated and may be collected, washed and dried. The complexity of these convolvulaceous resins has prevented, until recently, their isolation in a pure form and they have been studied by investigating the products of their hydrolysis (short-chain volatile fatty acids, hydroxy fatty acids and sugars). The main constituent of jalap resin is convolvulin, a substance with some 18 hydroxyl groups esterified with valeric, tiglic and exogonic acids. Exogonic acid is 3,6-6,9-dioxidodecanoic acid. (For its stereochemical structure see E. N. Lawson *et al.*, *J. Org. Chem*., 1992, **57**, 353).



Exogonic acid

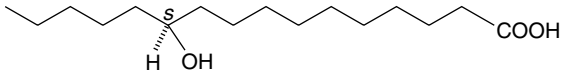
Jalap is a powerful hydragogue cathartic and was formerly extensively used either as standardized powder, as Jalap Resin or as Jalapin. The latter is the decolorized ether-insoluble portion of Jalap Resin.

Recently, using modern techniques, Japanese researchers have carried forward the investigation of those convolvulaceous species which are of relevance to the oriental market. Examples are given below.

Brazilian Jalap Rhizome

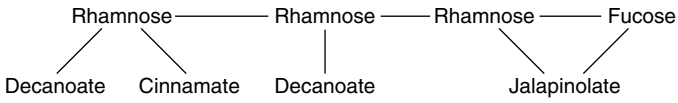
This is derived from *Ipomoea operculata* and constitutes a substitute for Mexican jalap. In a series of papers Ono and colleagues (see *Chem. Pharm. Bull*., 1992, **40**, 1400 and references cited therein) characterized 18 operculins (ether-soluble resin glycosides). These resemble the other known jalapins in that they are monomers with similar intramolecular macrocyclic ester structures in the glycosidic acid moieties. However, their component acids (*n*-decanoic and *n*-dodecanoic acids) are characteristically different from those of previously known resin glycosides (isobutyric, 2-methylbutyric and tiglic acids), see 'Jalap' above. On alkaline hydrolysis a particular operculin will give a characteristic operculinic acid along with *n*-decanoic and *n*-dodecanoic acids. Operculinic acid E, for example, is 11*S*-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-

(1→2)-β-D-glucopyranoside. The formula for 11*S*-jalapinolic acid (common to all operculinic acids) is given below:



11*S*-Jalapinolic acid

Ipomoea batatus. This species, the sweet potato, is widely cultivated as a food but it has also traditional (Brazilian) medicinal uses and a number of pharmacological claims have been made for it. In 1979 Kawasaki *et al*. reported the roots to contain a mixture of hexa-, hepta- and octa-decylferulates; Noda *et al*. (*Chem. Pharm. Bull*., 1992, **40**, 3163) isolated five new ether-soluble resin glycosides called simonins I–V. The arrangement of the acids in relation to the carbohydrate moieties of the molecule is illustrated by simonin I.



Simonin I

Similar compounds (stoloniferins) have been recorded in *Ipomoea stolonifera* (N. Noda *et al*., *Phytochemistry*, 1998, **48**, 837).

The roots of *Convolvulus scammonia* (*vide supra*) contain ether-soluble resin glycosides called scammonins; they possess a glycosidic acid, e.g. scammonic acid A, and have an intramolecular macrocyclic ester structure involving various sugars (see H. Kogetsu *et al*., *Phytochemistry*, 1991, **30**, 957).

VOLATILE OILS IN AROMATHERAPY

Aromatherapy is based primarily on the use of volatile oils, either singly or in admixture. They are administered in baths (drops of oil are added to the water and vigorously mixed), in compresses, in massage and as inhalations. For compress and massage usage the volatile oils are mixed with a suitable carrier (e.g. the fixed oils of apricot kernel, evening primrose, starflower, sweet almond to cite but a few) and for inhalation vaporizers and burners are available in addition to the traditional steam inhalation or use of the handkerchief or tissue.

A number of the oils used in aromatherapy have already been mentioned and include those from benzoin, black pepper, German chamomile, cinnamon leaf, clove, eucalyptus, fennel, frankincense, ginger, juniper berry, lavender, lemon, myrrh, neroli, orange, peppermint, pine, rose, sandalwood, spearmint, tea-tree and thyme. Others are listed below, citing: Botanical source; Geographical origin (but not necessarily the sole); Parts of the plant from which the oil is extracted; Extraction process; and Principal constituents. It must be remembered that volatile oils can contain over 100 constituents, most in very small amounts, and the principal components might not be those giving the oil its unique characteristics.

Basil. *Ocimum basilicum*, Labiatae; Egypt; Leaves and flowering tops; Distillation; Linalool, cineole, methyl chavicol.

Bergamot. *Citrus bergamia*, Rutaceae; Sicily; Peel; Expression; Linalyl acetate, linalool, limonene.

Cedarwood. *Cedrus atlantica*, Pinaceae; Morocco; Wood chips and shavings; Distillation; α-Cedrene, atlantone, atlantol.

Citronella. *Cymbopogon nardus*, Graminae; Sri Lanka; Grass leaves; Distillation; Geranyl acetate, citronellol, citronellal.

Clary sage. *Salvia sclarea*, Labiatae; Hungary; Leaves and flowering tops; Distillation; Linayl acetate, linalool.

Cypress. *Cupressus sempervirens*, Cupressaceae; France; Tree needles and cones; Distillation; Pinene, carene, terpinolene, camphene.

Grapefruit. *Citrus paradisi*, Rutaceae; USA; Peel; Expression; Limonene.

Jasmine. *Jasminum officinale*, Oleaceae; India; Flowers; Solvent extraction; Benzyl acetate, linalyl acetate, benzyl alcohol, linalool.

Lemongrass. *Cymbopogon citratus*, Gramineae; India; Grass leaves; Distillation; Citral, geraniol, linalool.

Lime. *Citrus medica*, Rutaceae; Peru; Fruit; Distillation; Citral, limonene, linalool, camphene, sabinene.

Mandarin. *Citrus reticulata*, Rutaceae; Spain; Fruit peel; Expression; Limonene, terpenene, myrcene.

Marjoram (Sweet). Origanum majorana, Labiateae; Egypt; Dried leaves and flowering tops; Distillation; Terpinene, terpineol, myrcene, ocimene, sabinene, cymene, geranyl acetate.

Melissa. *Melissa officinalis*, Labiatae; Cultivated UK; Flowering tops; Distillation; Citral.

Myrtle. *Myrtus communis*, Myrtaceae; Morocco; Leaves and twigs; Distillation; Limonene, linalool, geraniol, myrtenol.

Palmarosa. *Cymbopogon martini*, Gramineae; Comoros; Grass leaves; Distillation; Geraniol, linalool, geranyl acetate.

Patchouli. *Pogostemon patchouli*, Labiatae; Indonesia, India, Europe, USA; Dried leaves; Distillation; Patchoulol, pogostol.

Petitgrain. *Citrus aurantium*, Rutaceae; Paraguay, France; Leaves and twigs; Distillation; Linalyl acetate, linalool.

Pine. *Pinus sylvestris*, Pinaceae; Austria; Pine needles; Distillation; Terpenes, sesquiterpenes, bornyl acetate.

Ravensara. *Ravensara aromatica*, Lauraceae; Madagascar; Leaves; Distillation; Estragole, pinene, caryophyllene.

Rose Absolute. *Rosa centifolia*, Rosaceae; Morocco; Fresh flowers; Solvent extraction; Similar constituents to Rose otto.

Rose Otto. *Rosa damascena*, Rosaceae; Bulgaria; Fresh flowers; Distillation; Citronellol, geraniol, 2-phenylethanol, nerol.

Vetiver. *Vetiveria zizanioides*, Gramineae; Indonesia, Reunion; Grass roots; Distillation; Alcohols, ketones.

Ylang Ylang. *Cananga odorata* var. *genuina*, Annonaceae; Madagascar, Reunion; Fresh flowers; Steam distillation; Acetates of geranyl and benzyl alcohols, linalool, caryophyllene.

Further reading

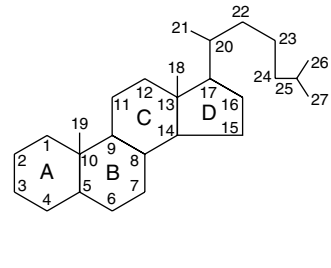
Lis-Balchin M 2006 Aromatherapy science, a guide for healthcare professionals. Pharmaceutical Press, London

23

Saponins, cardioactive drugs and other steroids

Plant materials containing saponins have long been used in many parts of the world for their detergent properties. For example, in Europe the root of *Saponaria officinalis* (Caryophyllaceae) and in South America the bark of *Quillaja saponaria* (Rosaceae). Such plants contain a high percentage of glycosides known as saponins (Latin *sapo*, soap) which are characterized by their property of producing a frothing aqueous solution. They also have haemolytic properties, and when injected into the blood stream, are highly toxic. The fact that a plant contains haemolytic substances is not proof that it contains saponins, and in the species examined by Wall (1961) only about half of those containing haemolytic substances actually contained saponins. When taken by mouth, saponins are comparatively harmless. Sarsaparilla, for example, is rich in saponins but is widely used in the preparation of non-alcoholic beverages.

Saponins have a high molecular weight and a high polarity and their isolation in a state of purity presents some difficulties. Often they occur as complex mixtures with the components differing only slightly from one another in the nature of the sugars present, or in the structure of the aglycone. Various chromatographic techniques have been employed for their isolation. As glycosides they are hydrolysed by acids to give an aglycone (sapogenin) and various sugars and related uronic acids. According to the structure of the aglycone or sapogenin, two kinds of saponin are recognized—the steroidal (commonly tetracyclic triterpenoids) and the pentacyclic triterpenoid types (see formulae below). Both of these have a glycosidal linkage at C-3 and have a common biogenetic origin via mevalonic acid and isoprenoid units.



Steroid skeleton Pentacyclic triterpenoid skeleton

A distinct subgroup of the steroidal saponins is that of the steroidal alkaloids which characterize many members of the Solanaceae. They possess a heterocyclic nitrogen-containing ring, giving the compounds basic properties (as an example see solasodine, Fig. 23.5).

STEROIDAL SAPONINS

The steroidal saponins are less widely distributed in nature than the pentacyclic triterpenoid type. Phytochemical surveys have shown their presence in many monocotyledonous families, particularly the Dioscoreaceae (e.g. *Dioscorea* spp.), Agavaceae (e.g. *Agave* and *Yucca* spp.) and Smilacaceae (*Smilax* spp.). In the dicotyledons the occurrence of diosgenin in fenugreek (Leguminosae) and of steroidal alkaloids in *Solanum* (Solanaceae) is of potential importance. Some species of *Strophanthus* and *Digitalis* contain both steroidal saponins and cardiac glycosides (q.v.). Examples of saponins and their constituent sugars are given in Table 23.1.

Steroidal saponins are of great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisone, diuretic steroids, vitamin D and the cardiac glycosides. Some are used as starting materials for the synthesis of these compounds. Diosgenin is the principal sapogenin used by industry but most yams, from which it is isolated, contain a mixture of sapogenins in the glycosidic form.

Table 23.1 Examples of steroidal saponins.

Steroidal saponin	Sugar components	Occurrence
Sarsaponin (Parillin)	3 glucose, 1 rhamnose	*Smilax* spp.
Digitonin	2 glucose, 2 galactose, 1 xylose	Seeds of *Digitalis purpurea* and *D. lanata*
Gitonin	1 glucose, 2 galactose, 1 xylose	Seeds and leaves of *D. purpurea* and seeds of *D. lanata*
Dioscin	1 glucose, 2 rhamnose	*Dioscorea* spp.

As with cardiac glycosides, the stereochemistry of the molecule is of some importance, although not so much so for cortisone manufacture. Natural sapogenins differ only in their configuration at carbon atoms 3, 5 and 25, and in the spirostane series the orientation at C-22 need not be specified (cf. steroidal alkaloids). Mixtures of the C-25 epimers—for example, diosgenin (Δ^5,25α-spirosten-3β-ol) and yamogenin (Δ^5,25β-spirosten-3β-ol)—are of normal occurrence and their ratio, one to the other, is dependent upon factors such as morphological part and stage of development of the plant. In some instances in the plant, the side-chain which forms ring F of the sapogenin is kept open by glycoside formation as in the bisdesmosidic saponin sarsaparilloside of *Smilax aristolochiaefolia*.

BIOGENESIS OF STEROIDAL SAPONINS

Steroidal saponins arise via the mevalonic acid pathway; the preliminary stages have been discussed in Chapter 18. A scheme for the subsequent cyclization of squalene to give cholesterol is illustrated in Fig. 23.1. Cholesterol, the wide distribution of which in plants has only relatively recently been shown, can be incorporated into a number of C_{27} sapogenins without side-chain cleavage (Fig. 23.2), although it is not necessarily an obligatory precursor. Extensive investigations involving whole plants, homogenates and cell cultures have been performed to elucidate these detailed pathways, including the origin of the 25-epimers (e.g. diosgenin and yamogenin).

As early as 1947 Marker and Lopez had postulated that steroidal saponins exist in plants in a form where the side-chain is held open by glycoside formation. However, direct evidence for the natural occurrence of these compounds was not forthcoming for another 20 years. It has been shown that such open-chain saponins are, like the more common ones, formed from cholesterol. In *Dioscorea* homogenates one such compound has been converted to dioscin (a diosgenin glycoside) (Fig. 23.3).

NATURAL STEROIDS FOR THE PRODUCTION OF PHARMACEUTICALS

Although *total* synthesis of some medicinal steroids is employed commercially, there is also a great demand for natural products which will serve as starting materials for their *partial* synthesis.

As indicated in Fig. 23.4, which illustrates the range of steroids required medicinally, cortisone and its derivatives are 11-oxosteroids, whereas the sex hormones, including the oral contraceptives, and the diuretic steroids have no oxygen substitution in the C-ring. Fig. 23.5 shows some of the more important natural derivatives which are available in sufficient quantity for synthetic purposes. Hecogenin with C-ring substitution provides a practical starting material for the synthesis of the corticosteroids, whereas diosgenin is suitable for the manufacture of oral contraceptives and the sex hormones. Diosgenin, however, can also be used for corticosteroid synthesis by the employment, at a suitable stage in the synthesis, of a microbiological fermentation to introduce oxygen into the 11α-position of the pregnene nucleus.

Efforts are constantly being made to discover new high-yielding strains of plants and to assure a regular supply of raw material by the cultivation of good-quality plants. Hardman in a review on steroids (*Planta Med.*, 1987, **53**, 233) recorded that, annually, the American *Chemical Abstracts* contained some 3000 references pertinent to plant steroids or related compounds. Some of the better-known examples of steroidal sapogenins and their sources are given in Table 23.2. (For a review, tabulating over 200 sapogenins, see A. V. Patel *et al., Fitoterapia*, 1987, **58**, 67.)

Dioscorea species

Tubers of many of the dioscoreas (yams) have long been used for food, as they are rich in starch. In addition to starch, some species contain steroidal saponins, others alkaloids. From a suitable source the sapogenins are isolated by acid hydrolysis of the saponin. Previous fermentation of the material for some 4–10 days often gives a better yield. The water-insoluble sapogenin is then extracted with a suitable

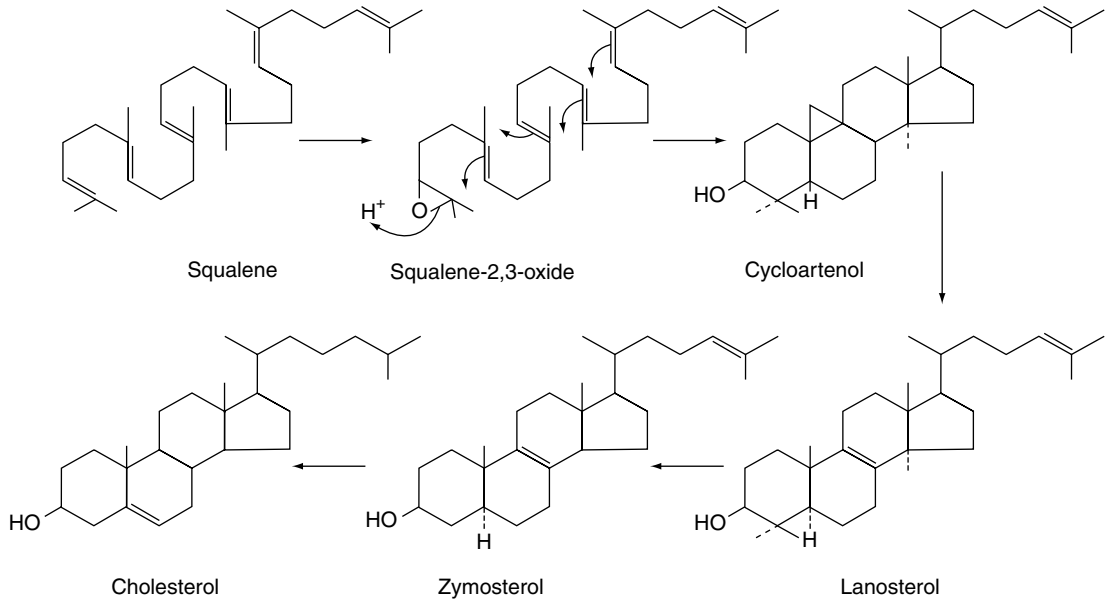


Fig. 23.1
Possible route for the formation of cholesterol in higher plants and algae.

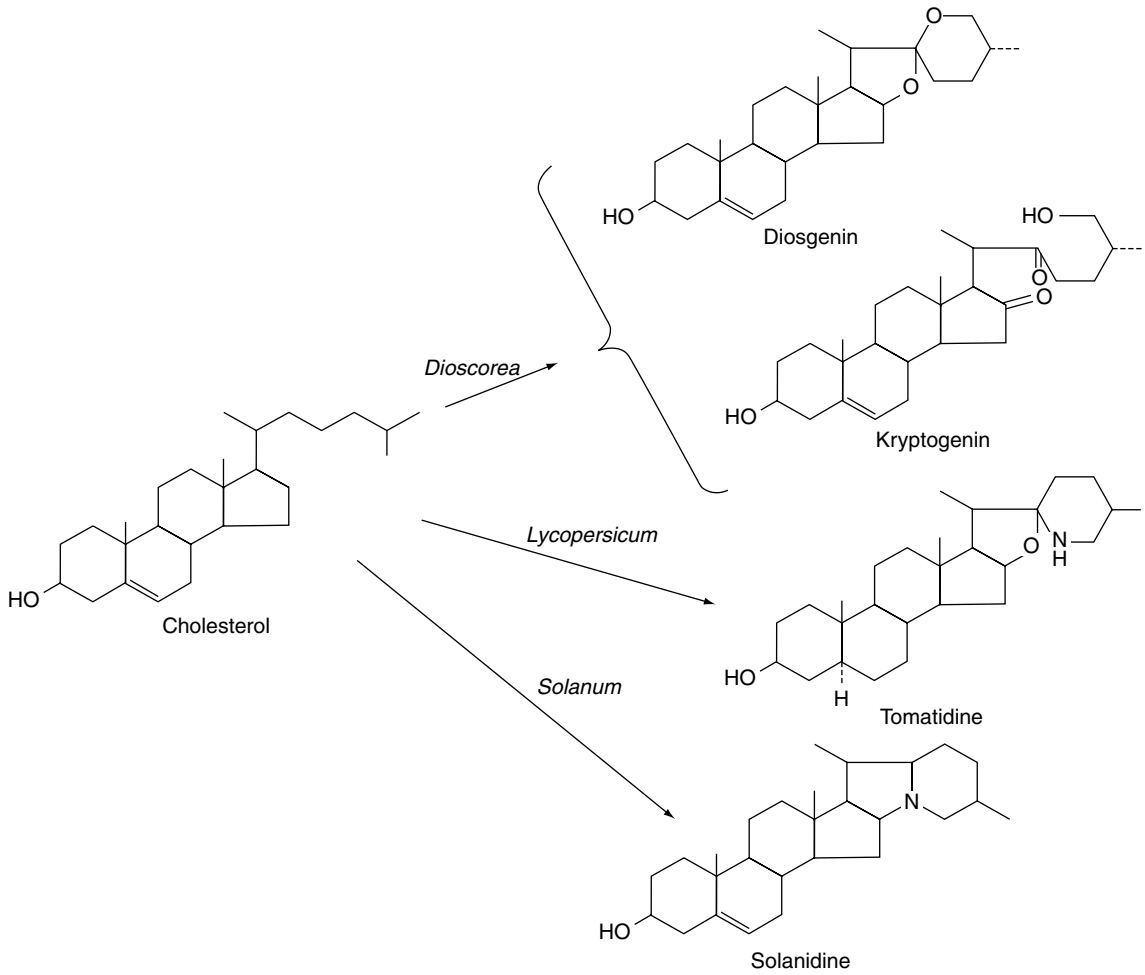


Fig. 23.2
Some plant metabolites of cholesterol.

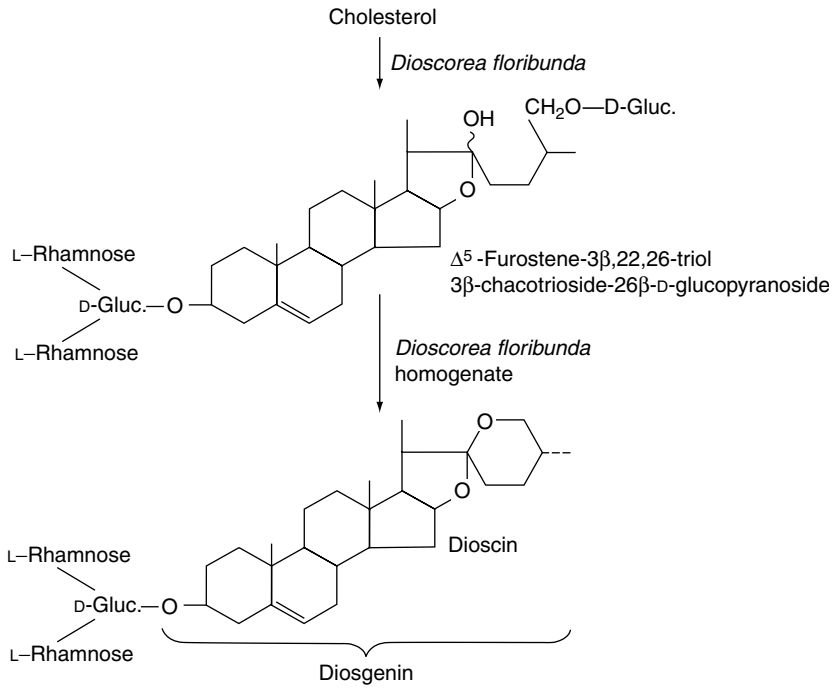


Fig. 23.3
Formation and metabolism of an open-chain saponin in *Dioscorea*.

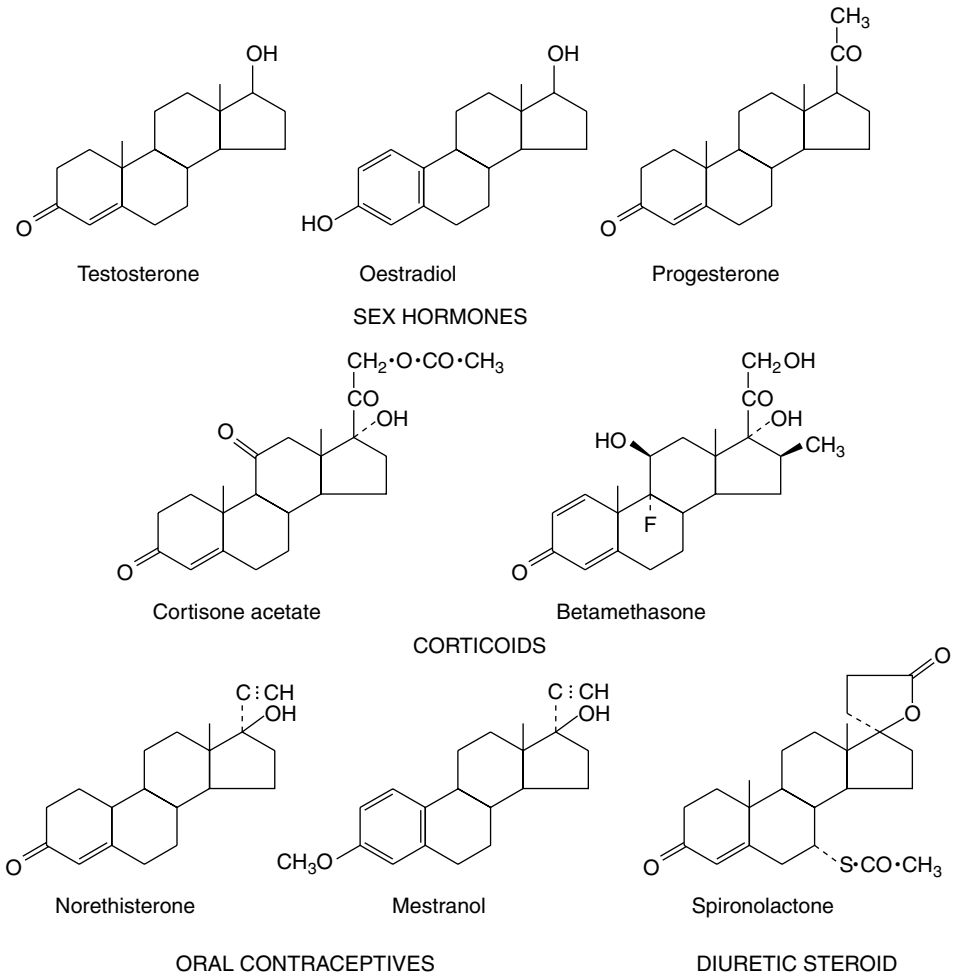


Fig. 23.4
Examples of structures of therapeutically active steroids.

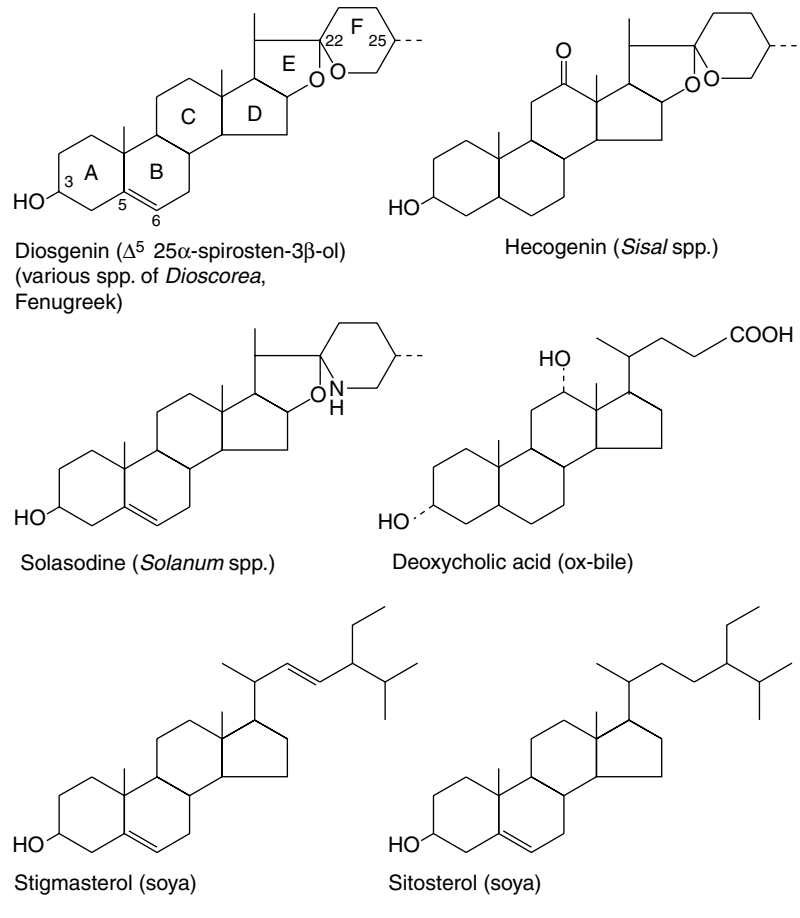


Fig. 23.5
Some naturally occurring steroids.

Table 23.2 Some steroidal sapogenins and their sources.

Sapogenin	Species	Location
Diosgenin	*Dioscorea sylvatica*	Transvaal and Natal
	D. mexicana and *D. composita*	Mexico and Central America
	D. collettii, D. pathaica and *D. nipponica*	China
	D. floribunda	Guatemala and cultivated in India
	D. deltoidea and *D. prazeri*	India
	D. tokoro	Japan
	Costus speciosus	India
	Kallstroemia pubescens	Tropical America; introduced into West Brazil
	Trillium spp.	North America
	Trigonella foenum-graecum	India, Egypt, Morocco
Hecogenin	*Agave sisalana*	Subtropical America and cultivated in Kenya for sisal and saponin
	A. rigida	Mexico
	Hechtia texensis	Central America
Sarsapogenin	*Yucca* spp., *Smilax* spp.	Central America
Sarmentogenin	*Strophanthus* spp.	Africa

organic solvent. Both wild and cultivated plants are used. Cultivation requires attention to correct soil and drainage, support for the vines and freedom from weeds, virus, fungus and insect attack. According to the species, the tubers reach maturity in 3–5 years and on average, yield 1–8% of total sapogenin.

Until 1970 diosgenin isolated from the Mexican yam was the sole source for steroidal contraceptive manufacture. With the nationalization of the Mexican industry, however, prices were increased to such an extent that manufacturers switched to hecogenin for corticosteroids, to other sources of diosgenin and to the use of the steroidal alkaloids of *Solanum* species. Total synthesis also became economically feasible and is now much used. More recently, the economics of steroid production have again changed in that China is now exporting large quantities of diosgenin; it is of high quality, being free of the 25β-isomer yamogenin, although this is of no commercial significance, and is reasonably priced. Three of the many *Dioscorea* spp. found in China and used commercially are given in Table 23.2; the tubers of these yield 2% of diosgenin, with the average content of diosgenin for the main areas of production (Yunnan Province and south of the Yangtze River) being 1%.

Sisal

Hecogenin is obtained commercially as the acetate in about 0.01% yield from sisal leaves (*Agave sisalana*). In East Africa, from leaf 'waste' stripped from the leaves during removal of the fibre, a hecogenin-containing 'sisal concentrate' is produced. From this the 'juice' is separated and allowed to ferment for 7 days. The sludge produced contains about 80% of the hecogenin originally present in the leaves; steam at 1380 kPa pressure is employed to complete the hydrolysis of the original glycosides. By filtration and drying a concentrate containing about 12% hecogenin and varying amounts of other sapogenins is produced. This crude material is shipped for further processing and cortisone manufacture. Hecogenin is also produced in Israel and China. A number of new steroidal saponins have been isolated from the dried fermented residues of Chinese *A. sisalana* forma Dong No. 1 (see Yi Ding *et al*., *Chem. Pharm. Bull*., 1993, **41**, 557).

A survey of 34 species of *Agave* by Blunden and colleagues in 1978 showed that the extracts of most yielded steroidal sapogenins. Previously it had been shown that certain commercial samples of crude sapogenins from *A. sisalana* also contained the dihydroxy steroid rockogenin, sometimes in appreciable quantity; this compound appears to be an artefact formed during processing and should be avoided. A dihydroxyspirostane, barbourgenin, has been described (G. Blunden *et al*., *J. Nat. Prod*., 1986, **49**, 687). *Agave* hybrids with a high hecogenin content and relatively free of tigogenin, with which it is usually associated, have been developed. Gbolade *et al*. (*Fitoterapia*, 1992, **63**, 45) reported on factors (season, geographical location) affecting steroidal sapogenin levels in Nigerian *Agave* and *Furcraea* species.

Another genus of the Agavaceae which has been systematically studied for the presence of steroidal compounds is *Cordyline* in which many sapogenins, including 1,3-dihydroxysapogenins, have been detected.

FENUGREEK

Although included in this section as a potential industrial source of diosgenin, the seeds of *Trigonella foenum-graecum* L. (Leguminosae) are also described in the *BP* and *EP*. However their principal current use is as a spice; India, Morocco and Egypt among others being important producers.

The very hard seeds have a strong characteristic odour and are irregularly rhomboidal and oblong or square in outline. They are somewhat flattened and are divided into two unequal parts by a groove in the widest surfaces. Their shape and size, and position of the embryo and hilum are shown in Fig. 23.6. The *BP*/*EP* describes the seeds as brown to reddish-brown but in commerce olive-green or yellow-brown samples are often

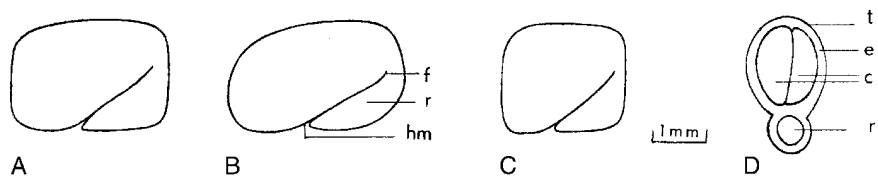
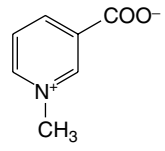


Fig. 23.6
Commercial fenugreek seed. A, Morocco, Israel; B, Ethiopia; C, India, Pakistan; D, transverse section of a seed. c, Cotyledons; e, endosperm; f, furrow; hm, hilum and micropyle region; r, radicle; t, testa. (After Fazli and Hardman, *Trop. Sci.*, 1968 **10**, 66.)

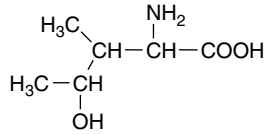
encountered. Microscopically the testa and hypodermis are characteristic and the mucilage-containing endosperm enables the swelling index (q.v.) of the seeds (not less than six) to be used as a test of purity.

Fenugreek contains the simple pyridine-type alkaloid trigonelline; this base, also reported in garden peas, hemp seed, coffee and many other products was first isolated and described by Jahns in 1885. Today it serves as the reference substance for the *BP*/*EP* TLC identification test for the drug. Pharmaceutical manufacturing interest lies in a number of steroidal sapogenins, particularly diosgenin which is contained in the oily embryo. Fazli and Hardman investigated a number of commercial samples of seed as possible commercial sources of diosgenin (see reference in Fig. 23.6) and reported contents of 0.8–2.2% expressed on a moisture-free basis. In 1986, Gupta and colleagues isolated a series of furostanol glycosides (F-ring opened) named trigofoenocides A–G. In a series of papers, further furostanol glycosides designated trigoneosides Ia, Ib–Xlla have been reported in Egyptian seeds (T. Murakami *et al*., *Chem. Pharm. Bull*., 2000, **48**, 994). As with dioscoreas, the yield of diosgenin is increased by fermentation of the seeds prior to acid hydrolysis. Although the diosgenin yield is lower than that of the dioscoreas, fenugreek is an annual plant which will also give fixed oil, mucilage, flavouring extracts and high-protein fodder as side-products. A number of Hardman's registered varieties have been subjected to field trials in the UK. However, as long as other cheap sources of diosgenin are available commercially, fenugreek must be regarded as a fall-back source for this sapogenin.

A non-essential amino acid, 4-hydroxyisoleucine, first identified by Fowden *et al*. in 1973, constitutes up to 80% of the free amino acid composition of fenugreek seeds and has been shown to possess insulin-stimulating properties both *in vitro* and *in vivo* (C. Haefelé *et al*., *Phytochemistry*, 1997, **44**, 563). Some 39 components of the volatile oil fraction have been identified.



Trigonelline



4-Hydroxyisoleucine

Based on mucilage content, the *BP*/*EP* sets a swelling index of not less than 6.0 for the powdered seeds.

In addition to its use as a spice and potential source of diosgenin fenugreek is widely employed in traditional systems of medicine. Its antidiabetic, cholesterol-lowering, anti-inflammatory, antipyretic, antiulcer and anticancer properties have been demonstrated.

Further reading

Petropoulos GJ (ed), Hardman R (series ed) 2002 Fenugreek: The genus *Trigonella*. CRC Press, Taylor and Francis Group, Boca Raton, FL, 200 pp. *646 refs*

Solanum species

This large genus (over 1000 spp.) is noted for the production of C_{27} steroidal alkaloids in many species. Some of these alkaloids are the nitrogen analogues of the C_{27} sapogenins (e.g. solasodine and diosgenin: Fig. 23.5). Another series of C_{27} compounds contain a tertiary nitrogen in a condensed ring system (e.g. solanidine; Fig. 23.2). These compounds can also be employed in the partial synthesis of steroidal drugs, and a number of companies have devoted considerable attention to commercial production. Species so exploited are *Solanum laciniatum, S. khasianum* (a nearly spineless variety has been produced) and *S. aviculare*; trials on the production of *S. marginatum* have been conducted in South America. Zenk and colleagues have assayed over 250 spp. of *Solanum* for solasodine. A number of new glycosides have been isolated from *S. dulcamara* leaves and include two based on tigogenin and two on soladulcidine.

The steroidal alkaloids were reviewed in 1993 by Atta-ur-Rahman and M. I. Choudhary (*Methods in Plant Biochemistry* (ed. P. G. Waterman) Vol. 8. Academic Press, London, p. 451).

Soya bean sterols

The soya (soy, soja) plant, *Glycine max* (*G. soya*) (Leguminosae) is extensively cultivated for its seeds, which are rich in oil and protein. The seeds also contain appreciable quantities of the phytosterols stigmasterol and sitosterol (Fig. 23.5). Although not sapogenins, they are included here because they are now used extensively for steroid synthesis. They are obtained as byproducts of soap-making, being components of the unsaponifiable matter of the fixed oil. Pure stigmasterol, with its unsaturated side-chain is amenable to chemical conversion to suitable starting materials and can replace diosgenin. But it was more recently that sitosterol, the saturated side-chain of which could not be removed chemically without ring fragmentation, became commercially useful as the result of the discovery of a suitable microbiological side-chain removal. Both phytosterols are now processed by microorganisms. Similar phytosterols are found in other products—for example, cotton-seed oil, tall-oil (from the wood-pulp industry) and sugarcane wax.

For details of the soya isoflavones and their dietary importance, see Chapter 32: *The plant nutraceuticals*.

Sarsaparilla root

Sarsaparilla consists of the dried roots and sometimes also of the rhizomes of species of *Smilax* (Liliaceae, modern authors, Smilacaceae). The determination of the exact geographic and botanical sources of the numerous varieties which have from time to time been imported has been a matter of some difficulty (see Table 23.3).

The plants produce numerous roots, 3 m or so long, which are attached to a short rhizome. The roots are cut, sufficient, however, remaining in the ground for the plant to resume its growth. Sometimes

Table 23.3 Varieties of sarsaparilla.

Variety and geographic source	Synonyms	Botanical source
Mexican (Southern Mexico, Guatemala, British Honduras)	Vera Cruz or Grey	*Smilax aristolochiaefolia*
Honduras (Guatemala, British Honduras, Honduras, cultivated in Jamaica)	Brown	*S. regelii*
Ecuadorian and Peruvian	Guayaquil	*S. febrifuga*
Central American	Costa Rica or 'Jamaican'	Undetermined spp.

the rhizomes as well as the roots are collected. After drying in the sun the drug is made into bundles and the bundles into bales.

Sarsaparilla is imported in large bales bound with wire. Each bale usually contains numerous bundles of approximately uniform size. These consist of long roots, with or without pieces of rhizome and aerial stems. The commercial varieties (Table 23.4) differ from one another in colour, ridges and furrows; in the presence or absence of rhizome and aerial stems; in the relative proportions of cortex, wood and pith, as seen in transverse section; in their microscopical structure. The drug is nearly odourless but has a somewhat sweetish and acrid taste. Owing to the presence of saponins, aqueous extractives froth readily.

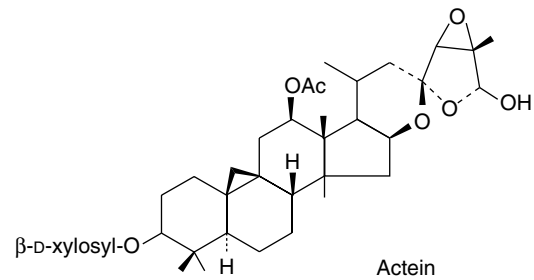
Much chemical work has been done on sarsaparillas without proper botanical identification of the material. Different species contain one or more steroidal saponins. Two isomeric genins are known: smilagenin and sarsasapogenin. These differ only in their configuration at C-25 and correspond to the reduced forms of diosgenin and yamogenin respectively. The principal crystalline glycoside of *Smilax aristolochiaefolia* is parillin (sarsasaponin, sarsasaponoside); it was first isolated from a sample of Jamaica sarsaparilla in 1913 by Power and Salway. On hydrolysis it gives sarsasapogenin, three molecules of glucose and one of rhamnose. Sarsaparilloside, contained in the same species, is a bisdesmosidic saponin (i.e. it possesses two distinct glycosyl groupings, in this case at C-3 and C-26) and represents the parillin molecule with an opened F-ring stabilized by glucosylation.

Uses. Sarsaparilla formerly enjoyed a high reputation in the treatment of syphilis, rheumatism and certain skin diseases. It is included in the *BHP* (1960) where it is indicated in the treatment of psoriasis and eczema, and for rheumatism and rheumatoid arthritis. Its action would appear to arise from the steroid content of the roots. Sarsaparilla is widely used as a vehicle, and large quantities are employed in the manufacture of non-alcoholic drinks. The genins are used in the partial synthesis of cortisone and other steroids.

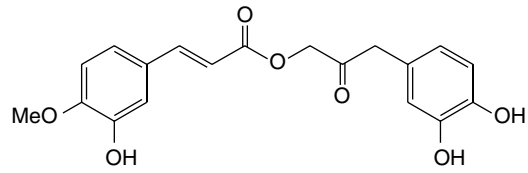
Black Cohosh

Black Cohosh, Cimicifuga *BHP* 1983, is the dried rhizome and roots of *Actea racemosa* L. syn. *Cimicifuga racemosa* [L.], Nutt., family Ranunculaceae. This perennial plant is native to Canada and the northern American states and was well-known to the native Americans of these areas. The drug contains substances with endocrine activity and extracts have been widely employed in herbal medicine to treat menopausal and other female disorders, as well as various rheumatic conditions. However, in the 1990s doubts arose over its safety following reports concerning its hepatotoxicity; restrictions on its use have now been applied in a number of countries.

The rhizomes contain a number of triterpenoid glycosides, including actein, 23-epi-26-deoxyactein and cimiracemoside C. Cimicifugoside M and cimifugin can specifically serve as indicators for species identification (K. He *et al.*, *Planta Med.*, 2002, **66**, 635). Phenyl propanoside esters are also present including cimiracemates A–D together with ferulic acid, isoferulic acid and methyl caffeate (S.-N. Chen *et al.*, *Phytochemistry*, 2002, **61**, 609). For other reports, see M. Nishida *et al.*, *Chem. Pharm. Bull.*, 2003, **51**, 1215; H. Yoshitsu *et al.*, *Chem. Pharm. Bull.*, 2006, **54**, 1322.



Actein



Cimiracemate A

BUTCHER'S BROOM

Butcher's broom, *Ruscus aculeatus* L. family Liliaceae, is a perennial, rigid, dark green much-branched bush, 2–3 feet in height. The leaf-like structures, twisted at the base and terminating in a sharp point, are actually cladodes—flattened stems or internodes that resemble and function as leaves. Small white flowers that arise in the axils of scarious bracts are followed by red berries, which appear situated on the surface of the cladodes.

The species is found in woods and dry places, extending across southern Europe to the Caucasus, and northward to Belgium. It is common in some southern areas of England.

The *BP*/*EP* drug consists of the dried, whole or broken roots and rhizomes of the plant. These are collected in autumn and dried to give yellow, knotty pieces of rhizome up to 10 cm in length, showing stem scars on the upper surface and roots and root-scars on the lower surface. A very hard, central cylinder can easily be removed from the outer layer.

Microscopical characteristics, as seen in the powder, include cells with thickened beaded walls with large oval pits, thin-walled

Table 23.4 Macroscopical characters of sarsaparilla.

	Mexican	Honduras	Ecuadorian	Central American
Bundles	Up to 65 m long. Rhizomes and stems up to 10%	50–75 cm long. Roots only	About 50 cm long. Rhizomes and stems up to 10%	About 45 cm long. Roots only
Diameter of roots	3.5–6 mm	2–5.5 mm	2–6 mm	1–4.5 mm
Colour	Greyish-reddish- or yellowish-brown	Reddish-brown to dark brown	Reddish-brown to purplish	Reddish-brown to yellowish brown
Hypodermal and endodermal cells	Horse-shoe thickening	Uniform thickening	Variable. Sometimes not uniform thickening	Uniform thickening

parenchyma containing calcium oxalate, thick-walled fibres, and small vessels.

The rhizomes of this plant contain saponins related to those of *Dioscorea*; thus, one sapogenin is 1β-hydroxydiosgenin (ruscogenin). The plant glycosides involve up to three sugars attached at the C-1 hydroxyl with glucose terminating an uncyclized side-chain at C-26 (for detailed structures see Bombardelli *et al.*, *Fitoterapia*, 1972, **43**, 3). In a series of publications on *Ruscus aculeatus* M. Yoshikawa *et al.* have described many more saponins of both the spirostanol and furostanol series and recently, a new saponin which is unique in having a diglucoside unit at C-23 of the spirostanol skeleton (*Phytochemistry*, 1999, **51**, 689). Both the alcoholic extract of the roots and the ruscogenins themselves have anti-inflammatory activity, produce diminished capillary permeability and exert a vasoconstrictor effect in the peripheral blood vessels. On the continent of Europe ointments and suppositories containing the active constituents are available for the treatment of conditions responding to the above effects.

The *BP*/*EP* TLC test for identity uses stigmasterol and ruscogenins as reference substances and a vanillin reagent for their visualization. A minimum of 1.0% total sapogenins, expressed as ruscogenins (mixture of neoruscogenin and ruscogenin) is specified; liquid chromatography is used for the assay.

Allied species. Various ruscogenins and a major new saponin have been detected in the rhizomes of *Ruscus colchicus* and *R. hypoglossum* (E. de Combarieu *et al.*, *Fitoterapia*, 2002, **73**, 583; 2003, **74**, 423, corrigendum).

ELEUTHEROCOCCUS

The drug Siberian Ginseng (*BP*/*EP*, *BHP*) consists of the dried, whole or cut organs of *Eleutherococcus senticosus* Maxim. [*Acanthopanax senticosus* (Rupr. et Maxim.) Harms], family Araliaceae. The plant is native to China and is now cultivated there and in Russia, Japan and Korea.

Characters. The pale brown, uneven rhizomes, up to 4 cm in diameter, bear the scars of aerial stems and on the lower surface are roots and root scars. The fracture is fibrous and the internal surface light brown to pale yellow. The cylindrical roots are of variable length, up to about 1 cm in diameter, knotty and somewhat branched. When dry the bark is not easily removed from the underlying xylem; commercially it may be removed at harvest and both wood and bark used. Features of the powder include lignified fibres, reticulate and border-pitted vessels, parenchymatous cells containing cluster crystals of calcium oxalate and secretory canals with brown contents. Odour faintly aromatic; taste bitter and persistent.

Constituents. The rhizomes and roots contain a number of constituents termed eleutherocides (A to G; M). These, however, are not all of the same chemical group but include the phenyl propane glycosides eleutheroside B (syringin) and its aglycone sinapyl alcohol; also the lignans (−)-syringaresinol diglucoside (E) and its stereoisomer (D) (formulae Table 21.7), together with caffeic acid and its ethyl ester, chlorogenicacid and coniferaldehyde. Coumarins include coumarin, isofraxidin 7-glucoside (B_1) and sesamin (B_4). A group of heteroglycans (eleutherans A to G) have been studied for their hypoglycaemic activity. Other constituents include hedera-saponin (M), daucosterol (A) and volatile oil, about 0.8%.

The *BP*/*EP* requires a minimum content of 0.8% for the sum of eleutheroside B and eleutheroside E determined by liquid chromatography using UV spectrophotometric absorption at 220 nm and measurement of peak areas. These eleutherosides are also characterized by the TLC test for identity.

DNA analysis has been used to authenticate Japanese and Chinese commercial samples of the drug (T. Maruyama *et al*., *Planta Medica*, 2008, **74**, 787).

Uses. Eleutherococcus has been used in Chinese medicine since antiquity for the treatment of rheumatoid complaints and for its revitalization properties. For many years, its adaptogenic qualities have been utilized in former USSR countries and now in W. Europe it is employed as a tonic in states of fatigue.

Further reading

Davydov M, Krikorian AD 2000 *Eleutherococcus senticosus* (Rupr. and Maxim.) (Araliaceae) as an adaptogen: a closer look. J. Ethnopharmacology 72: 345–393. *An extensive discussion including chemical constituents, numerous formulae, over 200 refs*

GINSENG

For some 2000 years the roots of *Panax ginseng* C. A. Meyer (Araliaceae) have held an honoured place in Chinese medicine. Today it is a product of world-wide usage. Production is principally confined to China, Korea and Siberia, although it is cultivated commercially on a small scale in Holland, England, Germany and France (Champagne district).

The most expensive ginseng is that derived from Korean root. The plant, about 50 cm tall with a crown of dark green verticillate leaves and small green flowers giving rise to clusters of bright red berries, is cultivated under thatched covers and harvested when 6 years old. Sun-drying of the root, after removal of the outer layers, produces white ginseng, whereas the red ginseng is obtained by first steaming the root, followed by artificial drying and then sun-drying. Rootlets are numerous on the lower surface of white ginseng but normally absent from red ginseng. The roots are graded and packed. Nineteenth-century descriptions record the care then taken with the preparation, silk, cotton and paper wrappings being used according to the quality of the drug; the wrapped roots were finally stored in containers with quicklime. Small roots are processed separately and form a separate article of commerce. The *BP*/*EP* recognizes both red and white ginseng.

The scraping of the roots before drying would appear to be disadvantageous because histochemical tests and GLC analysis show the active saponins to be located outside the root cambium.

Constituents. *P. ginseng* roots have been thoroughly studied by modern methods of analysis and, of the many compounds isolated, the medicinal activity appears to reside largely in a number of dammarane-type saponins termed ginsenosides by Japanese workers and panaxosides by Russian workers. These two series of compounds, all now generally termed ginsenosides, are glycosides respectively derived from the diol 20(S)-protopanaxadiol and the triol 20(S)-protopanaxatriol. Examples of the former are ginsenosides R_{b1}, R_{b2} and R_{b4} and of the latter ginsenosides (panaoxosides) R_e, R_f, R_{g1}, R_{g2} (see Fig. 23.7). Acid hydrolysis of these saponins involves ring closure of the aglycone giving either panaxadiol or panaxatriol (Fig. 23.7). Some 30 ginsenosides have been named, although not all fit into the above scheme, e.g. ginsenoside R_o is an oleanolic acid derivative. Glucose is the principal sugar involved with some input of arabinose and rhamnose.

The *BP*/*EP* specifies a minimum of 0.40% for the sum of ginsenosides R_{g1} and R_{b1}; this is determined by liquid chromatography of a methanolic extract using a reference solution containing the two ginsenosides to he assayed, with absorption measurements at 203 nm. TLC is used as a test for identity and to exclude substitution with *P. quinquifolium*, which contains no ginsenoside Rf.

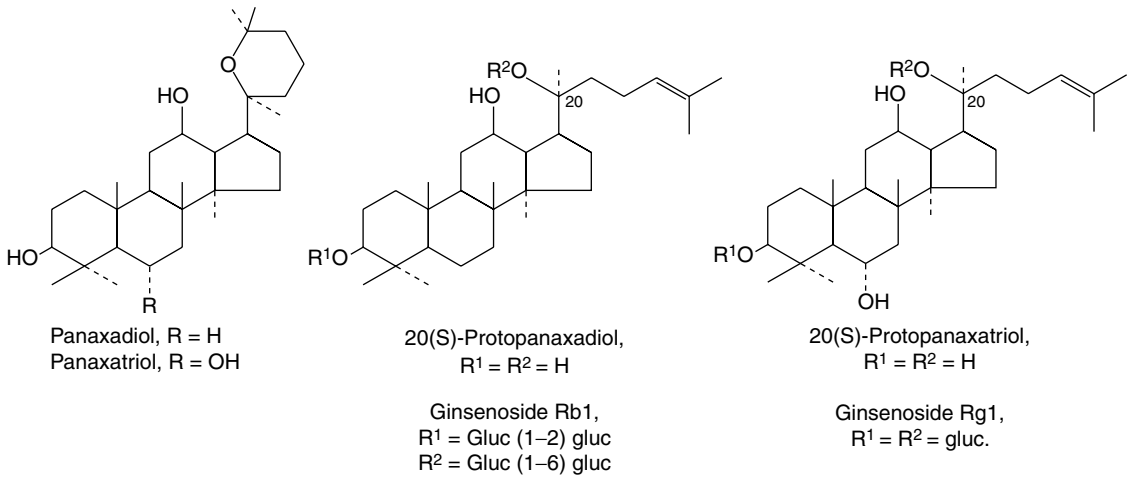


Fig. 23.7
Steroids associated with ginseng.

Two other groups of compounds present in the root which have known therapeutic activity are high molecular weight polysaccharides (glycans) and acetylenic compounds. The glycans of *P. ginseng* have been named panaxans (A–U); panaxans A and B have been shown to be constituted mainly of α-(1 → 6) linked D-glucopyranose units with C-3 branching and a small component of peptide. (For the isolation of other polysaccharides termed ginsenans see M. Tomoda *et al.*, *Biol. Pharm. Bull.*, 1993, **16**, 1087.) Those glycans tested have hypoglycaemic, antiulcer and immunological properties. One acidic polysaccharide MW 150000, originally isolated in 1993, and composed of 3.7% protein, 47.1% hexoses and 43.1% galacturonic acid, has antineoplastic immuno-stimulant properties.

A considerable number of mainly C_{17}, but also C_{14}, polyacetylenic alcohols have been isolated from the roots in recent years and are typified by panaxynol and panaxydol (Fig. 23.8). These compounds have been shown to have antitumour properties and Japanese patents exist for their isolation and derivatization. The cytotoxic activity of the C_{17}-polyacetylenes against leukaemia cells has been shown to be almost 20 times greater than that for the C_{14}-compounds (Y. Fujimoto *et al.*, *Phytochemistry*, 1992, **31**, 3499). K. Hirakura *et al.* have characterized a series of polyacetylenes named ginsenoynes A–K (see *Phytochemistry*, 1992, **31**, 899 and references cited therein) and subsequently (*ibid.*, 1994, **35**, 963) three new linoleoylated polyacetylenes.

Other constituents include sesquiterpenes (panacene, β-elemene, panasinsanol A and B, ginsenol, etc.) to be found in the volatile oil (0.05–0.1%), together with various monoterpenes and monoterpene alcohols. Three minor sesquiterpenes recently identified are panaxene, panaginsene and ginsinsene (R. Richter *et al.*, *Phytochemistry*, 2005, **66**, 2706). Lignans of the dibenzocyclooctadiene type have been isolated from Korean Red Ginseng (Fig. 23.8). Minor components isolated from ginseng roots include sterols, vitamins of the D group, flavonoids and amino acids.

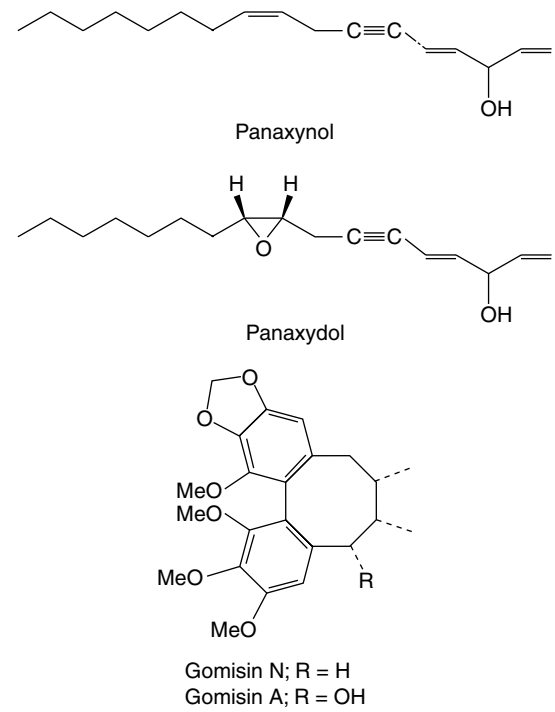


Fig. 23.8
Acetylenes and lignans of ginseng.

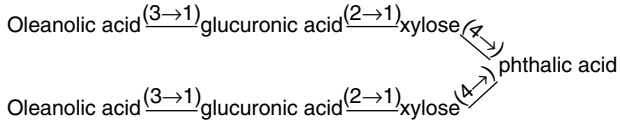
Uses. In Asia the drug is held in esteem for the treatment of anaemia, diabetes, gastritis, sexual impotence and the many conditions arising from the onset of old age. In the West, too, it has in recent years become an extremely popular remedy particularly for the improvement of stamina, concentration, resistance to stress and to disease; in this sense the action of the drug is described as 'adaptogenic'.

Many 'ginseng' products are available as OTC products either for oral administration or as cosmetic preparations. In the US mainstream market for herbal sales, for the first eight months of 1999 ginseng stood at third place, with retail sales valued at over $60 million. Ginseng is included in the *BHP* (1996) and 29 references covering its constituents and actions are given in the *British Herbal Compendium*, Vol. 1 (1992).

Allied species. *Panax quinquefolium* root is one of the major drugs of US foreign trade. It is produced in the eastern USA and Canada, 90% of the US cultivated drug coming from north-central Wisconsin. Some of the ginsenosides of this species are the same as those of the Chinese and Korean drug; others appear to differ. In addition to 14 known dammarane-type saponins, M. Yoshikawa *et al.* (*Chem. Pharm. Bull.*, 1998, **46**, 647) identified a further five new compounds designated quinquenosides I–V. D. Don *et al.* (*Chem. Pharm. Bull.*, 2006, **54**, 751) have reported on a new dammarane-type saponin, ginsenoside R_{g8}, together with other known ginsenosides. In 1997, Kitanaka *et al.* showed the hybrid *P. ginseng* × *P. quinquefolium* to be superior

to either parent in production of ginsenosides. However, as the plant is sterile, root cultures were established and these showed a comparable ginsenoside production to the field grown material (D. Washida *et al.*, *Phytochemistry*, 1998, **49**, 2331).

Panax pseudoginseng ssp. *himalaicus* var. *augustifolius* (Himalayan ginseng). The roots contain active saponins and ginsenosides R_0 and R_{b1}; chikusetsusaponins IVa and VI have been recorded. Shukla and Thakur (*Phytochemistry*, 1990, **29**, 239) characterized pseudoginsenoside-RI_2 which consists of oleanolic acid, phthalic acid, glucuronic acid and xylose moieties arranged as below:



Panax notoginseng roots (Sanchi-ginseng) contain dammarane saponins, identical or similar to those of ginseng. M. Yoshikawa *et al*. (*Chem. Pharm. Bull.*, 1997, **45**, 1039; 1056) have isolated, in addition to 14 known saponins, nine new dammarane-type oligoglycosides named notoginsenosides A–J. The roots also contain a polysaccharide (sanchinan-A) having a branched structure with a galactose backbone and side-chains containing arabinose and galactose; this glycan contains a small amount of protein and possesses reticuloendothelial activating properties (K. Ohtani *et al.*, *Planta Med.*, 1987, **53**, 16).

Panax japonicum and *P. japonicum* var. *major* contain chikusetsusaponins, ginsenosides and glycans.

Panax vietnamensis (Vietnamese ginseng). The roots are a secret remedy of the Sedang ethnic minority and contain a number of known and new ginsenosides (N. M. Duc *et al*., *Chem. Pharm. Bull*., 1993, **41**, 2010; 1994, **42**, 115, 634).

Further reading

Court WE (ed), Hardman R (series ed) 2000 Medicinal and aromatic plants—industrial profiles, Vol 15. Ginseng: the genus *Panax*. Harwood Academic, Amsterdam

PENTACYCLIC TRITERPENOID SAPONINS

Unlike the steroidal saponins, the pentacyclic triterpenoid saponins are rare in monocotyledons. They are abundant in many dicotyledonous families, particularly the Caryophyllaceae, Sapindaceae, Polygalaceae and Sapotaceae. Among the many other dicotyledonous families in which they have been found are the Phytolaccaceae, Chenopodiaceae, Ranunculaceae, Berberidaceae, Papaveraceae, Linaceae, Zygophyllaceae, Rutaceae, Myrtaceae, Cucurbitaceae, Araliaceae, Umbelliferae, Primulaceae, Oleaceae, Lobeliaceae, Campanulaceae, Rubiaceae and Compositae. Altogether some 80 families are involved.

In these saponins the sapogenin is attached to a chain of sugar or uronic acid units, or both, often in the 3-position, as in the examples above. Biosynthesis, as with the steroids, involves ring-closure of squalene and is illustrated in Fig. 23.9.

Triterpenoid saponins may be classified into three groups represented by α-amyrin, β-amyrin and lupeol.

The related triterpenoid acids are formed from these by replacement of a methyl group by a carboxyl group in positions 4, 17 or 20 (Fig. 23.10).

Plant materials often contain these saponins in considerable amounts. Thus, primula root contains about 5–10%; liquorice root about 2–12% of glycyrrhizic acid (and a correspondingly larger amount of glycyrrhizin, the potassium calcium salt); quillaia bark up to about 10% of the mixture known as 'commercial saponin'; the seeds of the horse-chestnut up to 13% of aescin. As some plants contain more than one saponin and purification is often difficult, the structures of even some of the well-known saponins given in Table 23.5 have only recently been established. Oleanolic acid also occurs as a saponin in sugar beet, thyme, *Guaiacum* spp. (also in the nor-form), and in the free state in olive leaves and clove buds.

Further reading

Tan N, Zhou J, Zhao S 1999 Advances in structural elucidation of glucuronide oleane-type triterpene carboxylic acid 3,28-*O*-bisdesmosides (1962–1997). Phytochemistry 52(2): 153–192

LIQUORICE ROOT

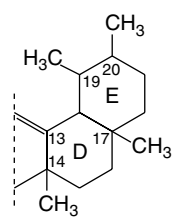
The pharmacopoeial drug is now defined as the dried unpeeled or peeled, whole or cut root and stolons of *Glyrrhiza glabra* L. and/or of *G. inflata* Bat. and/or *G. uralensis* Fisch. Varieties of *G. glabra* traditionally yielding the commercial drug are:

1. *Glycyrrhiza glabra* var. *typica* Reg. et Herd., a plant about 1.5 m high bearing typical papilionaceous flowers of a purplish-blue colour. The underground portion consists of long roots and thin rhizomes or stolons. The principal root divides just below the crown into several branches which penetrate the soil to a depth of 1 m or more. A considerable number of stolons are also given off, which attain a length of 2 m but run nearer the surface than the roots. The plant is grown in Spain, Italy, England, France, Germany and the USA.
2. *G. glabra* L. var. *glandulifera* Wald. et Kit. is abundant in the wild state in Galicia and central and southern Russia. The underground portion consists of a large rootstock, which bears numerous long roots but no stolons.
3. *G. glabra* var. β-*violacea* Boiss. yields the 'Persian' liquorice, which is collected in Iran and Iraq in the valleys of the Tigris and Euphrates; it bears violet flowers.

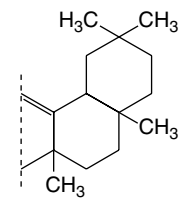
Much commercial extract is now obtained from the other species cited above.

Primula-saponin = L-rhamnose / D-glucose-D-galactose > D-glucuronic acid-*O*-3-primulagenin

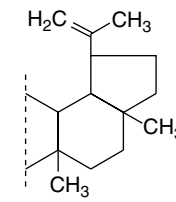
Glycyrrhizic acid = D-glucuronic acid-D-glucuronic acid-*O*-3-glycyrrhetinic acid



α-Amyrin



β-Amyrin



Lupeol

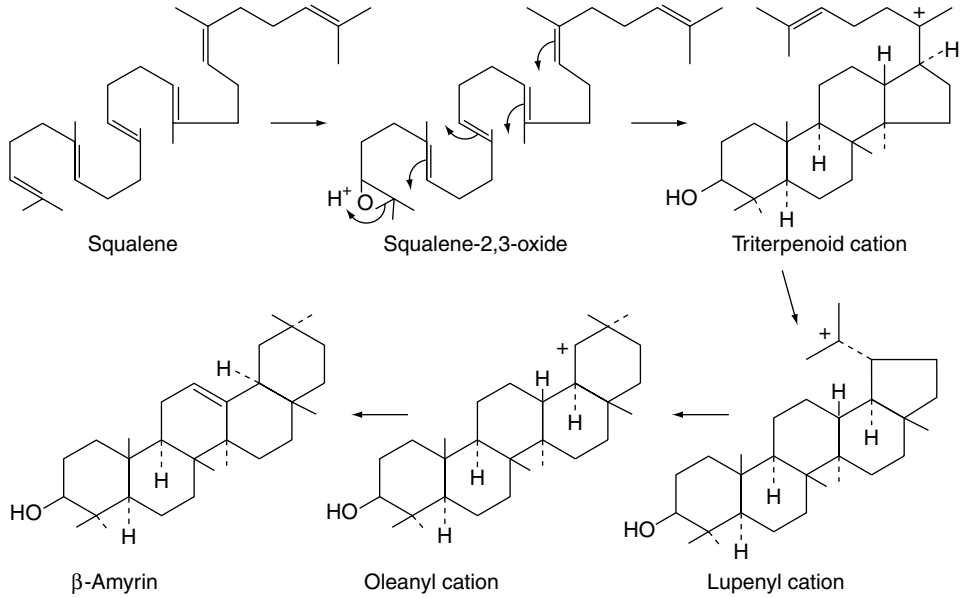
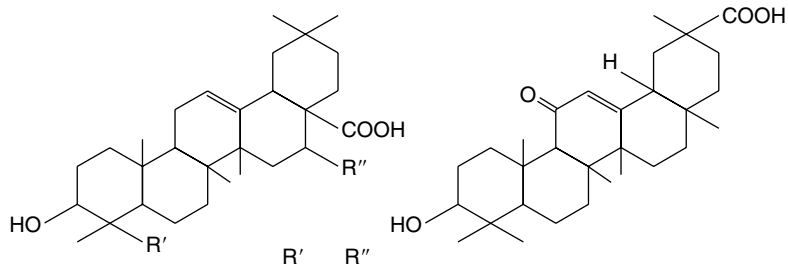


Fig. 23.9
Biosynthetic pathway of triterpenoids.



	R′	R″
Oleanolic acid	CH_3	H
Hederagenin	CH_2OH	H
Gypsogenin	CHO	H
Quillaic acid	CHO	OH

Fig. 23.10
Some triterpenoid acids of saponins.

Table 23.5 Pentacyclic triterpenoid saponins.

Saponin	Genin	Sugar components	Occurrence
Aescin	Aescigenin	2 glucose, 1 glucuronic acid (1 tiglic acid)	*Aesculus hippocastanum*
Aralin	Aralidin	2 arabinose, 1 glucuronic acid	*Aralia japonica*
Calendulasaponin A	Oleanolic acid	2 glucose, 1 galactose, 1 glucuronic acid	*Calendula officinalis*
Glycyrrhizic acid	Glycyrrhetinic acid	2 glucuronic acid	*Glycyrrhiza* spp.
Guaianin	Noroleanolic acid	1 rhamnose, 1 glucose, 1 arabinose	*Guaiacum* spp.
Gypsophilasaponin	Gypsogenin	1 galactose, 1 xylose, 1 arabinose, 1 fucose, 1 rhamnose	*Gypsophila* spp. and other Caryophyllaceae
Hederacoside A (hederin)	Hederagenin	1 glucose, 1 arabinose	*Hedera helix* (ivy) and other Araliaceae and Sapindaceae
Symphytoxide A	Hederagenin	1 arabinose, 2 glucose	*Symphytum officinale* roots
Primula-saponin	Primulagenin	1 rhamnose, 1 glucose, 1 galactose, glucuronic acid	*Primula* spp.
Quillaja-saponin	Quillaic acid (hydroxygypsogenin)	Glucuronic acid, 6 sugars and acyl moieties	*Quillaia saponaria*
Saikosaponin a	Saikogenin F	1 glucose, 1 fructose	*Bupleurum* spp.

History. Liquorice is referred to by Theophrastus. The Roman writers referred to it as *Radix dulcis*, but it does not appear to have been cultivated in Italy until about the thirteenth century. Its cultivation in England, now commercially ceased, has been traced back as far as the sixteenth century.

Cultivation and collection. In western Europe liquorice is cultivated, but the 'Russian' and 'Persian' drugs are obtained from wild plants. In China, large-scale cultivation is replacing collection from the wild. The plants usually grow well in deep, sandy but fertile soil, near streams. They are usually propagated by replanting young pieces of stolon but may be grown from seed. The underground organs are developed to a sufficient extent by the end of the third or fourth year, when they are dug up and washed. Some are peeled and cut up into short lengths before drying, but much is now used unpeeled. The drug is imported in bales. In southern Italy and the Levant a large proportion of the crop is made into stick or block liquorice. This is prepared by the process of decoction, the liquid being subsequently clarified and evaporated to the consistency of soft extract. The latter is made into blocks or sticks, stamped with the maker's name (e.g. Solazzi), dried, and exported in cases, which often contain bay laurel leaves. Chinese blocks weighing 5 kg each are available.

Macroscopical characters. The Spanish and Italian drugs are derived from the variety *typica*. They are sold as 'Spanish' liquorice irrespective of their exact geographical source. Typical '*Spanish*' liquorice occurs in straight pieces from 14 to 20 cm or more in length and from 5 to 20 mm in diameter. If unpeeled, they have a dark, reddish-brown cork, and the runners, which are more numerous than the roots, bear buds. The peeled drug has a yellow, fibrous exterior. Fracture, fibrous; odour, faint, but characteristic; taste, sweet and almost free from bitterness.

Unpeeled 'Russian' liquorice occurs in somewhat tapering pieces up to 30 cm long and 5 cm in diameter. It is of less regular appearance than the Spanish and consists of rootstock and roots. The surface is covered with a somewhat scaly, purplish cork. The pieces of rootstock often bear buds and have a pith, but the roots may be distinguished from the stolons of the Spanish drug by the absence of buds. Fracture, very fibrous, the strands of fibres tending to separate from one another. This variety is sometimes peeled. The taste is sweet but usually not entirely free from bitterness or acridity. 'Persian' liquorice from Iran closely resembles the Russian variety and is generally unpeeled. Anatolian or Turkish liquorice may be peeled or unpeeled and some pieces may have a diameter of up to 8 cm.

Much commercial liquorice root currently available in Britain is of Chinese origin. It is imported in bundles of stolons each bundle being about 15 cm long, 15 cm diameter, and bound with wire. The bundles are packed in plaited wood containers. Generally, the stolons have a smaller diameter than the European drug.

Microscopical characters. Both roots and runners show secondary thickening—the absence of a medulla in the root and its tetrarch structure (Fig. 41.8A–C) serving to distinguish the sections. The epidermis and most of the cortex are absent, being thrown off by the development of cork. The outer surface of the unpeeled drug is bounded by some 10 rows of narrow cork cells. Within the cork is a phelloderm or secondary cortex composed of parenchymatous cells, some of which may become collenchymatous. These cells contain simple starch grains about 10 μm diameter; a few contain prisms of calcium oxalate. The secondary phloem is composed of alternating zones of groups of fibres and sieve tissue. The phloem fibres are very thick-walled, are lignified and occur in cylindrical bundles surrounded by a sheath of parenchymatous cells each of which contains a single prism of calcium oxalate 10–35 μm in length. The sieve-tube tissue suffers partial obliteration but remains functional in the cambial region. The cambium is an incomplete line composed of about three layers of flattened cells. The secondary xylem is composed of large vessels, wood fibres and wood parenchyma. The vessels are 80–200 μm in diameter and show reticulate or pitted walls. The pits are slit-like bordered pits.

The vessels occur singly in small groups and alternate with bundles of wood fibres resembling the phloem fibres in form and in being enclosed in a sheath of parenchyma containing calcium oxalate. The parenchyma of the xylem has lignified pitted walls. The secondary tissues are divided by radial medullary rays 3–5 cells wide in the xylem and funnel-shaped in the phloem. These rays are up to 100 cells high (Fig. 23.11).

The characters of *G. uralensis* are more fully discussed in the next monograph.

Constituents. Since 1990 considerable research has been published on the constituents of liquorice mainly by Japanese workers in whose country the drug, imported from China, is an important traditional medicine. Unfortunately, the Chinese commercial drug, as investigated, may be derived from a number of species, e.g. *Glycyrrhiza uralensis, G. inflata* and *G. glabra*, so that it is not always possible to assign particular reported constituents to a specific source.

Liquorice owes most of its sweet taste to glycyrrhizin, the potassium and calcium salts of glycyrrhizinic acid. Glycyrrhizinic acid is the diglucopyranosiduronic acid of glycyrrhetic (glycyrrhetinic) acid, which has a triterpenoid structure (Fig. 23.12). Other hydroxy- and deoxy-triterpenoid acids related to glycyrrhetic acid have been isolated; the C-20 epimer of glycyrrhetic acid is named liquiritic acid.

The yellow colour of liquorice is due to flavonoids, which received further considerable study when in 1978 the antigastric effect of flavonoid-rich fractions was recognized. They include liquiritin, isoliquiritin (a chalcone, which occurs as a glycoside), liquiritigenin, isoliquiritigenin (chalcone form) and other compounds. Isoliquiritigenin is reported to be an aldose-reductase inhibitor and may be effective in preventing diabetic complications. Rhamnoliquiritin was isolated from the roots in 1968. Many flavonoids and isoprenoid-substituted flavonoids from *G. glabra* of various origins have since been reported. These include the pyranoisoflavan, glabridin (Fig. 23.12) and, more recently, two minor isoflavones, glabriso flavone A and B, and glabrocoumarone (Fig. 23.12) (T. Kinoshita *et al.*, *Chem. Pharm. Bull.*, 2005, **53**, 847). Various 2-methylisoflavones have been isolated from indigenous Indian roots together with an unusual coumarin (liqcoumarin), 6-acetyl-5-hydroxy-4-methyl-coumarin. An examination of liquorice from five countries has shown the flavonoid content to be geographically consistent, varying only in the relative proportions of constituents. Japanese traditional (kampo) extracts prepared by boiling show a high content of flavonoid aglycones which may be pharmacologically more active than the parent glycosides.

Other active constituents of liquorice are polysaccharides with a pronounced activity on the reticuloendothelial system. Research on these, at first devoted to *G. uralensis*, has been extended to *G. glabra* var. *glandulifera* from which glycyrrhizan GA has been characterized as the representative polysaccharide with immunological activity (K. Takada *et al*., *Chem. Pharm. Bull*., 1992, **40**, 2487). It has an estimated mass of 85 000 with a core structure which includes a backbone chain of β-1,3-linked D-galactose residues 60% of the units carrying side chains (composed of β-1,3- and β-1,6-linked D-galactosyl residues) at position 6.

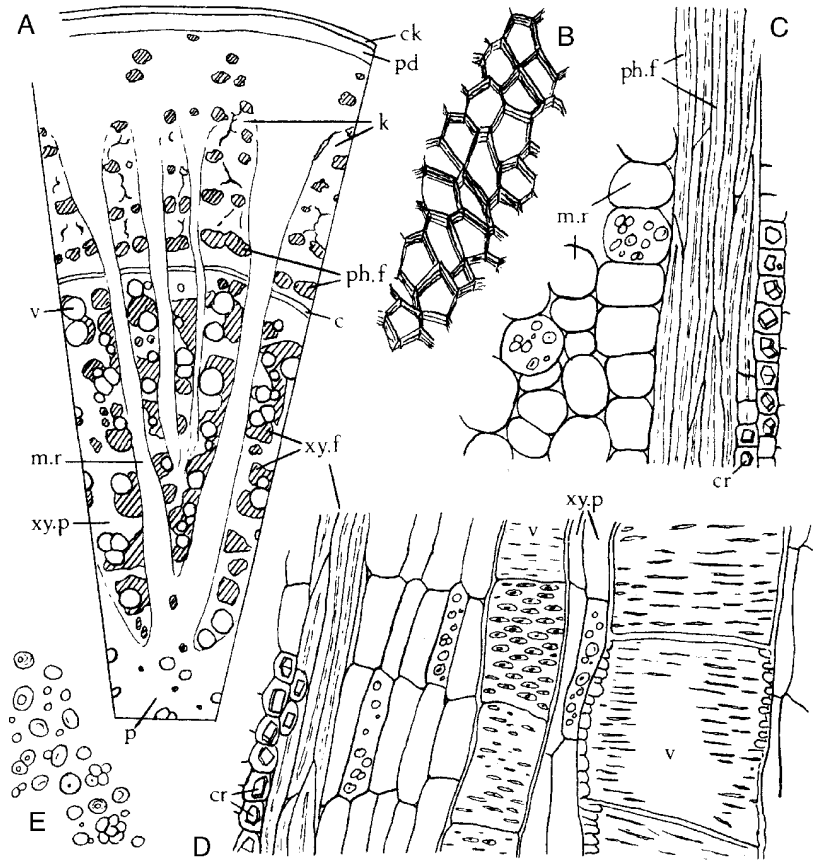


Fig. 23.11
Glycyrrhiza glabra. A, Transverse section of stolon (×25); B, fragment of cork layer from powder, in surface view; C, portion of longitudinal section through phloem; D, longitudinal section of wood; E, starch granules (all ×200). c, Cambial zone; ck, cork layer; cr, calcium oxalate crystals; k, non-functional sieve tissue (keratenchyma); m.r, medullary ray; p, pith; pd, phelloderm; ph.f, phloem fibres; v, vessel; xy. f, xylem fibres; xy. p, xylem parenchyma.

The roots also contain about 5–15% of sugars (glucose, sucrose); about 1–2% of asparagine (amide of aspartic or aminosuccinic acid); 0.04–0.06% volatile compounds; β-sitosterol; starch; protein; bitter principles (glycyramarin). The latter are particularly abundant in the outer tissues and are therefore largely removed in the peeled variety of liquorice.

Published analytical figures for the amount of glycyrrhizin in liquorice vary considerably. These differences are due partly to different analytical methods and partly to actual variations in the percentages present in different commercial varieties and samples; 6–13% is the usual range. Glycyrrhizin is confined mainly to the roots but falls rapidly in concentration in organs above soil level. In a field study on *G. glabra* growing in Uzbekistan, H. Hayashi *et al.*, (*Chem. Pharm. Bull.*, 2003, **51**, 1338) found that, based on the relative occurrence of rutin and isoquercetin, the populations could be divided into two distinct types.

Cell cultures. Suspension cell cultures of liquorice do not appear to produce either glycyrrhizin or glycyrrhetic acid but soyasaponins I and II, the amounts depending on culture strain and influence of plant hormones. The principal isoflavonoid produced is formononetin (C. Arias-Castro *et al.*, *Plant Cell Tissue and Org. Cult*., 1993, **34**, 63). Glycyrrhetic acid added to the cell culture undergoes a different mode of glycosylation to that normally occurring in the roots.

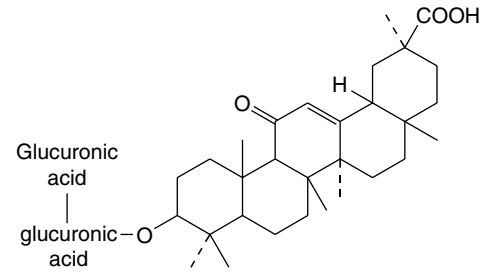
From hairy root cultures, initiated by *Agrobacterium rhizogenes*, two new prenylated flavonoids (licoagrochalcone A and licoagrocarpin) together with eight known flavonoids, have been isolated (Y. Asada *et al.*, *Phytochemistry*, 1998, **47**, 389).

Allied drug. Other species which have been investigated, some of which find domestic use, include *G. aspera*, *G. echinata*, *G. hirsuta*, *G. inflata*, *G. macedonia*, *G. pallidiflora* and *G. yunnanensis*. (For research papers on these species see K. Ohtani *et al.*, *Phytochemistry*, 1994, **36**, 139; T. Fukai *et al.*, *ibid*., 1994, **36**, 233.)

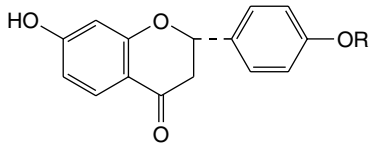
Standardization. The *BP*/*EP* requires a minimum content of 4.0% glycyrrhizic acid determined by liquid chromatography using monoammonium glycyrrhizate as a reference and absorption measurements at 254 nm. The total ash should not exceed 10.0% (unpeeled drug) or 6.0% (peeled drug) and the ash insoluble in hydrochloric acid similarly 2.0% (unpeeled drug), 0.5% (peeled drug).

Action and uses. Liquorice has long been employed in pharmacy as a flavouring agent, demulcent and mild expectorant. Gibson (*Lloydia*, 1978, **41**, 348) in a summary of the uses of liquorice from 2100 BC, pointed out that many of the early claims for a broad spectrum of uses for this drug appear to be borne out by modern pharmacological research; a view that has been further substantiated during the last decade. The recognition of the deoxycorticosterone effects of liquorice extracts and glycyrrhetinic acid has led to its use for the treatment of rheumatoid arthritis, Addison's disease and various inflammatory conditions. Interestingly, the flavonoid component of the root, which possesses antimicrobial properties, also exerts spasmolytic and antiulcerogenic activity. A Japanese patent (*Chem. Abs*., 1992, **117**, 55948) describes the formulation of a liquiritin cream as beneficial, with no adverse effects, for the removal of skin stains in patients with chloasma, senile melanoderma, etc.

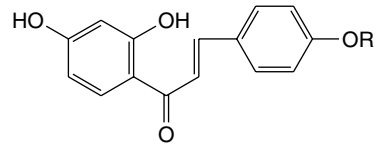
Unlike cortisone, liquorice may give symptomatic relief from peptic ulcer pain. It has been reported that glycyrrhizin gel can act as a useful vehicle for various drugs used topically; not only are the anti-inflammatory and antiviral effects relevant but also glycyrrhizin enhances skin penetration by the drug. Excessive consumption of



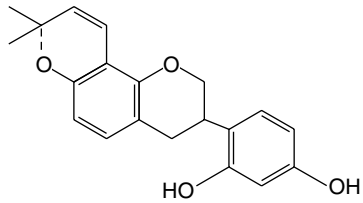
Glycyrrhizinic acid
(Glycyrrhizin = mixture of calcium and sodium salts of glycyrrhizinic acid)



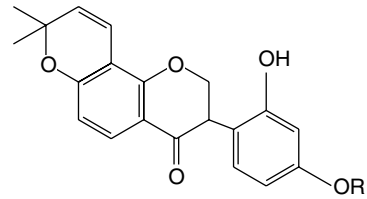
Liquiritin; R = Glucosyl
Liquiritigenin; R = H



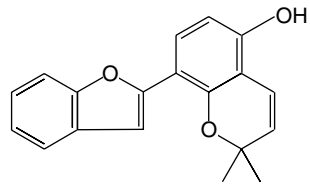
Isoliquiritin; R = Glucosyl
Isoliquiritigenin; R = H



Glabridin



R = H; Glabroisoflavone A
R = CH_3; Glabroisoflavone B



Glabrocoumarone

Fig. 23.12
Some constituents of liquorice root.

liquorice leads to hypertension and hypokalaemic alkosis; the hospitalization of an individual taking 200 g liquorice daily was reported in 1998. Most of the liquorice imported is used in the tobacco trade and in confectionery.

Further reading

Fiore C *et al* 2005 A history of the therapeutic use of liquorice in Europe. J Ethnopharmacology 99(3): 317–324

Hostettmann K, Marston A 1995 Saponins. Cambridge University Press, Cambridge

LIQUORICE ROOT FOR USE IN TRADITIONAL CHINESE MEDICINE

The *BP* 2007 included the above as a monograph involving the dried unpeeled roots and rhizomes of *Glycyrrhiza uralensis* Fisch., *G. inflata* Bat. or *G. glabra* L.

Unlike Liquorice Root *BP/EP*, the commercial drug consists of the roots and rhizomes sliced transversely or longitudinally giving irregularly circular to ovoid pieces up to about 3 mm thick. The outer surface is dark reddish-brown and longitudinally wrinkled. The general microscopical features of the powder resemble those for *G. glabra*.

G. uralensis, Manchurian liquorice, as present in a commercial sample bears a chocolate-brown, exfoliating cork and differs from *G. glabra* in internal structure, the medullary rays being curved and lacunae being present in the wood. It appears to contain about as much glycyrrhizin as the other varieties, together with a number of new oleane-type triterpene oligosaccharides called licorice saponins (I. Kitagawa *et al.*, *Chem. Pharm. Bull.*, 1993, **41**, 1567). Only traces of sugars are present and it gives an unpleasantly pungent extract. As with *G. glabra*, the yellow colouring matter contains the flavonoid glycoside liquiritin, a glycoside involving liquiritigenin, apiose and glucose, and a new chalcone oligoglycoside isoliquiritin apioside. Like *G. glabra*, *G. uralensis* contains polysaccharides showing immunological activities; glycyrrhizan UA is composed of L-arabinose, D-galactose, L-rhamnose and D-galacturonic acid.

As the supply of wild plants of *G. uralensis* is practically exhausted there is now large scale cultivation of the drug in the Inner Mongolian

area of China. A new biflavonoid named lichobichalcone and twelve known flavonoids from the cultivated roots have been reported (H. Bal *et al.*, *Chem. Pharm. Bull.*, 2003, **51**, 1095).

Actions. In common with *G. glabra*, many pharmacological activities have been cited for *G. uralensis*; these include: antihepatotoxic, antimutagenic, antimicrobial, antitumour and antiulcer. Glycyrrhisoflavone and glyasperin C have been reported as tyrosinase inhibitors (H. J. Kim *et al.*, *Planta Medica*, 2005, **71**, 785).

PRIMULA ROOT

Primula root *BP*/*EP* consists of the dried rhizome and root of *Primula veris* (L.) (cowslip) or *P. elatior* Hill. (oxlip), Primulaceae. These species occur wild throughout Europe with Bulgaria and Turkey the principal commercial sources. British herbal medicine has however traditionally used the leaves, flowers and roots of *P. vulgaris* (the common primrose).

The greyish-brown drug may be whole or cut with pieces of rhizome up to 5 cm in length and bearing the remains of stems and leaves together with numerous roots. Microscopical features include parenchymatous cells, reticulately thickened vessels and simple and compound starch granules. *P. elatior* is differentiated by the possession of groups of pitted sclereids.

Constituents include a mixture of triterpenoid saponins of the oleane type (5–10%) and phenolic glycosides such as primulaverin (primulaveroside). The latter, by enzyme hydrolysis during the drying process, forms the disaccharide primeverose and methyl 5-methoxysalicylate, the latter being responsible for the odour of the drug.

The pharmacopoeia includes a chromatographic test to detect *Vincetoxicum hirundinaria*, Aesclepiadaceae, a poisonous plant with similar-looking roots to those of *Primula* spp. The substitute also differs microscopically in its vascular structure and possesses numerous calcium oxalate crystals.

Primula root, like senega, is used as an expectorant for the treatment of bronchial conditions.

QUILLAIA BARK

Quillaia bark (*Soap Bark, Panama Wood, Quillaia*) is the dried inner bark of *Quillaja saponaria* Molina and of other species of *Quillaja* (Rosaceae). *Quillaja saponaria* is a tree about 18 m high found in Chile, Peru and Bolivia. It has been introduced into India and California. The generic name is derived from the Chilean word *quillean*, to wash, from the use made of the bark.

Macroscopical characters. Quillaia bark occurs in flat strips about 1 m long, 20 cm broad and 3–10 mm thick. It consists almost entirely of phloem, the outer region in which successive cork cambia develop having been more or less completely removed. A few, reddish- or blackish-brown patches of rhytidome adhere to the outer surface, which is otherwise of a brownish-white colour and reticulated. The rhytidome consists of dead portions of secondary phloem enclosed by secondary cork layers. The inner surface is yellowish-white and fairly smooth. The bark breaks with a splintery fracture and is inclined to laminate (between the zones of hard and soft phloem). Large crystals of calcium oxalate may be seen with the naked eye. The powdered drug is very sternutatory and produces an abundant froth when shaken with water. Taste, acrid and astringent.

Microscopical characters. A transverse section of quillaia bark has a chequered appearance which is caused by the crossing of the medullary rays by alternating bands of lignified and non-lignified phloem. The medullary rays are usually 2–4 cells wide. The phloem fibres are tortuous and often accompanied by small groups of rectangular sclereids. The parenchyma contains numerous starch grains up to 20 μm diameter and single prisms of calcium oxalate up to 20 μm long.

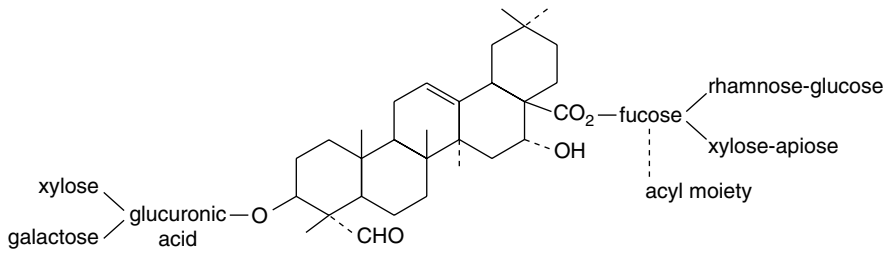
Constituents. The bark contains a mixture of saponins which on hydrolysis yield the principal sapogenin quillaic acid (hydroxygypsogenin) and gypsogenin (Fig. 23.10) together with sugars, uronic acids and acyl moieties. It is little wonder that the constitution of quillaia saponin proved difficult to unravel by classical procedures as it has now been shown to contain at least 60 different saponins (D. C. van Setten *et al.*, *Anal. Chem*., 1998, **70**, 4401). Di- and tri-saccharides are attached at C-3 and various complex oligosaccharides at C-28, the fucosyl moiety of which may be substituted with C_9-aliphatic acids or an *O*-acetyl group (S. Guo *et al.*, *Phytochemistry*, 2000, **53**, 861; **54**, 615). A typical structure is shown below.

Quillaia contains about 10% of saponins, *BP* ethanol (45%)-soluble extractive not less than 22.0%, and also sugars, starch and calcium oxalate.

Uses. Quillaia is used as an emulsifying agent, particularly for tars and volatile oils.

SENEGA ROOT

Senega of the *BP* and *EP* consists of the dried root crown and root of *Polygala senega* L. (Polygalaceae) or of closely related species of *Polygala* or a mixture of these. A variety found in N. America and now cultivated in Japan is *P. senega* var. *latifolia* Torr. et Gray, a robust perennial herb some 20–30 cm tall. Formerly abundant in eastern Canada and eastern USA it is now collected further westward.



Component of quillaia saponin

History. Senega was used by the North American Indians as a snake-bite remedy. It was employed by Tennent in 1734 for pleurisy and pneumonia, and its value was made known in London in 1738.

Macroscopical characters. Senega occurs in pieces 5–10 cm long and 2–12 mm diameter. The lower part is yellowish but the crown is somewhat darker. The latter is knotty and bears numerous, often purplish buds and the remains of aerial stems, which should not exceed about 2%. The tapering and often curved root frequently divides into two or more branches. Some, but by no means all, of the pieces bear a keel or ridge in the form of a rapidly descending spiral. The drug frequently has a marked odour of methyl salicylate. Taste, at first sweet, afterwards acrid. The saponins present cause the drug to have sternutatory properties and to froth when shaken with water.

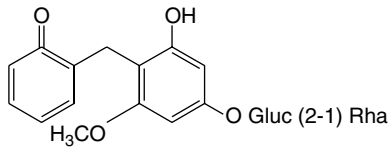
Transverse sections of different senega roots, or the same root cut at different levels, have widely different appearances. Some have a normal bark, which occupies nearly half the radius, and a uniform central wood with narrow medullary rays. In others, however, an abnormal local development of phloem gives rise to the keel, while one or more exceptionally wide, V-shaped, medullary rays give a very characteristic appearance to the wood. This is well seen in sections stained with phloroglucinol and hydrochloric acid.

For illustrations, see the 14th edition of this book, p. 309.

Constituents. Senega contains 6–12% of triterpenoid saponins. Earlier work based on the hydrolysis of the crude saponin mixture (senegin) produced a variety of products but during the 1970s Japanese workers characterized a number of individual saponins based on the aglycone presenegenin with glucose at C-3 and a number of sugars and a cinnamic acid derivative forming a branched chain at C-28; the units comprising the principal glycoside seneginin II are shown in Fig. 23.13. It is interesting to note the involvement of fucose, a deoxy sugar also found in certain cardioactive glycosides (Fig. 23.15) and in the saikosaponins (q.v.).

A number of oligosaccharide multi-esters have recently been identified in the roots (see H. Saitoh *et al.*, *Chem. Pharm. Bull.*, 1993, **41**, 1127, 2125) and named senegoses A–I. They appear to be di-and tetrasaccharides involving glucose and fructose esterified with acetic, benzoic, and *trans*- and *cis*-ferulic acids. A further series, senegoses J–O, are pentasaccharides (*Idem.*, *ibid*., 1994, **42**, 641). These compounds are structurally similar to the tenuifolioses previously isolated by the same group of workers from *P. tenuifolia* (*vide infra*).

Allied species and substitutes. *P. tenuifolia* is used in China and Japan as an expectorant, tonic and sedative. It contains constituents similar to those of *P. senega*, including saponins, xanthones, phenolic glycosides (tenuifolisides A–D) and oligosaccharides (tenuifolioses A–F), see Y. Ikeya *et al.*, *Chem. Pharm. Bull*., 1994, **42**, 2305). The bark, also used medicinally in China, Korea and Japan, has sedative, expectorant and anti-inflammatory properties. A number new phenones, tenuiphenones A–D, have recently been reported (Y. Jiang and P. Tu, *Chem. Pharm. Bull.*, 2005, **53**, 1164).



Tenuiphenone A

Many new triterpene saponins of *P. japonica* and *P. fallax* named polygalasaponins have been reported. The aglycone of a number of these saponins is bayogenin (Fig. 23.13). For recent new isolations, see H. Wang *et al.*, *Chem. Pharm. Bull.*, 2006, **54**, 1739; W. D. Zhang *et al.*, *Fitoterapia*, 2006, **77**, 336.

Southern or *White senega* is collected in the southern USA from *P. alba* and *P. boykini*. The roots are smaller than those of *P. senega* and have a normal wood.

Uses. Senega is used as a stimulant expectorant in chronic bronchitis. It is often prescribed with other expectorants such as ipecacuanha and ammonium carbonate.

EUROPEAN GOLDENROD

It is necessary to distinguish between two commercial sources of goldenrod used in medicine: one is native to Europe and Asia and the other involves two species of *Solidago* native to N. America, which are now naturalized and cultivated in Europe. Although generally similar in morphological form and constituents, there is variation between the two sources and the *BP*/*EP* includes them in separate monographs.

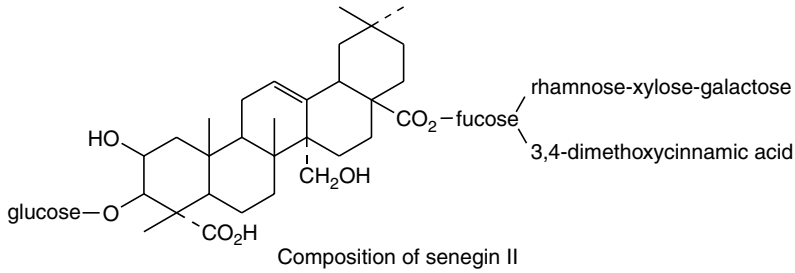
European Goldenrod *BP*/*EP*, Goldenrod *BHP* 1996 consists of the whole or cut, dried flowering aerial parts of *Solidago virgaurea* L. It is common in Britain, preferring dry woods and grassland, rocky areas, dunes, etc. The species is polymorphic with many named varieties.

A perennial herb, it is up to 1 m in height with stems, often reddish-brown at the base, somewhat branched and leafy. The lower leaves are up to 10 cm in length, obovate to lanceolate, toothed and narrowing at the base to a winged petiole. The stem leaves are alternate becoming progressively smaller, entire, inconspicuously toothed, sessile to amplexicaul, glabrous or slightly pubescent. The short-stalked yellow flowerheads are arranged around the stem in a terminal raceme or panicle. The involucre of greenish-yellow imbricate bracts is arranged in two to four rows; each capitulum possesses six to twelve ray florets and many tubular florets, both yellow. The fruits are brown, ribbed achenes 2–4 mm in length.

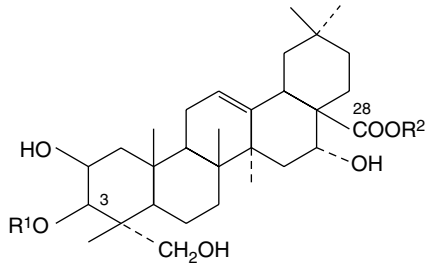
Features observable in the powdered drug include epidermal leaf fragments with striated cuticle and anomocytic stomata, various uniseriate clothing trichomes with some having an extended pennant-like terminal cell, glandular trichomes, cluster crystals of calcium oxalate, pappus hairs; compositae pollen grains.

Constituents. The principal constituents of European goldenrod are oleane-type saponins based on polygalacic acid-3-glucoside (Fig. 23.13). Four such compounds, subsequently designated virgaureasaponins B, C, D and E were 3, 28-bisdesmosidic glycosides involving glucose, rhamnose, xylose and fucose with acylation of the fucose with a chain of two or three β-hydroxybutyric acid moieties (G. Bader *et al.*, *Planta Med.*, 1995, **61**, 158); see Fig. 23.13 and *cf* Senega. Compounds B and C were identical with solidagosaponins XIV and XVIII belonging to a series of saponins (solidagosaponins I–XXIX) isolated from fresh plants of the Asian variety of *S. virgaurea* (Y. Inose *et al.*, *Chem. Pharm. Bull.*, 1991, **39**, 2037; 1992, **40**, 946; T. Miyase *et al.*, *Chem. Pharm. Bull.*, 1994, **42**, 617).

A number of flavonoids have been identified in the drug including chlorogenic acid and rutin, which are characterized in the pharmacopoeial TLC test; no quercitrin is evident, distinguishing this species from *S. gigantea* and *S. canadensis*. Other flavonoids include kaempferol, hyperoside and isoquercitrin. The presence of a diglucoside, leiocarposide (0.4–0.8%) is a further distinction from the above two species. Caffeic acid derivatives and phenolic acids in small amount are also present.



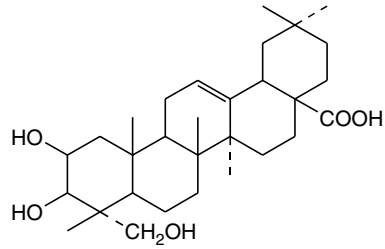
Composition of senegin II
(*Polygala senega*)



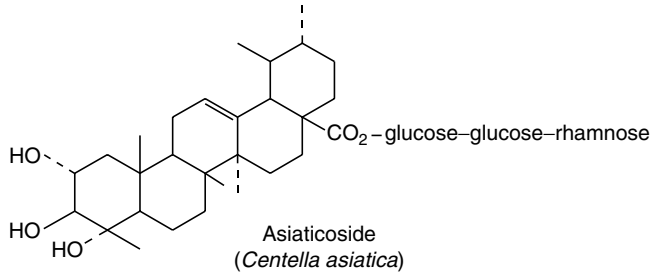
Polygalacic acid: $R^1 = H$, $R^2 = H$

Virgaureasaponins: R^1 = glucose, R^2 = fucose — rhamnose-xylose-rhamnose; β-hydroxybutyric acid (×2 or ×3)

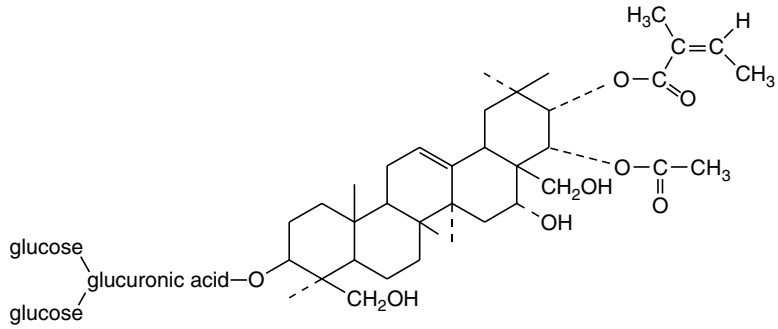
(*Solidago virgaurea*)



Bayogenin
(*Polygala japonica, P. fallax*)



Asiaticoside
(*Centella asiatica*)



Component of 'aescin'
(*Aesculus hippocastanum*)

Fig. 23.13
Triterpenoids of *Polygala, Solidago Centella* and *Hippocastanum*.

Uses. Used principally in traditional medicine for the treatment of urinary tract infections; also for catarrh and whooping cough and externally for the treatment of insect bites, wounds, etc.

GOLDENROD

Goldenrod *BP*/*EP* consists of the whole or cut, dried, flowering parts of *Solidago gigantea* Ait or *S. canadensis* L., their varieties or hybrids and/or mixtures of these. Family Compositae.

These species are two of some 100 spp. of *Solidago* native to America and were introduced into Europe as ornamentals, being much larger and showier than the native *S. virgaurea*; as garden escapes they have become naturalized along streams, rivers, lakes and waste places.

S. canadensis (*S. altissima*), tall goldenrod, is a native of eastern N. America. It is a vigorous erect plant 1–2.5 m with a spread of about 1 m. The stems are pubescent throughout with lanceolate, sharply pointed, three-veined leaves, roughish above, pubescent below. Broadly plumose heads of yellow flowers form a large pyrimidal panicle from August to November making for an attractive autumn border plant.

S. gigantea (*S. serotina*) resembles the above but below 1 m the stems are glabrous and the flowers are larger in compact erect corymbose panicles.

The pharmacopoeial description relates to both species and their hybrids. In the powder the absence of multicellular trichomes with a bent-over terminal cell should be noted, cf. European goldenrod.

Constituents. As with European goldenrod, bidesmosidic saponins are present in both *S. gigantea* and *S. canadensis*. Bayogenin (Fig. 23.13) is the aglycone common to both. The *canadensis* and *gigantia* saponins differ from one another in the length and structure of the saccharide chains, particularly in those units bonded to the C-3 position of bayogenin. Unlike *S. virgaurea*, there is no acylation of the sugar chains. Eight such saponins were isolated from *S. canadensis* and four from *S. gigantea* (M. Sovova *et al.*, *Ceska Slov. Farm.*, 1999, **48**, 113; *Chem. Abs.*, 1999, **131**, 240355 m).

Other constituents of *S. canadensis* include sesquiterpenes, di- and tri-terpenes and a very low content (1 mg/kg) of a new phenolic diglucoside related to that found in *S. virgaurea* (J. S. Zhang *et al.*, *Fitoterapia*, 2007, **78**, 69). Two new quercetin and kaempferol glycosides have recently been reported (B. Wu *et al.*, *Chem. Pharm. Bull.*, 2007, **55**, 815).

In common with European goldenrod the drug is assayed for its flavonoid content, a minimum of 2.5% flavonoids, expressed as hyperoside (dried drug), being required by the *BP*/*EP*. This high value may reflect the large proportion of flowers in collections of these plants. Quercitrin, chlorogenic acid and rutin are detected in the official TLC test (quercitrin is absent in the same test involving *S. virgaurea*).

Uses. In European phytotherapy for much the same purposes as European goldenrod—urolithiasis, cystitis, rheumatism and as an antiphlogistic.

Saikosaponins

This group of oleanene saponins occurs in the roots of *Bupleurum falcatum* (Umbelliferae), a drug long-used in Chinese medicine for the treatment of hepato-biliary disorders and known to possess anti-inflammatory properties. For a fuller description and formulae see Chapter 29.

Horse chestnut seed

Horse chestnut seeds and the tree *Aesculus hippocastanum* (Hippocastanaceae) need no description; native to western Asia the species is now widely distributed over the world as an ornamental.

Medicinally the seeds have long been used for their saponin content, the principal component being aescin (in recent publications termed 'escin') which occurs in concentrations of up to 20% in the dried seeds. As with a number of these well-known triterpenoid saponins it is only recently that it has been possible to elucidate completely their chemical structures and, as with other crude saponins, aescin itself has been shown to be a mixture of many closely related compounds. Acid hydrolysis of the aescin complex gives the saponin aescigenin and the sugars glucose, xylose, galactose and glucuronic acid together with esterifying acetic, butyric, isobutyric, angelic and tiglic acids. From 1996–1998 M. Yoshikawa *et al*. described nine new such acylated polyhydroxyoleane triterpene oligoglycosides (escins Ia, Ib, IIa, etc.) together with three isoescins (*Chem. Pharm. Bull*., 1998, **46**, 1764). As an example of one such structure see Fig. 23.13.

The seeds also contain flavones (quercitin, kaempferol and their glycosyl derivatives, Fig. 23.13), coumarins and tannins.

Extracts of horse chestnut have been traditionally employed both in the West and East for the treatment of peripheral vascular disorders including haemorrhoids, varicose veins, leg ulcers and bruises. OTC products are now available, their efficacy supported by a number of scientific reports. Thus some of the escins are anti-inflammatory, inhibiting the activity of lysosomal enzymes that damage capillary walls; coumarins cause a thinning of the blood, so much so that horse chestnut is contraindicated with anticoagulants such as warfarin; tannins tone the blood vessel walls and flavonoids are anti-inflammatory.

Further reading

Bombardelli E, Morazzoni P, Griffini A 1996 *Aesculus hippocastanum* L. Fitoterapia 67(6): 483–511. *A review with 143 references*

CENTELLA

Centella (Indian pennywort, gotu kola, Indian water navelwort, tiger grass) consists of the dried fragmented aerial parts of *Centella asiatica* (L.) Urban, family Umbelliferae syn. *Hydrocotyle asiatica*. It is typically found in moist situations throughout the pantropics including Pakistan, India, S.E. Asia and Africa. It has important traditional uses in India and Africa for the treatment of leprosy, and in the former for meditation purposes under the name *brahmi*.

The plant resembles the European marsh pennywort as a creeping perennial with kidney-shaped leaves, grouped at the stem nodes, up to 5 cm broad with small pink flowers giving fruits in umbels of two to four bicarpellate schizocarps.

The drug consists of a grey–green, compressed mass involving stems, leaves, flowers and fruits. Microscopical examination shows polygonal leaf epidermal cells with a striated cuticle, paracytic stomata and long, often twisted unicellular trichomes on the petiole epidermis. Also prisms of calcium oxalate crystals, bundles of septate fibres and fragments of the fruit with a parquetry arrangement of some cells.

Constituents. As an important Ayurvedic drug, Centella has been investigated over a long period of time and numerous constituents have been recorded. Among the most active are triterpenoid saponins, principally asiaticoside (Fig. 23.13), together with brahmoside, brahminoside, centelloside and medecasside, based on corresponding terpinic acids that also occur in the free state.

A minimal content of volatile oil consists principally of sesquiterpenes. O. A. Oyedej and A. J. Afolayan (*Pharm. Bull.*, 2005, **43**, 249), in a comparison of the Japanese oil with that from plants grown in S. Africa, have identified by GC-MS some 40 constituents, the chief of which are α-humulene (21.06%), β-carophyllene, myrcene, germacrene B and bicyclogermacrene. Samples varied in composition and showed broad-spectrum antibacterial activity.

Among other constituents of centella are flavonoids (quercetin, kaempferol, etc.) phytosterols, amino acids and a bitter principle, vallerin.

Uses. The *BHP* (1983) lists centella as a mild diuretic, antirheumatic, dermatological agent and peripheral vasodilator; topically as vulnerary. As such, it is used for rheumatic conditions and as a skin tonic in wound healing. Employed for indolent wounds it is also included in medicinal creams and some cosmetic preparations. These uses have largely been supported by pharmacological data.

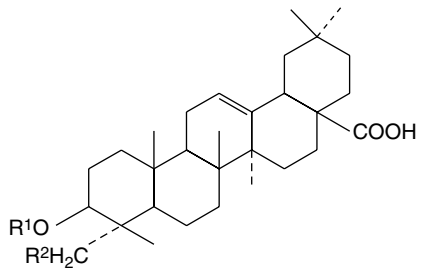
IVY

Ivy does not feature strongly in British herbal medicine but, with a considerable number of other European drugs, it is now included in the *BP* as a result of its *EP* status. The drug consists of the whole or cut aerial leaves of *Hedera helix* L. family Araliaceae collected in the spring. This familiar climber and creeper is widely distributed throughout Europe and Asia. Non-flowering shoots produce alternate palmate three- to five-lobed leaves with conspicuous pale veins; leaves of the flowering shoots are often larger, ovate or rhombic and entire. Microscopical features include: epidermal cells with wavy anticlinal walls, occasionally anisocytic but mainly anomocytic stomata on the lower epidermis, mucilage cells of the mesophyll and cluster crystals of calcium oxalate about 40 μm in diameter.

Important constituents of ivy are saponins involving the pentacyclic triterpenoid genins hederagenin, bayogenin and oleanolic acid. Examples are given in Fig. 23.14; see also F. Delmas *et al.*, *Planta Medica*, 2000, **66**, 343. Other constituents are flavonoids (rutin, quercetin), caffeic acid derivatives (chlorogenic, rosmarinic acid, etc.), sterols, polyacetylenes and volatile oil.

The *BP*/*EP* TLC test for identity indicates the presence of α-hederin and heteracoside C; a minimum concentration of 3.0% heteracoside C determined using liquid chromatography is specified.

Ivy-leaf extracts have been traditionally used as an expectorant for the treatment of various chest conditions, such as bronchitis and whooping cough; also for gout and rheumatic pains. Like most saponins, those of ivy are toxic in excess causing diarrhoea, vomiting and allergy. Externally, ivy is used cosmetically and for a variety of skin conditions. Molluscidal, antibacterial and antileishmanial properties have been reported for the saponins.



	R^1	R^2
α-Hederin	Ara 2–1 Rha	OH
β-Hederin	Ara 2–1 Rha	H
Hederacolchiside A_1	Ara [Glc 4–1] 2–1 Rha	H

Fig. 23.14
Saponins of Ivy.

CARDIOACTIVE DRUGS

A considerable number of plants scattered throughout the plant kingdom contain C_{23} or C_{24} steroidal glycosides which exert on the failing heart a slowing and strengthening effect. In Western medicine it is the glycosides of various *Digitalis* species that are extensively employed. The introduction of the foxglove (*D. purpurea*) into British medicine for treatment of dropsy by the Birmingham physician and botanist William Withering, in 1785, constitutes one of the fascinating stories of medicine. It was not realized at that time, however, that dropsy could be the result of a heart condition. Before the introduction of digitalis it was treated by the oral administration of dried and powdered toad-skins; later investigations were to show that this treatment too, did not lack a pharmacological basis. The action of the digitalis glycosides on the heart is discussed in Chapter 6.

The heart-arresting properties of these glycosides also render them most effective as arrow poisons and a number of tropical plants are better-known in this respect than for their medicinal use.

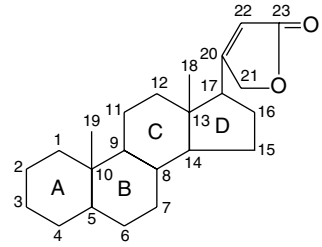
Distribution in nature

In plants cardiac glycosides appear to be confined to the Angiosperms. Cardenolides (see below) are the more common and are particularly abundant in the Apocynaceae and Asclepiadaceae, but are also found in some Liliaceae (e.g. *Convallaria*), and in the Ranunculaceae, Moraceae, Cruciferae, Sterculiaceae, Euphorbiaceae, Tiliaceae, Celastraceae, Leguminosae and Scrophulariaceae. The bufanolides occur in some Liliaceae (e.g. *Urginea*) and in some Ranunculaceae (e.g. *Helleborus*). In toad venoms the genins are partly free and partly conjugated with suberylarginine.

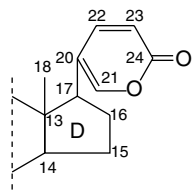
Some of the main genera containing cardiac glycosides are as follows: Apocynaceae: *Adenium*, *Acokanthera*, *Strophanthus*, *Apocynum*, *Cerbera*, *Tanghinia*, *Thevetia*, *Nerium*, *Carissa* and *Urechites*; Asclepiadaceae: *Gomphocarpus*, *Calotropis*, *Pachycarpus*, *Asclepias*, *Xysmalobium*, *Cryptostegia*, *Menabea* and *Periploca*; Liliaceae: *Urginea*, *Bowiea*, *Convallaria*, *Ornithogalum* and *Rohdea*; Ranunculaceae: *Adonis* and *Helleborus*; Moraceae: *Antiaris*, *Antiaropsis*, *Naucleopsis*, *Maquira* and *Castilla*; Cruciferae: *Erysimum* and *Cheiranthus*; Sterculiaceae: *Mansonia*; Tiliaceae; seeds of *Corchorus* spp.; Celastraceae: *Euonymus* and *Lophopetalum*; Leguminosae: *Coronilla*; and Scrophulariaceae. In the latter family cardiac glycosides have been found only in the genus *Digitalis* if we include in this the plant which some botanists call *Digitalis canareniensis* and others place in the genus *Isoplexis*.

Structure of glycosides

Two types of genin may be distinguished according to whether there is a five-or six-membered lactone ring. These types are known respectively as cardenolides (e.g. digitoxigenin) and bufanolides or bufadienolides (e.g. scillarenin). The following formulae indicate their structure and ring numbering:



Cardenolide



Bufadienolide

Substitution patterns for some typical genins are given in Table 23.6.

The sugar moieties, attached to the aglycone by a C-3,β-linkage, are composed of up to four sugar units which may include glucose or

Table 23.6 Genins of some cardioactive glycosides.

	Carbon ring numbering							
Genins	1	3	5	10	11	12	14	16
Cardenolides								
Digitoxigenin		OH		CH_3			OH	
Gitoxigenin		OH		CH_3			OH	OH
Gitaloxigenin		OH		CH_3			OH	OCHO
Digoxigenin		OH		CH_3		OH	OH	
Diginatigenin		OH		CH_3		OH	OH	OH
Strophanthidin		OH	OH	CHO			OH	
Ouabagenin	OH	OH	OH	CH_2OH	OH		OH	
Dienolides								
Scillaridin A*		OH		CH_3			OH	
Scilliphaeosidin*		OH		CH_3		OH	OH	
Hellebrigenin		OH	OH	CHO			OH	

*Double bond at C4–C5

rhamnose together with other deoxy-sugars whose natural occurrence is, to date, known only in association with cardiac glycosides. A number of the deoxy-sugars are 2,6-dideoxyhexoses (e.g. digitoxose) or their 3-*O*-methyl ethers (e.g. cymarose). In addition to rhamnose and fucose, a number of other 6-deoxyhexose derivatives have more recently been discovered together with 2-*O*-methyl and 2-*O*-acetyl sugars. In the case of fucose, the D-form is known only in cardiac glycosides, whereas the L-form is widely distributed in nature. Cardiac glycosides involving cyclic sugars are known in *Calotropis* spp. and probably occur in other members of the Asclepiadaceae.

A characteristic arrangement of the carbohydrate side-chain at C-3 is: aglycone-(characteristic cardiacglycoside sugars, or rhamnose)$_x$-(glucose)$_y$; *X* and *Y* may = 0 and there are some modifications of this general pattern. Some examples of the sugars found in these glycosides are given in Fig. 23.15.

Biogenesis of cardiac glycosides

Aglycones of the cardiac glycosides are derived from mevalonic acid but the final molecules arise from a condensation of a C_{21} steroid with a C_2 unit (the source of C-22 and C-23). Bufadienolides are condensation products of a C_{21} steroid and a C_3 unit (Fig. 23.16).

Progesterone, which is formed with cardiac glycosides, in *Digitalis lanata* as a result of feeding pregnenolone, is itself a precursor of the cardiac glycosides. Work, involving *Strophanthus kombé*, on the intermediates between progesterone and the cardenolides affords evidence consistent with the pathway: progesterone → 5β-pregnanolone → 5β-hydroxypregnanolone → cardenolides (Fig. 23.17). Similar transformations have also been demonstrated in *Digitalis purpurea* cultures but alternative pathways exist depending on whether hydroxylation of the nucleus occurs before or after the essential acetate condensation for the butenolide ring formation. Indeed, work involving some nine enzymes has helped to demonstrate that cardenolide genins arise as a result of a complex interlinking multi-dimensional system of pathways rather than by a single route leading directly to the end product.

As is evident from Fig. 23.17, a key enzyme in the biosynthesis of cardiac glycosides is progesterone 5β-reductase. Such an enzyme is also involved in the production of animal steroids so it is of interest to note that I. Gavidia *et al*. (*Phytochemistry*, 2007, **68**, 853) have shown that with *Digitalis purpurea* this enzyme is not homologous with the

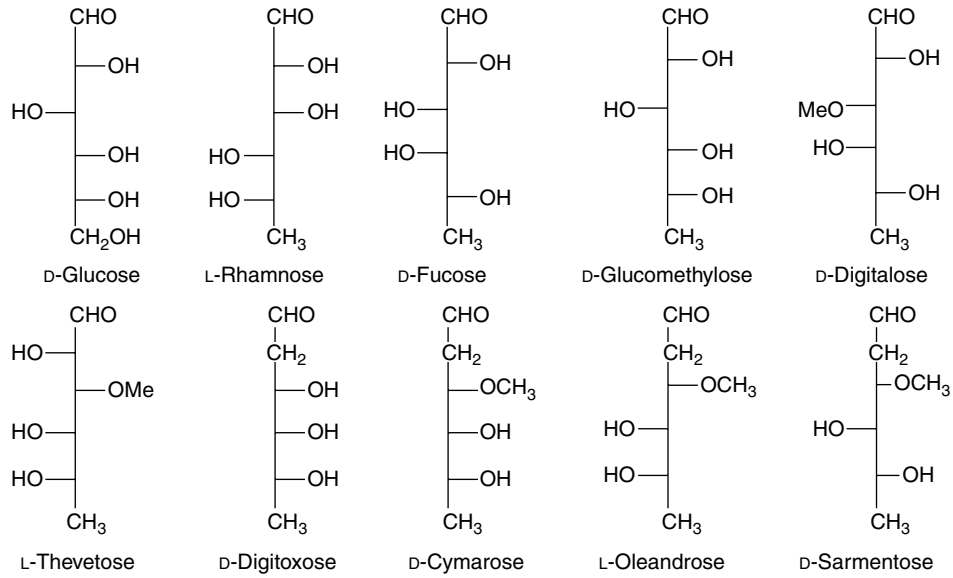


Fig. 23.15
Some examples of sugars found in cardiac glycosides (Fischer representation).

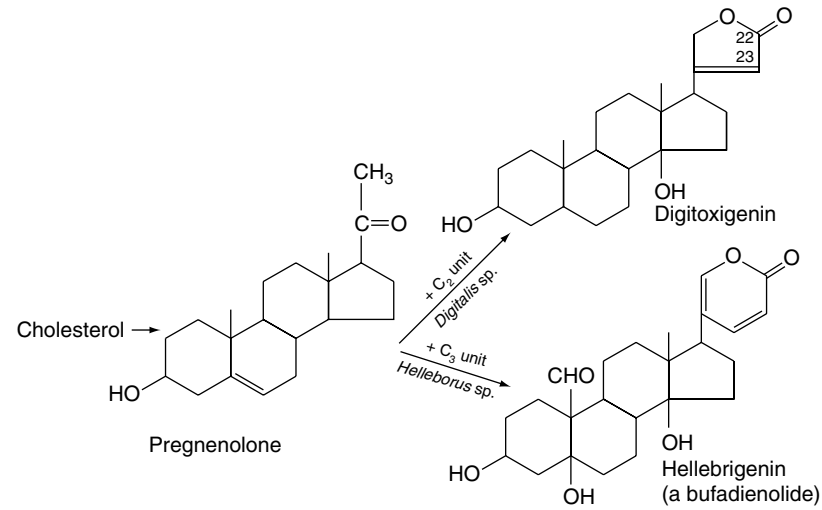


Fig. 23.16
Formation of aglycones of cardiac glycosides from a C_{21} steroid.

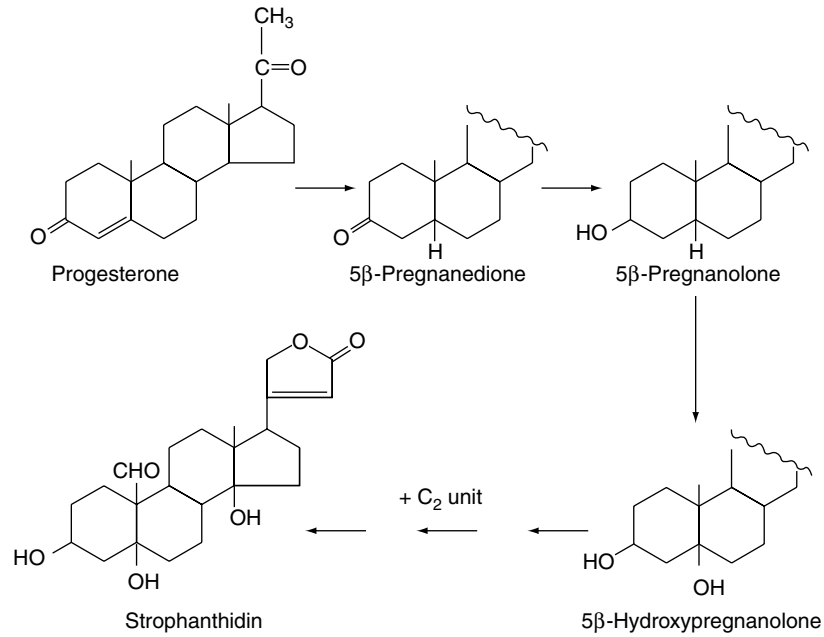


Fig. 23.17
Suggested intermediates in the metabolism of progesterone to cardiac glycosides.

corresponding enzyme in animals, suggesting that the steroid pathway evolved independently in plants and animals. Apparently, a similar situation occurs with 3β-hydroxy-Δ5-steroid dehydrogenase, an enzyme preceding the above in the cardenolide pathway.

Biogenetic studies involving the side-chain indicate that glucose is the most effective precursor of digitoxose and of the sugar side-chain of the *Nerium oleander* glycosides. Some ten enzymes have now been shown to be involved in the sugar side-chain biosynthesis in *Digitalis* species.

Tests and assays

The tests and assays available for cardioactive medicinals depend on biological activity, reactions of the sugar side-chain of the glycosides, and properties of the butenolide side-chain. Traditionally, the *BP* employed a biological assay for Digitalis (and formerly Strophanthus) on the basis that, compared with chemical and physical assays, it offered the best indication of the combined activity of the complex mixture of glycosides present. Now no biological assays are included for cardioactive drugs.

For digitalis leaves, the *EP* and *BP* utilize the red-violet colour (λ_{max} 540 nm) produced by the interaction of cardenolides and 3,5-dinitrobenzoic acid. Other colour reactions based on the butenolide moiety are the red-orange (λ_{max} 495 nm) given with an alkaline sodium picrate reagent (*EP* assay for digitoxin and digoxin), the red colour with xanthydrol reagent (*EP* test for digitalis leaf) and the red colour (λ_{max} about 470 nm) produced with sodium dinitroprusside. In the ultraviolet region the butenolide side-chain exhibits λ_{max} 217 nm and with purified substances, for example the eluted glycoside zones produced by TLC which contain little extraneous material, this can be used for rapid evaluation. These spectroscopic tests do not, in themselves, distinguish between glycosides and their corresponding aglycones.

A colour test specific for the digitoxose moiety is the Keller–Kiliani test; for details see 'Digitalis Leaf'. The test is employed by the *EP* for the identification of digitoxin and digoxin and by the *BP* as an assay for digoxin injection and tablets (λ_{max} 590 nm).

The separation and determination of individual glycosides by TLC, electrophoresis and GLC (derivatized glycosides) has received much attention over the years.

CARDENOLIDES

Of the cardioactive glycosides, those of the cardenolide group (Table 23.6) are the most important medicinally. Synthesis of these compounds has presented obvious difficulties but in recent years a large number of synthetic monosides have been prepared; one of these, a mannoside of strophanthidin, has proved extremely potent. All the medicinal preparations are derived from natural sources.

DIGITALIS LEAF

Digitalis (*Purple Foxglove Leaves*) consists of the dried leaves of *Digitalis purpurea* L. (Scrophulariaceae). It is required to contain not less than 0.3% of total cardenolides calculated as digitoxin.

History. Foxglove leaves appear to have been used externally by the Welsh 'Physicians of Myddfai' but the plant had no name in Greek or Latin until named digitalis by Fuchs (1542). The poisonous nature of the leaves was well known, and the drug was recommended by Parkinson in 1640; it was introduced into the *London Pharmacopoeia* of 1650. William Withering published his work on the clinical use of foxglove in 1785. For further reading, of largely historical interest, see J. K. Aronson (1985), *An Account of the Foxglove and its Medical Uses 1785–1985*, Oxford, Oxford University Press. Groves and Bisset have reviewed the topical use of *Digitalis* prior to William Withering pointing out the evidence for effective transdermal passage of the glycosides (*J. Ethnopharmacol*., 1991, **35**, 99).

Plant. The foxglove is a biennial or perennial herb which is very common in the UK and most of Europe, including some Mediterranean regions of Italy, and is naturalized in North America. It is produced commercially in Holland and Eastern Europe. In the first year the plant forms a rosette of leaves and in the second year an aerial stem about 1–1.5 m in height. The inflorescence is a raceme of bell-shaped flowers of the floral formula K(5), C(5), A4 didynamous, G(2). The common wild form of the plant has a purple corolla about 4 cm long, the ventral side of which is whitish but bears deep purple eyespots on its inner surface. Many horticultural varieties exist, but these are of low therapeutic potency. The fruit is a bilocular capsule which contains numerous seeds attached to axile placentae.

Digitalis grows readily from seed. In the wild state it is usually found in semi-shady positions. It grows well in sandy soil, provided that a certain amount of manganese is present, this element being apparently essential and is always to be found in the ash.

Collection. Either first- or second-year leaves are permitted by the pharmacopoeias.

There has been a long-standing general belief that the pharmacological activity of leaves increases during the course of a day to reach a maximum in the early afternoon. Biological assays have given some support to this supposition and variations involving individual glycosides have also been reported.

After collection the leaves should be dried as rapidly as possible at a temperature of about 60°C and subsequently stored in airtight containers protected from light. Their moisture content should not be more than about 6%.

Macroscopical characters. Digitalis leaves (Fig. 23.18) are usually ovate-lanceolate to broadly ovate in shape, petiolate and about 10–30 cm long and 4–10 cm wide. The dried leaves are of a dark greyish-green colour. The lamina is decurrent at the base; apex subacute. The margin is crenate or dentate and most of the teeth show a large water pore. Both surfaces are hairy, particularly the lower, and a fringe of fine hairs is found on the margin. The veins are depressed on the upper surface but very prominent on the lower. The main veins leave the midrib at an acute angle, afterwards branching and anastomosing repeatedly. The drug has no marked odour, but a distinctly bitter taste.

Microscopical characters. A transverse section of a foxglove leaf shows a typical bifacial structure and a midrib strongly convex on the lower surface (Fig. 23.18). Stomata and hairs are present on both surfaces, but are more numerous on the lower one. Calcium oxalate is absent. The palisade tissue is interrupted at the midrib. A zone of collenchyma underlies both epidermi in the midrib region. The crescent-shaped midrib bundle is enclosed in an endodermis one or two cells thick developed as a starch sheath. The pericycle is parenchymatous above and collenchymatous below. Sclerenchymatous fibres are absent.

Surface preparations (Fig. 23.18C, D) show that the upper epidermis consists of polygonal, relatively straight-walled cells, and bears both clothing and glandular hairs. The cells of the lower epidermis are wavy, and the stomata and hairs much more numerous than on the upper surface of the leaf. The stomata are small and slightly raised above the surrounding cells. The clothing hairs are uniseriate, two- to seven-celled, bluntly pointed, smooth or finely warty, with cells often collapsed alternatively at right angles (Fig. 23.18). The glandular hairs have a unicellular or occasionally a short uniseriate pedicel, with a unicellular or bicellular terminal gland (Fig. 23.18E). The cuticle of the hairs and epidermal cells may be stained red with a solution of Sudan Red in glycerin.

Prepared digitalis. This was a standardized powder of the *BP* (1989); it was adjusted to strength with weaker powdered digitalis or with powdered grass.

Constituents The chemistry of digitalis has engaged the attention of many workers since about 1820. Important progress was made by Nativelle (1868), Kiliani (1891), Stoll (1938) and Haack *et al*. (1956).

The primary (tetra) glycosides (purpurea glycoside A, purpurea glycoside B and glucogitaloxin) all possess at C-3 of the genin a linear chain of three digitoxose sugar moieties terminated by glucose. Purpurea glycosides A and B, first characterized by A. Stoll in 1938, constitute the principal active constituents of the fresh leaves. On drying, enzyme degradation takes place with the loss of the terminal glucose to give digitoxin, gitoxin and gitaloxin, respectively. Digitoxin and gitoxin are therefore the main active components of the dried drug. Poor storage conditions will lead to further hydrolysis and complete loss of activity. The gitaloxigenin series with its formyl group at C-16 is less stable than the other two series and was not discovered until 1956; the glycosides of this series are claimed to have similar or greater activities than those of the digitoxigenin group. The aglycones digitoxigenin and gitoxigenin are produced by acid hydrolysis of the respective glycosides but they are not found in quantity in the fresh or dried leaves. The aglycones, the formulae of which are shown in Fig. 23.19 are formed in the plant via the acetate-mevalonate pathway as already indicated in Fig. 23.16.

Other glycosides, present in small proportions, and involving the same genins contain digitalose and glucose; they exist as mono- and diglycosides. In this group verodoxin is claimed to have a toxicity of three times that of gitaloxin. These series are listed in Table 23.7. In 1961 small yields of yet other glycosides were reported from digitalis and include those with an acetylated side-chain (see Table 23.8).

Over the years much attention has been given to the variation of glycoside content in digitalis both throughout the 2-year life cycle and during the

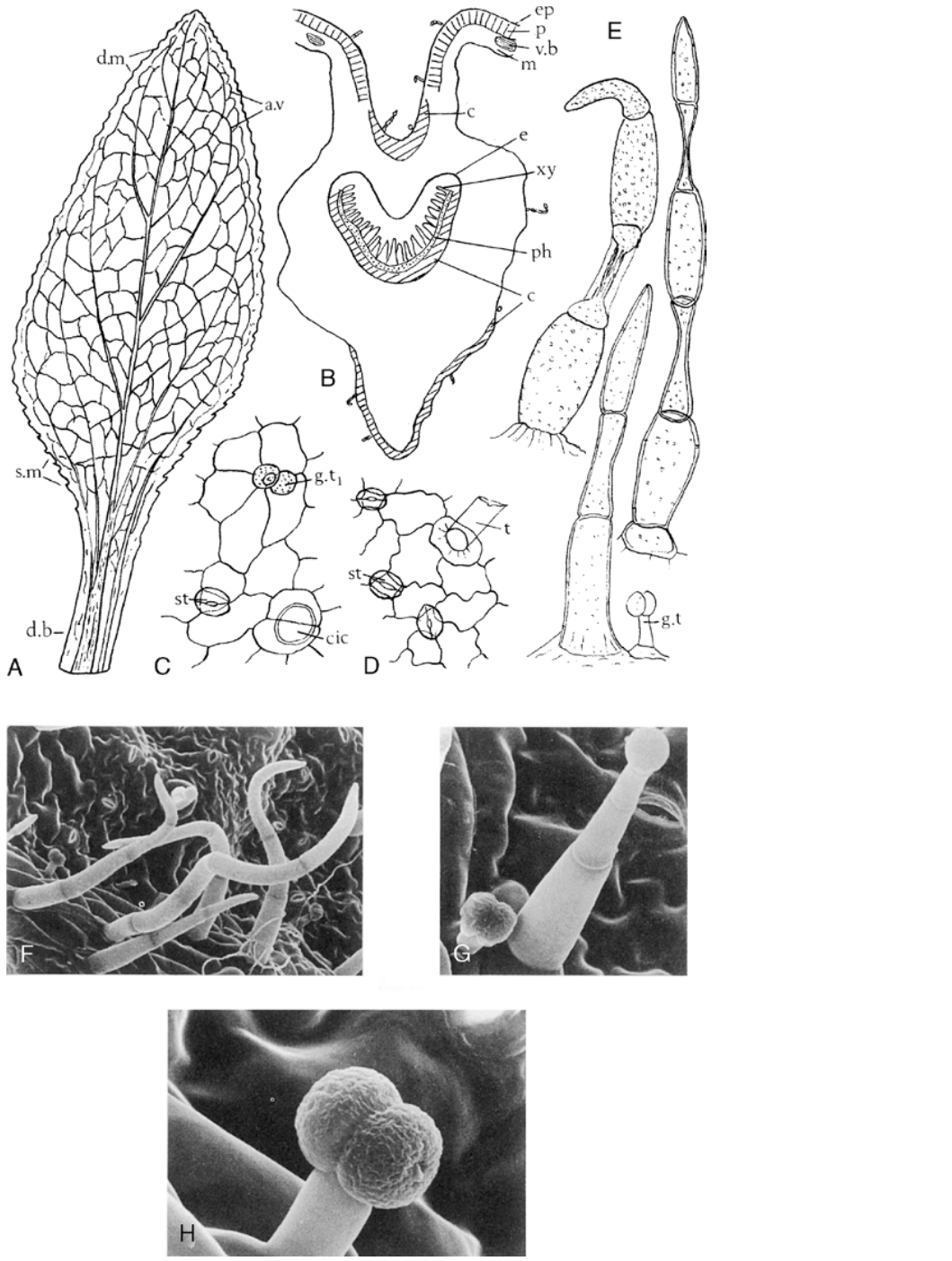
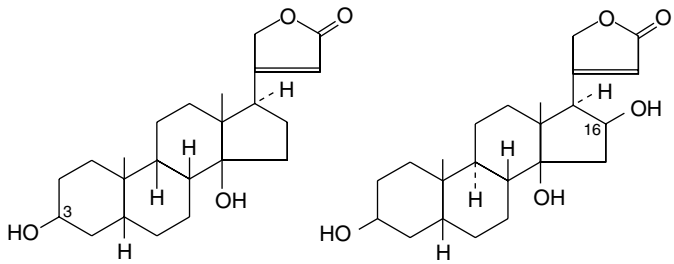


Fig. 23.18
Digitalis purpurea leaf. A, First-year leaf (×0.25); B, transverse section midrib of first-year leaf (×15); C, upper epidermis; D, lower epidermis; E, trichomes (all ×200); F–H, scanning electron micrographs: F, lower surface or leaf and G, H ditto showing glandular trichomes. a.v, Anastomosing veins; c, collenchyma, cic, cicatrix; d.b, decurrent base; d.m, dentate margin; e, endodermis; ep, epidermis; g.t, glandular trichome; g.t_1, ditto surface view; m, mesophyll; p, palisade; ph, phloem; s.m, serrate margin; st, stoma; t, trichome base; xy, xylem. (Photos: Lorraine Seed and R. Worsley.)

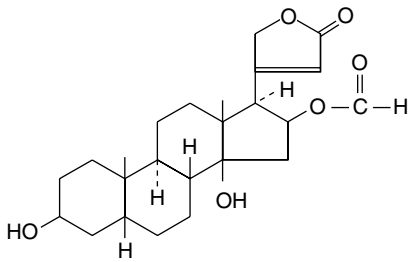
course of a single day. General conclusions are not easily drawn, because of the apparently contradictory results obtained by different workers. These contradictions probably arise from the different methods of assay employed, the different environmental conditions of the plants studied, and the possible use of different chemical races. It is generally agreed that first-year leaves collected July–August have the highest content of total glycosides and that after a fall during the winter months, another peak, but not as high as the first-year one, is reached at the time of flowering.

For plants of Belgian origin, Lemli showed, in 1961, by chromatographic analysis that digitalinum verum and glucoverodoxin are formed first in the young leaves and then cease to accumulate further. At this stage small amounts only of purpurea glycoside B and glucogitaloxin are present but these steadily increase, finally to reach 40% of the total glycosides. Purpurea glycoside A is formed last and eventually becomes the major component at 50% of the total glycoside mixture.



Digitoxigenin
(Series A)

Gitoxigenin
(Series B)



Gitaloxigenin
(Series E)

Fig. 23.19
Aglycones of *Digitalis purpurea* cardioactive glycosides.

Chemical races with respect to glycosides derived from digitoxin and gitoxin have been identified.

Digitalis purpurea leaves also contain anthraquinone derivatives, which include: 1-methoxy-2-methylanthraquinone, 3-methoxy-2-methylanthraquinone, digitolutein (3-methylalizarin-1-methylether), 3-methylalizarin, 1,4,8-trihydroxy-2-methyl-anthraquinone, etc. Saponins have also been isolated from the leaves, the sapogenins being produced more readily than cardenolides towards the end of the growing season. A number of leaf flavonoids have been described.

Keller–Kiliani test for digitoxose. Boil 1 g of powdered digitalis leaf with 10 ml of 70% alcohol for 2–3 min, filter; to 5 ml of filtrate add 10 ml of water and 0.5 ml of strong solution of lead acetate; shake and filter. Shake the filtrate with 5 ml of chloroform, allow to separate, pipette off the chloroform and remove the solvent by gentle evaporation in a porcelain dish. Dissolve the cooled residue in 3 ml of glacial acetic acid containing two drops of 5% ferric chloride solution. Carefully transfer this-solution to the surface of 2 ml of concentrated sulphuric acid; a reddish-brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish-green, darkening with standing.

Digitalis seeds. The seeds of *D. purpurea* contain different glycosides from those of the leaves. When extracted and standardized, they are known as 'Digitalin' (Digitalinum Purum Germanicum or amorphous Digitalin). This consists of the physiologically active 'digitalinum verum', with other water-soluble glycosides, including the saponins digitonin and gitonin.

Allied drugs. *Digitalis thapsi* is found in Spain and Italy. The leaves have a crenate margin and decurrent lamina. The leaves are characterized by the absence of non-glandular hairs, the presence of glandular hairs of two types, some consisting of a bicellular gland and unicellular stalk, others having a unicellular gland and a three- to four-celled stalk, the presence of a striated cuticle, pericyclic fibres and small prisms of calcium oxalate. The vein-islet number is higher than the other *Digitalis* species, varying from 8.5 to 16. *D. lutea* has a potency similar to those of *D. purpurea* and *D. ferruginia* and is cultivated in the former USSR. *D. ferruginea* ssp. *ferruginea* is the most widespread of the nine *Digitalis* spp. which grow in Turkey. For a report on the isolation of phenylethanoid glycosides from the aerial parts see Ihsan Çalis *et al*., *Chem. Pharm. Bull*., 1999, **47**, 1305.

Adulterants. The characters described above, particularly the margin, venation and trichomes, distinguish the official leaves from all the adulterants which have been recorded. For example, mullein leaves (*Verbascum thapsus*) are densely covered with large branched woolly hairs (see Fig. 42.4I). Other possible adulterants are comfrey (*Symphytum officinale*; see Fig. 42.3G), primrose (*Primula vulgaris*; see Fig. 42.5G, H), elecampane (*Inula helenium*), ploughman's spikenard (*Inula conyza*) and nettle (*Urtica dioica*).

Uses. Digitalis preparations are mainly used for their action on cardiac muscle (see Chapter 6).

Table 23.7 Aglycone and sugar components of digitalis leaves.

	Aglycone		
Sugar components (attached at C-3)	Digitoxigenin (Series A)	Gitoxigenin (Series B)	Gitaloxigenin (Series E)
Glucose–(digitoxose)$_3$–	Purpurea glycoside A	Purpurea glycoside B	Glucogitaloxin
(Digitoxose)$_3$–	Digitoxin	Gitoxin	Gitaloxin
Glucose–digitalose–	Gluco–odoroside H	Digitalinum verum	Glucoverodoxin
Digitalose–	Odoroside H	Strospeside	Verodoxin

Table 23.8 Acetylated side-chain glycosides of *Digitalis purpurea*.

Glycoside	Aglycone	Sugar moieties
Acetyl glucogitoroside	Gitoxigenin	Glucose–acetyldigitoxose–
Acetyl digitalinum verum	Gitoxigenin	Glucose–acetyldigitalose–
Purlanoside A	Digitoxigenin	Glucose–(digitoxose)$_2$–acetyldigitoxose–
Purlanoside B	Gitoxigenin	Glucose–(digitoxose)$_2$–acetyldigitoxose–

Further reading

Aronson JK 1985 An account of the foxglove and its medical uses 1785–1985. Oxford University Press, Oxford

Groves MJ, Bisset NG 1991 A note on the use of *Digitalis* prior to William Withering. Journal of Ethnopharmacology 35: 99–103

DIGITALIS LANATA LEAF

The plant, *Digitalis lanata* (Scrophulariaceae), the leaves of which are used as a source of the glycosides digoxin and lanatoside C, is a perennial or biennial herb about 1 m high, indigenous to central and south-eastern Europe. It is also cultivated in Holland, Ecuador and the USA. Some 1000 tonnes of plant material are required annually to meet world demand.

Characters. The leaves are sessile, linear-lanceolate to oblong-lanceolate and up to about 30 cm long and 4 cm broad. The margin is entire, the apex is acuminate and the veins leave the midrib at a very acute angle. The distinctive microscopical characters are the beaded anticlinal walls of the epidermal cells, the 10–14-celled non-glandular trichomes which are confined almost exclusively to the margin of the leaf, and the glandular hairs found on both surfaces; some have bicellular heads and unicellular stalks, while others have unicellular heads and 3–10-celled, uniseriate stalks. As in *D. purpurea*, pericyclic fibres and calcium oxalate are absent.

Constituents. First isolated by Stoll in 1933, the primary glycosides resemble those of *D. purpurea* but are acetylated at the digitoxose moiety next to the terminal glucose. This confers crystalline properties on the compounds, making them more amenable to isolation. Partial hydrolysis of the glycosides occurs during the drying and storage of leaves, and deacetylation will produce products the same as in *D. purpurea*. In addition to the above series of glycosides, two others, involving digoxigenin and diginatigenin (Table 23.6 and Fig. 23.20), are found in the leaves.

The principal glycosides of *D. lanata* leaves are listed in Table 23.9. In a series of papers by D. Krüger and colleagues (*Planta Med*., 1984, **50**, 267 and references cited therein) nine other glycosides based on digitoxigenin and digoxigenin are described; two sugars involved, not listed in Table 23.9, are xylose and 2,6-dideoxyglucose.

Using radioimmunoassay techniques (q.v.), Weiler and Westerkamp have been able to assay many thousands of individual plants and by selection have obtained races yielding 2–4 times the quantity of digoxin found in normal mixed populations (see Chapter 14 for further details). Lehtola *et al*. (1981) found that digoxigenin glycoside levels do not appear to be influenced by collection date (June 28–September 9) or by time of collection (a.m. or p.m.). However, total glycoside levels are higher in first-year leaves but the important medicinal glycosides (e.g. lanatoside C) attain their highest levels in the second-year plants. Rather similar results have been recorded for a Brazilian cultivar (F. C. Braga *et al*., *Phytochemistry*, 1997, **45**, 473). The primary glycosides appear to be stored exclusively in the vacuoles of cells.

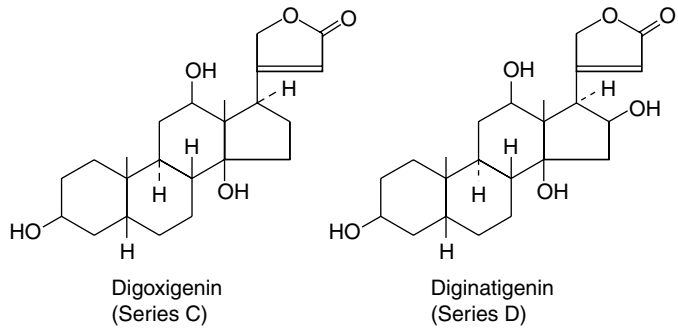


Fig. 23.20
Aglycones of *Digitalis lanata* cardioactive glycosides. See Fig. 23.19 for those also found in *D. purpurea*.

Anthraquinone derivatives, similar to those found in *D. purpurea*, have been recorded in the leaves and a number of flavonoid glycosides characterized.

Cell and organ culture. Both cell and hairy root cultures of *D. lanata* have proved disappointing as a source of cardioactive glycosides. Green tissues appear to be a requisite and green hairy roots produced by light exposure give a 600-fold increase in cardenolide accumulation over roots cultivated in the dark. Luckner and colleagues have studied the development and cardenolide formation of somatic embryos; they established the optimum conditions for the regeneration of shoots from shoot tips and the subsequent adaptation of the regenerated *D. lanata* plants to open ground (*Planta Med*., 1990, **56**, 53; 175).

Table 23.9 Some cardioactive glycosides of *Digitalis lanata* leaves.

Glycoside	Aglycone	Sugar moieties
Lanatoside A	Digitoxigenin	Glucose–acetyldigitoxose–$(digitoxose)_2$–
Acetyldigitoxin	Digitoxigenin	Acetyldigitoxose–$(digitoxose)_2$–
Digitoxin	Digitoxigenin	$(Digitoxose)_3$–
Glucoevatromonoside	Digitoxigenin	Glucose–digitoxose–
Digitoxigenin-*O*-glucosyl-6-deoxyglucoside	Digitoxigenin	Glucose–glucomethylose–
Glucodigifucoside	Digitoxigenin	Glucose–fucose–
Lanatoside B	Gitoxigenin	Glucose–acetyldigitoxose-$(digitoxose)_2$–
Glucogitoroside	Gitoxigenin	Glucose–digitoxose–
Digitalinum verum	Gitoxigenin	Glucose–digitalose–
Lanatoside C	Digoxigenin	Glucose–acetyldigitoxose-$(digitoxose)_2$–
Acetyldigoxin	Digoxigenin	Acetyldigitoxose–$(digitoxose)_2$–
Deacetyl-lanatoside C	Digoxigenin	Glucose–$(digitoxose)_3$–
Digoxin	Digoxigenin	$(Digitoxose)_3$–
Digoxigenin–glucosyl–bis–digitoxoside	Digoxigenin	Glucose–$(digitoxose)_2$–
Lanatoside D	Diginatigenin	Glucose–acetyldigitoxose-$(digitoxose)_2$–
Lanatoside E	Gitaloxigenin	Glucose–acetyldigitoxose-$(digitoxose)_2$–
Glucolanadoxin	Gitaloxigenin	Glucose–digitoxose–
Glucoverodoxin	Gitaloxigenin	Glucose–digitalose–

Uses The leaves are used almost exclusively for the preparation of the lanatosides and digoxin. Over the past decades digoxin has become the most widely used drug in the treatment of congestive heart failure. In long-term treatments patients require about 1 mg day^{-1} and the world-wide use of the drug now amounts to several thousand kilograms per year.

Proprietary preparations of the lanatoside complex, lanatoside C and lanatoside A are available in various countries but the glycoside from *D. lanata* most widely used is digoxin. Acting similarly to digitalis leaf, digoxin is more rapidly absorbed from the gastrointestinal tract than are the purpurea glycosides, which renders it of value for rapid digitalization in the treatment of atrial fibrillation and congestive heart failure. Lanatoside C is less well absorbed than digitoxin but it is less cumulative and for rapid digitalization the deacetyl derivative is preferable.

CARDIAC GLYCOSIDES OF THE APOCYNACEAE

At least a dozen genera of the Apocynaceae are known to contain cardioactive glycosides. Although all parts of the appropriate plants contain the glycosides, the latter often become concentrated in particular structures (e.g. seeds, barks, etc.). Extracts of a number are used as arrow poisons.

Strophanthus

Seeds of East African *Strophanthus kombé* were formerly official in the *BP* and a tincture prepared from them was used similarly to digitalis. The principal glycosides are K-strophanthoside, K-strophanthin-b and cymarin, all based on the genin strophanthidin (Table 23.6). Many minor glycosides have also been isolated. The seeds also contain about 30% of fixed oil, the bases trigonelline and choline, resin and mucilage.

For a full description of the seeds of this and other species, see earlier editions.

Strophanthus gratus seeds contain 4–8% of ouabain (G-strophanthin), a rhamnose glycoside more stable than those present in other species. It can be isolated in a pure crystalline form, and has been used as a standard in biological assays and for the preparation of ouabain injections. Ouabain is also the principal glycoside of the wood of the African *Acokanthera schimperi* (*A. ouabaio*). For the structure of the aglycone see Table 23.6.

Strophanthus sarmentosus seeds yield a number of glycosides with sarmentogenin as the aglycone. See also Chapter 14 for other information on these seeds.

The oleander glycosides

Nerium oleander, the oleander plant, and related species contain glycosides having a similar action to that of digitalis. Of Mediterranean origin, this evergreen flowering tree is widely cultivated in Japan and other countries as a garden and roadside ornamental.

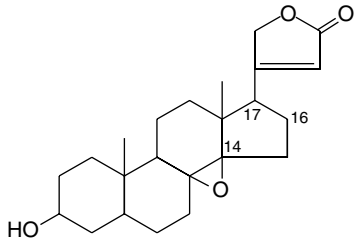
The principal constituents of the leaves are oleandrin and digitalinum verum. Oleandrin is the monoside, comprising oleandrigenin (16-acetylgitoxigenin) and L-oleandrose. Other components are interesting, as they demonstrate modification of the cardiac activity with change of structure. Thus, the uzarigenin glycosides have a *trans*-fusion of the A/B rings at C-5 (uzarigenin = 5α-digitoxigenin) and have a lowered activity. And the adynerigenin and presumably also the Δ^{16}-dehydroadynerigenin glycosides involving diginose and digitalose are inactive. Adynerigenin is digitoxigenin in which the 14-OH group has been replaced with an 8,14-ββ-epoxy group.

The leaves also contain gitoxigenin and digitoxigenin glycosides. A new glycoside, neridiginoside, has recently been obtained by activity directed isolation using the CNS depressant effect of a methanolic extract of the leaves on mice; it is the 3β-*O*-(D-diginosyl)-glycoside of a 5β,14β-cardenolide. Three other known constituents nerizoside, neritaloside and odoroside-H were also obtained (S. Begum *et al.*, *Phytochemistry*, 1999, **50**, 435 and references cited therein). For the isolation of three new triterpenes, see L. Fu *et al.*, *J. Nat. Prod.*, 2005, **68**, 198. From the bark and twigs, new pregnanes in addition to the known neridienone have been described and their anti-inflammatory and cytotoxic properties studied (L. Bai *et al.*, *J. Nat. Prod.*, 2007, **70**, 14).

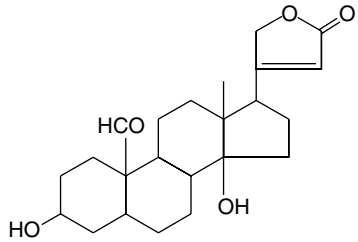
Two glycosides isolated from *N. odorum*, in addition to others, are oleandrigenin-β-D-glucosyl-β-D-diginoside and gentiobiosyloleandrin.

The seeds of *Thevetia peruviana (T. neriifolia)* the yellow oleander, are a rich source of the glycoside thevetin A, which by partial hydrolysis and the loss of two glucose units yields peruvoside, the therapeutic cardioactive properties of which are well-known. Peruvoside consists of L-thevetose linked to the aglycone cannogenin. Thevetin has found use in continental Europe, and is considered particularly useful in cases of mild myocardial insufficiency and where digitalis intolerance exists.

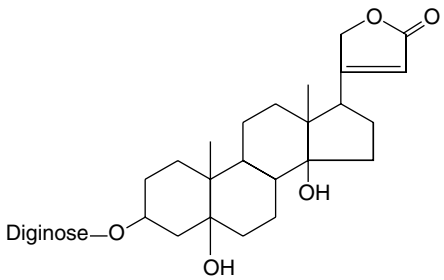
Oleander ingestion causes many cases of poisoning world-wide; in 1994, 303 cases were reported in Texas, and in Australia during 1972–8 it was responsible for 27% of paediatric plant poisonings. Fatal cases have been reported elsewhere and in Sri Lanka the use of the



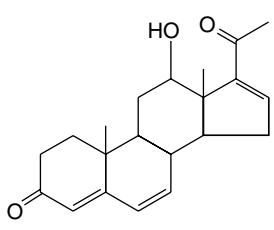
Adynerigenin



Cannogenin



Neridiginoside



Neridienone

seeds in suicide attempts, particularly among teenagers, poses a problem (see M. Eddleston *et al*., *Lancet*, 2000, **355**, 967 and references cited therein).

MISCELLANEOUS SOURCES

Plants which contain medicinally useful cardenolides have been obtained from other families (Asclepiadaceae, Cruciferae, Euphorbiaceae, Liliaceae, Moraceae, Leguminosae, Ranunculaceae, Sterculiaceae).

Convallaria

The lily of the valley, *Convallaria majalis* (Liliaceae) is much used on the continent of Europe and in herbal medicine for its cardioactive properties which are similar to those of digitalis but much less cumulative. Either the aerial parts, collected when the flowers are beginning to open, or the rhizomes and roots, are used.

The principal glycoside is convallatoxin which on hydrolysis gives strophanthidin (Fig. 23.17) and (−)-rhamnose. The plant contains many minor cardenolides, about 40 glycosides associated with nine different aglycones having been identified. Sugars not recorded elsewhere for cardiac glycosides are allose and the disaccharide rhamnosido-6-deoxyallose. (For the isolation of novel cardenolides see V. K. Saxena *et al.*, *J. Nat. Prod*., 1992, **55**, 39.)

The glycosides appear to be formed in the leaves and a turnover apparently takes place towards the end of the vegetative period. Bioconversions which lead to the formation of minor glycosides have been studied, along with the utilization of digitoxigenin and digitoxin by the plant for production of convallatoxin.

Convalloside, a glycoside of the seeds, when acted on by strophanthobiase yields convallatoxin and D-glucose. A number of flavonoid glycosides are also present in the leaves, and the roots contain a saponin, convallamaroside, which is a 22-hydroxyfuranostanol saponin with three independent sugar chains at C-1, C-3 and C-7.

Japanese lily of the valley, *Convallaria keiskei*, contains glycosides of convallagenin.

The dried aerial roots of *Adonis vernalis* (Ranunculaceae) contain more than 30 cardenolides, acting similarly to those of strophanthus; cymarin is the major constituent. (See B. Kopp *et al.*, *Phytochemistry*, 1992, **31**, 3195.)

The aerial parts of *Erysimum canescens* and other species of *Erysimum* (Cruciferae) are used in the former USSR, and contain glycosides based on strophanthidin. Erysimin, for instance, gives on hydrolysis strophanthidin and digitoxose. Another drug, used in Russia similarly to digitalis, is derived from the bark of the silk-vine *Periploca graeca* (Asclepiadaceae). Its principal glycoside, periplocin, affords on hydrolysis glucose, cymarose and periplogenin.

BUFADIENOLIDES

The bufadienolides (Table 23.6) are less widely distributed in nature than are the cardenolides; they are found in some Liliaceae and Ranunculaceae, and in the toad venoms the genins are partly free and partly combined with suberyl arginine. Therapeutically they find little use as cardioactive drugs because of their low therapeutic index and their production of side-effects. However squill (q.v.) has a time-honoured place as an expectorant and has been widely used in the treatment of cough.

In a review (see 'Further reading') Krenn and Kopp have listed 267 bufadienolides for the period 1967–1995 found in six plant families—Crassulaceae, Hycinthaceae (Liliaceae), Iridaceae, Melianthaceae, Ranunculaceae and Santalaceae—and in animals in the Bufonidae, Cilucridae and Lampyridae.

SQUILL

Squill *BP* consists of the dried sliced bulbs of *Drimia maritima* (L.) Stearn [*Urginea maritima* (L.) Baker], Liliaceae collected after the plant has flowered and from which the membranous outer scales have been removed. *D. maritima* occurs wild as an aggregate of at least six species of varying chromosome number, not all giving a bulb with an acceptable glycoside content. *D. maritima* (L.) Baker *sens. str*. is hexaploid and is a variety from which the commercial drug can be obtained and which has recently been the most studied. It is known in commerce as white squill. That grown in the Mediterranean area (Italy, Malta) is now almost unobtainable and the principal source is Indian squill (see below). Red squill, which is also derived from a variety of *U. maritima*, is collected in Algiers and Cyprus, and differs from the white in containing red anthocyanin pigment and the glycoside scilliroside.

Collection and preparation The bulbs are collected in August, a month in which the plant has finished flowering and is without aerial leaves. After the dry outer scales have been removed, the bulbs are cut transversely into thin slices. These are dried in the sun or by stove heat, when they lose about 80% of their weight. The dried slices are packed in bags (containing about 50 kg) or in barrels.

History. Squill was well known to the early Greek physicians and to the Egyptians. A vinegar of squills was known to Dioskurides and an oxymel of squills to the Arabian physicians.

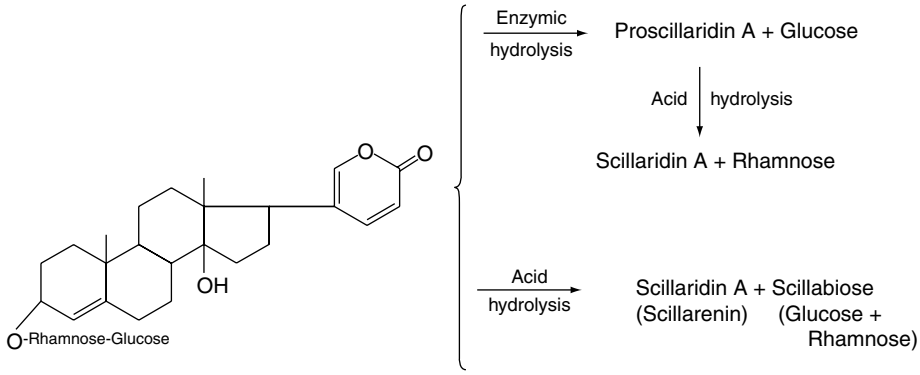
Macroscopical characters. Squill bulbs are pear-shaped and about 15–30 cm diameter. Whole bulbs are rarely imported because they tend to start growing unless stored in a refrigerator.

The dried drug occurs in yellowish-white, translucent strips about 0.5–5 cm in length and tapering at both ends. The drug is brittle when perfectly dry, but it readily absorbs moisture and becomes tough and flexible. The hygroscopic nature is particularly noticeable in the powdered drug, which, if carelessly stored, tends to cake into solid masses and develop mould. It should be stored in an atmosphere free from moisture. Odour, slight; taste, bitter and acrid.

Microscopical characters. Under the microscope squill shows abundant large polygonal parenchymatous cells of the mesophyll, many of which contain mucilage, surrounding a bundle of many raphides of calcium oxalate (see Fig. 42.9G). The individual crystals are 50–**250**–**500**–900 μm long and 1–6 μm diameter. The mucilage sheath is stained by corallin soda. Very occasional small, rounded, starch grains, about 10 μm diameter, are present in the mesophyll cells. The epidermis is composed of rectangular cuticularized cells, in surface view polygonal and usually axially elongated. Stomata are absent or very rare on the adaxial surface; a few are constantly present on the abaxial surface. They are anomocytic, circular in outline, with wide guard cells. The mesophyll is traversed by numerous small vascular bundles, which account for the presence in the powdered drug of occasional small spiral and annular xylem vessels.

Constituents. Pure glycosides were not isolated until in 1923 Stoll separated a crystalline glycoside, scillaren A, and an amorphous mixture of glycosides, scillaren B. Scillaren A, the most important constituent of squill, is readily hydrolysed by an enzyme scillarenase or by acids as shown at top of p. 331.

Small quantities of glucoscillaren A (a triglycoside) also occur in the bulb. Other glycosides with 12α- and 12β-OH substitution have similar sugar side-chains at C-3. Other minor glycosides and the isolation of new bufadienolides are described by B. Kopp and L. Krenn (*Phytochemistry*, 1996, **42**, 513; *J. Nat. Prod*., 1996, **59**, 612). *D. maritima* collected



Scillaren A

in Egypt is markedly different in chemical constitution to bulbs of *U. aphylla* from Greece and Turkey, and *U. numidica* from Tunisia.

Many flavonoids have been detected in extracts of the bulb of *U. maritima*; they include quercetin derivatives and kaempferol polyglycosides together with *C*-glycosides such as vitexin and isovitexin. The drug also contains sinistrin, a fructan resembling inulin; it is composed largely of β-D-fructofuranosyl residues. M. Iizuka *et al.* have recorded 33 compounds isolated from the squill bulb—ten were new natural compounds, nine were bufadienolides and one was a lignan (*Chem. Pharm. Bull.*, 2001, **49**, 282). Idioblasts (see above) contain calcium oxalate crystals embedded in mucilage consisting mainly of glucogalactans. Anthocyanins are present in small amount.

Tests. The *BP* specifies an ethanol (90%) extractive of not less than 68.0%, an acid-insoluble ash limit of not more than 1.5%, mucilage staining red with alkaline corallin solution and no purple stain with 0.01-M iodine solution.

Action and uses. The glycosides are poorly absorbed from the gastrointestinal tract, they are of short-action duration and they are not cumulative. In small doses the drug promotes mild gastric irritation causing a reflex secretion from the bronchioles. It is for this expectorant action that it is widely used; in larger doses it causes vomiting.

Red squill

See 'Rodenticides', Chapter 40.

INDIAN SQUILL OR URGINEA

This consists of the dried, usually longitudinally sliced, bulb of *Drimia indica* (Roxb.) J. P. Jessop [*Urginea indica* (Roxb.) Kunth.]. The drug is used in India and has now been reintroduced into the *BP* and is described in the *BHP*. It occurs in curved pieces of a slightly darker colour than the European squill, which it closely resembles. The strips are sometimes united in groups of about four to eight to a portion of the axis, such pieces being seldom found in the European drug. Also in contrast to the latter, the mucilage of the mesophyll cells stains red with alkaline corallin solution and reddish-purple with iodine solution.

The constituents appear to be similar to those of white squill.

Uses. Indian squill has a digitalis-like action on the heart and in small doses is used as an expectorant in the same way as European squill.

Black hellebore rhizome

Black hellebore rhizome is obtained from *Helleborus niger* (Ranunculaceae), a perennial herb indigenous to Central Europe. It contains three crystalline cardiac glycosides: helleborin, helleborein and hellebrin. Of these, the last two have a digitalis-like action, hellebrin being approximately 20 times more powerful than helleborein. The aglycone hellebrigenin (Fig. 23.16) is the bufadienolide analogue of strophanthidin. The drug which has abortifacient as well as cardiotonic properties is considered dangerous and is now obsolete in ordinary medicine.

Further reading

Krenn L, Kopp B 1998 Bufadienolides from animal and plant sources. Phytochemistry 48(1): 1–29

Steyn PS, van Heerden FR 1998 Bufadienolides of plant and animal origin. Natural Product Reports 15(4): 397–414

OTHER STEROIDS

There are few other types of steroidal compounds in addition to those already discussed that have, at the moment, any pharmaceutical significance.

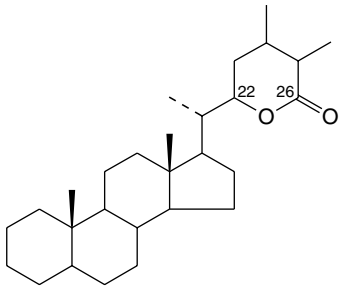
The phytosterols (e.g. stigmasterol and sitosterol) have been mentioned in connection with steroid synthesis, and the role of cholesterol in the biosynthesis of other steroids has been noted (Figs 23.2, 23.16). Steroidal alkaloids in which the nitrogen may be either cyclic or noncyclic are considered in Chapter 26.

WITHANOLIDES

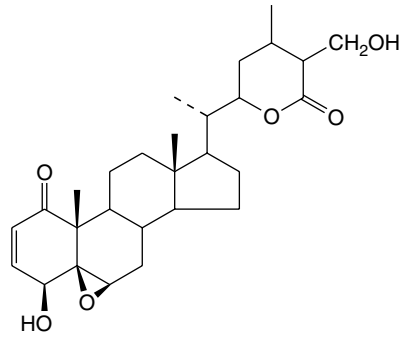
This class of steroidal lactones involves an ergostane-type framework in which C–22 and C–26 are appropriately oxidized to form a δ-lactone ring.

Chemists had long been interested in the constituents of *Withania somnifera* (Solanaceae), an Indian plant well-known in traditional medicine, and in 1968 Israeli workers determined the structure of a constituent lactone (withaferin A) of the plant; since then many more compounds of this now large class, have been characterized. They are subdivided into nine groups: withanolides, withaphysalins, physalins, nicandrenones, jaborols, ixocarpalactones, perulactones, acnistins and miscellaneous withasteroids (see M. Leopoldina *et al.*, *Planta Medica*, 2004, **70**, 551).

In addition to their potential pharmacological value as sedatives, hypnotics, antiseptics and antimitotics, some withanolides are cell differentiation inducers—compounds of a new type of antitumour agent (M. Kuroyanagi *et al.*, *Chem. Pharm. Bull.*, 1999, **47**, 1646). Withanolides have proved of interest because of their occurrence as chemical races of *Withania* and because of their structural variation in hybrids of different races (q.v.). Other genera of the family in which they occur include *Acnistus*, *Datura*, *Deprea*, *Hyoscyamus*, *Iochroma*, *Jaborosa*, *Lycium*, *Nicandra*, *Physalis* and *Solandra*.



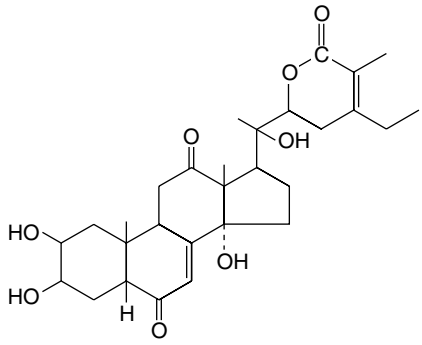
Withanolide skeleton



Withaferin A

The first report of a withanolide from the Labiatae concerns the characterization of a new compound, ajugin, from *Ajuga parviflora* whole plants (P. M. Khan *et al*., *Phytochemistry*, 1999, **51**, 669). Previously the steroidal lactone, ajugalactone, had been isolated from *A. decumbens*; this compound has an α,β-unsaturated carbonyl group and inhibits insect metamorphosis (contrast the ecdysones below). A number of other species have been used by various cultures to treat a variety of ailments. There are rare reports of withanolides of the Taccaceae and Leguminosae.

The isolation of new withanolides continues to be an active area of research.



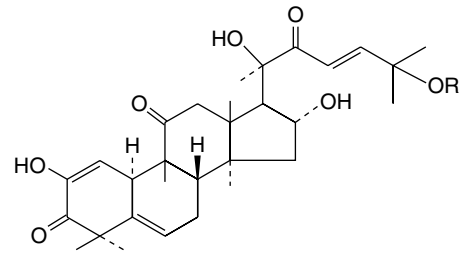
Ajugalactone

Ecdysones. The ecdysis of insects relates to the moults which larvae undergo during their transformation into an adult, a process controlled by complex hormonal mechanisms. Ecdysones, or insect-moulting hormones, are substances which stimulate these changes, and one example is ecdysone itself, which was first isolated from silk-worm pupae.

Only a few such compounds have been isolated from arthropods, but in plants they occur in much greater variety and abundance. Ecdysterone (20-hydroxyecdysone) is also an example of one which has been obtained from both plant and insect sources; whether a plant–insect relationship exists with regard to this substance and whether such compounds have a function in the plant is at present not known. It is perhaps significant, however, that insects do not themselves biosynthesize steroids *de novo* and rely on plant materials for suitable precursors.

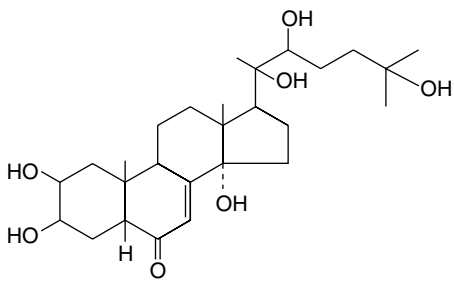
In the plant, cholesterol is a precursor of insect-moulting hormones, and in one morphological group of *Helleborus* they are formed together with bufadienolides (q.v.) and saponins.

Cucurbitacins. These tetracyclic triterpenoids are of interest because of their cytotoxic and antitumour properties. They occur in the Cucurbitaceae and other families such as the Euphorbiaceae and Cruciferae. Cucurbitacin A was isolated in 1953 and its structure determined in 1963. They may occur in the glucosidic form and are hydrolysed by the enzyme elaterase. The formulae of two examples are given below.

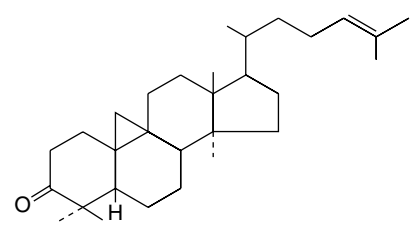


Cucurbitacin E (elaterin); R = CH_3CO
Cucurbitacin I (elaterin B); R = H

Cycloartanes. As was indicated previously (see Fig. 23.1) the ring closure of squalene 2,3-oxide yields cycloartenol as an intermediate in plant sterol biosynthesis. However, these phytosterols are also found in the free state in a wide range of plants; medicinal examples include the neem and olive plants, *Euphorbia* spp., *Hypericum*, the woody nightshade and a number of members of the Cucurbitaceae. The formula of one such compound is given below.



Ecdysterone



Cycloartenone

24

Miscellaneous isoprenoids

In addition to the groups of compounds considered in Chapters 22 and 23, there exists in nature a tremendous range of other isoprenoids, some of which have become of increasing interest as medicinal agents. There are also those plant metabolites of 'mixed' biogenetic origin which contain an isoprenoid moiety (e.g. some indole alkaloids, the cannabinoids and chlorophylls) and these are considered in other appropriate chapters.

MONOTERPENES

As illustrated in Figures 18.18 and 18.19, the monoterpenes are derived from the C_{10} geranyl pyrophosphate and constitute important components of volatile oils. Other examples are given below. Monoterpenoid compounds are reviewed regularly in *Natural Product Reports* (for coverage of the 1990 literature see D. H. Grayson, *ibid*., 1994, **11**, 225).

IRIDOIDS

The iridoids are cyclopentan-[*c*]-pyran monoterpenoids and constitute a group of which the number of known members is constantly increasing. The name derives from *Iridomyrmex*, a genus of ants which produces these compounds as a defensive secretion. In a series of reviews covering the years up to December 1989 several hundred iridoids, classified originally into 10 groups, have been listed. Junior (*Planta Med*., 1990, **56**, 1) has reviewed (146 refs) the isolation and structure elucidation of these compounds. Most occur as glycosides; some occur free and as bis compounds. There are many seco-iridoids, see secologanin, in which the pyran ring is open, and in a few the pyran ring oxygen is replaced by nitrogen, Fig. 24.1.

For a review covering new naturally occurring iridoids reported during 1994–2005, see B. Dinda *et al.*, *Chem. Pharm. Bull.*, 2007, **54**, 159–222; for Part 2 covering the identification of 158 new plant seco-iridoids from 1994 to 2005 and the bioactivity of the two groups, see B. Dinda *et al.*, *Chem. Pharm. Bull.*, 2007, **55**, 689–728.

Of pharmaceutical significance is their presence in Valerian, Gentian and Harpagophytum and the involvement of loganin (Chapter 26) as a precursor of the non-indole portion of some alkaloids.

GENTIAN

Gentian (Gentian Root *BP, EP, BHP*) consists of the dried fermented rhizomes and roots of the yellow gentian, *Gentiana lutea* L. (Gentianaceae), a perennial herb about 1 m high found in the mountainous districts of central and southern Europe and Turkey. Important districts for its collection are the Pyrenees, the Jura and Vosges Mountains, the Black Forest, former Yugoslavia and the Carpathians.

As it is now a protected plant in some areas, attempts are being made to cultivate it in some EU countries (France, Italy, Germany); for this, the initial selection of plant material is of vital importance.

History. Gentian, possibly not derived from the species now official, was known to Dioskurides and Pliny. The drug was commonly employed during the Middle Ages.

Collection and preparation. When the plants are 2–5 years old, the turf is carefully stripped around each and the rhizomes and roots are dug up. This usually takes place from May to October, collection in the autumn being more difficult on account of the hardness of the soil, although possibly preferable from the medicinal point of view. There is no UK demand for 'white' or unfermented gentian, the commercial

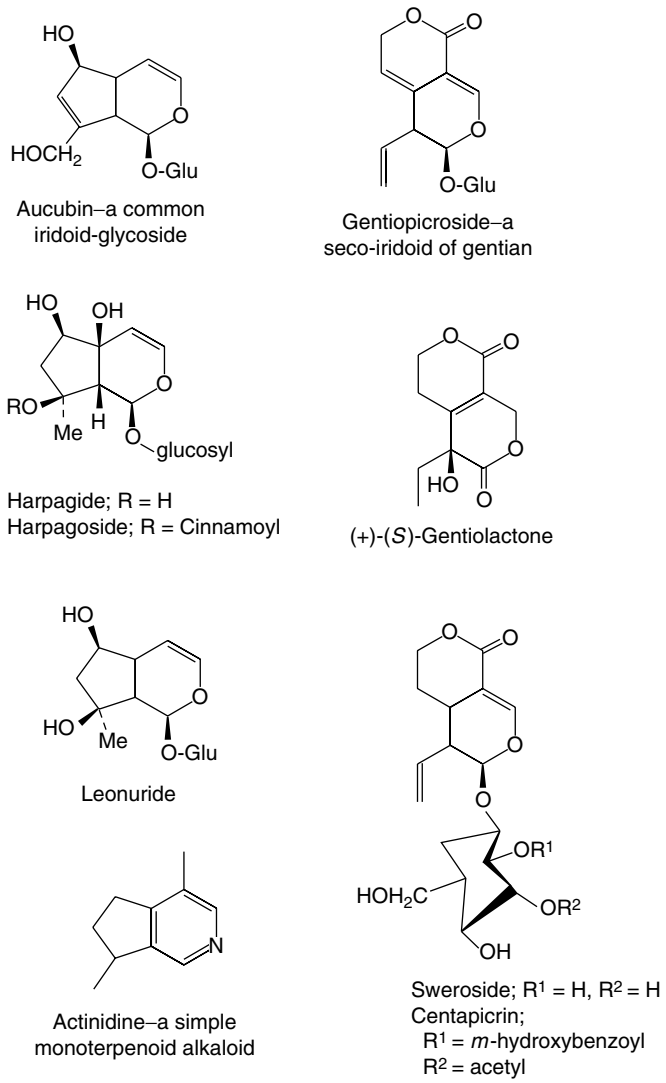


Fig. 24.1
Examples of iridoids.

drug consisting of 'red' or fermented gentian. The method of preparing this varies somewhat in different districts. Usually, the drug is made into heaps, which are allowed to lie on the hillside for some time and may even be covered with earth. After it is washed and cut into suitable lengths the drug is dried, first in the open air and then in sheds. Prepared in this way the drug becomes much darker in colour, loses some of its bitterness and acquires a very distinctive odour.

Macroscopical characters. The plant has a cylindrical rhizome which may attain a diameter of 4 cm and give off roots more than 1 m in length. The crown bears 1–4 aerial stems. The fresh root is whitish and fleshy internally and practically odourless.

The commercial drug consists of simple or branched, cylindrical pieces up to 20 cm long and 1–3 cm diameter. The outer surface is covered with a yellowish-brown cork. The rhizomes are usually of larger diameter than the roots and frequently bear one or more apical buds and encircling leaf scars. On drying, the rhizomes wrinkle transversely, whereas the roots wrinkle longitudinally. The drug is brittle when perfectly dry, but readily absorbs moisture from the air and becomes very tough. It has a characteristic odour and a sweet taste, which later becomes bitter.

Microscopical characters. A transverse section shows an orange–brown bark separated by a darker cambium line from the porous, very indistinctly radiate wood. Only the rhizomes show a pith. More detailed examination shows about 4–6 rows of thin-walled cork cells between which and the cambium is a somewhat thick-walled phelloderm and wide zone of brown, thin-walled parenchyma containing oil globules and minute needles of calcium oxalate. Small groups of soft phloem are seen but phloem fibres are absent.

Examination of the wood and pith shows abundant parenchyma having similar cell contents to those of the bark. The vessels occur either isolated or in small groups and show mainly reticulate or scalariform thickening; a few spiral and annular vessels occur. Groups of soft phloem ('phloem islands', 'interxylary phloem') occur in the xylem. The drug contains very little starch and no sclerenchymatous cells or fibres.

Allied drugs. The roots of other species of *Gentiana* (e.g. *G. purpurea, G. pannonica* and *G. punctata*) have been imported. They appear to have similar medicinal properties to the official drug but are usually of smaller size. In India the roots of *G. kurroa* and *Picrorhiza kurroa* are used as gentian substitutes under the name of 'kathi roots'. *G. kurroa*, however is now considered to be a threatened species and shoot multiplication and root formation in *in vitro* cultures have been studied for its possible propagation. Similarly, work in Japan has focused on the mass production of *G. triflora* plants by the cultivation of tissue segments in artificial media in the presence of growth hormones. The *BP*/*EP* includes a TLC test to differentiate between the official drug and related species.

Adulterants. Adulteration, probably due to careless collection, sometimes occurs. The rhizomes of *Rumex alpinus*, which give the test for anthraquinone derivatives, have been reported; also a dangerous but easily detected admixture with the rhizomes of *Veratrum album*.

Constituents. Gentian contains bitter glycosides, alkaloids, yellow colouring matters, sugars, pectin and fixed oil.

The seco-iridoid *gentiopicroside* (also known as gentiopicrin and gentiamarin; formula see Fig. 24.1) is the principal constituent and was isolated from fresh gentian root in 1862. It occurs to the extent of about 2% and on hydrolysis yields a lactone (gentiogenin) and glucose. A biphenolic acid ester of gentiopicroside, amarogentin, which occurs in small amount (0.025 to 0.05%) has a bitterness value some 5000 times greater than that of gentiopicroside and is therefore an important constituent of the root; other bitters isolated are sweroside and swertiamarin. The isoprenoid gentiolactone has been separated into its enantiomers (Fig. 24.1) by HPLC involving a chiral column (R. Kakuda *et al.*, *Chem. Pharm. Bull.*, 2003, **51**, 885) and the same group of workers (J. Toriumi *et al.*, *Chem. Pharm. Bull.*, 2003, **51**, 89) has reported on new triterpenes in addition to α-amyrin, β-amyrin and lupeol.

The yellow colour of fermented gentian root is due to xanthones (Chapter 21) and includes gentisin (also known as gentiamarin) (Fig. 21.16), isogentisin and gentioside (a 3β-primeverosidoisogentisin). Gentian also contains *gentisic acid* (2,5-dihydroxybenzoic acid) and about 0.03% of the alkaloids gentianine and gentialutine, which may be artefacts of the preparation process.

The bitter principles of *G. lutea* and *G. purpurea* have been assayed by HPLC and separated preparatively by overpressure layer chromatography. The official *BP bitterness value* of the root should be not less than 10 000 when determined by comparison with quinine (200 000).

Gentian is rich in sugars, which include the trisaccharide *gentianose*, the disaccharides *gentiobiose* and *sucrose*. During the fermentation

process these are partially hydrolysed into glucose and fructose. If fermentation is allowed to proceed too far, the hexose sugars are converted into alcohol and carbon dioxide. Gentian should yield 33–40% of water-soluble extractive (*BP* not less than 33%), but highly fermented root yields much less.

For references to the chemical composition and to the seasonal variations in the content of secondary metabolites, in the aerial parts of *G. lutea*, see N. Menković *et al*., *Planta Medica*, 2000, **66**, 178.

Three monoamine oxidase inhibitors have been located in the bark (H. Haraguchi *et al.*, *Phytochemistry*, 2004, **65**, 2255).

Uses. Gentian is used as a bitter tonic. In traditional medicine it has been employed to treat various gastrointestinal conditions, as an anti-inflammatory and wound-healing agent. It is also reported to have choleretic, antioxidative, hepatoprotective and antifungal activities (see A. Mathew *et al.*, *Pharm. Biol.*, 2004, **42**, 8).

CENTAURY

Centaury (*BP*/*EP, BHP*), family Gentianaceae consists of the dried flowering aerial parts of *Centaureum erythraea* Rafn, including *C. majus* and *C. suffruticosum*.

The biennial plant, some 30 cm in height, is widely distributed throughout Europe, N. America, N. Africa and W. Asia; it is exported from Morocco, Bulgaria and Hungary.

As seen in the dried drug, the hollow stems are yellowish-green with distinct ribs; the sessile leaves, 1–5 cm long, are light green in colour, obovate or spathulate in outline with an entire margin and an obtuse apex; the inflorescence consists of a tubular five-toothed calyx and a joined five-lobed, white-pinkish corolla, five stamens and a cylindrical ovary having parietal placentation and several small brown seeds.

Features of the above are seen in the powder and include fragments of leaf having sinuous epidermal cells with striated cuticles and prisms, occasionally clusters, of calcium oxalate in the mesophyll cells; pollen grains are about 25–30 μm in diameter with three pores and a pitted exine.

The drug has a very bitter taste due to small amounts of seco-iridoid glycosides. Compounds characterized include centapicrin, swertiamarin, sweroside and gentiopicroside. The *BP* TLC test for identity uses a swertiamarin/rutin test solution. Other constituents include flavonoids (up to 0.4%), methylated xanthone derivatives, traces of pyridine and actinidine alkaloids (Fig. 24.1), triterpenoids and various acids.

Centaury is employed as a bitter, stimulating the appetite and increasing the secretion of bile and gastric juice.

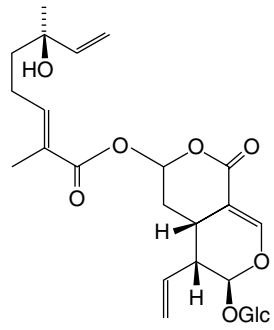
BOGBEAN LEAF

Bogbean Leaf *BP*/*EP, BHP* is the dried, entire or fragmented leaf of *Menyanthes trifoliata* L., family Menyanthaceae. It is a perennial glabrous aquatic or bog plant up to 30 cm in height with leaves and flowers raised above the surface of the water. It grows widely throughout Europe, northern Morocco and N. America. Commercial supplies come largely from central Europe.

The trifoliate leaves have petioles (7–20 cm) with a long, sheathing base. The leaflets are obovate or elliptic with an entire or sometimes sinuous margin and spathulate base. The taste is very bitter and persistent.

Microscopical features include thin-walled sinuous epidermal cells with anomocytic stomata, cuticular striations and characteristic aerenchyma in the leaf lamina and petiole.

The constituents include: the bitter seco-iridoid glycosides menthiafolin and loganin; flavonoids (hyperin, kaempferol, quercetin, rutin and trifolioside); small amounts of tannin; triterpenes including the betulinic acid derivative menyanthoside which is the principal saponin of the rhizome; phenol-carboxylic acids; coumarins.



Menthiafolin

Loganin is used as a reference in the *BP* TLC examination and a minimum of 3000 is specified for the bitterness test.

Bogbean leaf is used for its bitter and diuretic properties and for the treatment of various rheumatic conditions.

PLANTAIN

Three common European plantains are *Plantago major* L. (common plantain), *P. media* L. (hoary plantain) and *P. lanceolata* L.s.l. (ribort plantain, ribwort). They are distributed generally throughout Europe and temperate Asia and have become naturalized in the US and elsewhere; they are common weeds of lawns and cultivated ground. The dried leaves of *P. major* collected at the time of flowering are included in the *BHP* 1983 and the leaves and scape of *P. lanceolata* in the *BP*/*EP* (the scape is the leafless ridged pedicel bearing the terminal spike.

The leaves of *P. major* are 10–30 cm in length, ovate or elliptic, entire or irregularly toothed with the blade abruptly contracting into the long petiole. In the dried drug the leaves are brittle and often folded. *P. lanceolata* has strongly ribbed, ovate to lanceolate leaves up to 30 cm in length and 4 cm wide with the blade gradually narrowing into the petiole which is about half as long as the blade. The leaf margin is distinctly toothed. The deeply furrowed five- to seven-ribbed scape usually exceeds the leaves in length and terminates in a characteristic spike of bracts and small white flowers, the long stamens of which in the fresh plant are particularly conspicuous.

Both species have similar microscopical features and these, particularly the clothing and glandular trichomes, are fully described in the pharmacopoeias.

Constituents. The constituents appear similar for both species and include the iridoid aucubin and derivatives (Fig. 24.1), flavonoids, e.g. apigenin and luteolin (see Table 21.5), sugars, mucilage and various organic acids. The *BP*/*EP* requires a minimum of 1.5% of total *o*-dihydroxycinnamic acid derivatives expressed as acteoside and detects contamination of the drug with *Digitalis lanata* leaves by TLC.

Uses. Traditional uses of plantain depend on its stated expectorant, diuretic and antihaemorrhagic properties.

VALERIAN ROOT

Valerian consists of the rhizome, stolons and roots of *Valeriana officinalis* L.s.l. (Valerianaceae), collected in the autumn and dried at a temperature below 40°C. The plant is a perennial about 1–2 m high. It is obtained from wild and cultivated plants in The Netherlands, Belgium,

France, Germany, eastern Europe and Japan. It is also cultivated in the USA. Polyploidy occurs in *V. officinalis* and there are diploid, tetraploid and octoploid types. British valerian is usually octoploid and central European usually tetraploid.

Cultivation, collection and preparation. Valerian-growing in England has now ceased. Much drug is still produced in Europe, particularly in Holland; trials carried out in 1971–74 at Poznán on light soil showed that propagation by sowing seed, as distinct from the more laborious planting of seedlings, is fully justified.

History. The word '*Valeriana*' is first met with in writings of the ninth and tenth centuries. The drug is mentioned in Anglo-Saxon works of the eleventh century, and was much esteemed not only for its medicinal properties, but also as a spice and perfume. Spikenard ointment, which was used by the Romans and has long been used in the East, was prepared from young shoots of *Nardostachys jatamansi*.

Macroscopical characters. The drug consists of yellowish-brown rhizomes, stolons and roots. The rhizomes are erect, 2–4 cm long and 1–2.5 cm wide, and may be entire or sliced. The roots, which are up to 10 cm long and 2 mm diameter, are more or less matted and broken. In some samples of the drug they almost completely envelop the rhizome, while in others they are mainly separated from it. The drug breaks with a short and horny fracture and is whitish or yellowish internally. The development of the characteristic odour during drying and storage results from a breakdown of the unstable valepotriates and the hydrolysis of esters of the oil to give isovaleric acid as a product, see below. The taste is camphoraceous and slightly bitter.

Microscopical characters. A transverse section of the rhizome shows a thin periderm, a large parenchymatous cortex which is rich in starch and an endodermis containing globules of volatile oil. Within a ring of collateral vascular bundles lies a large pith containing scattered groups of sclerenchymatous cells.

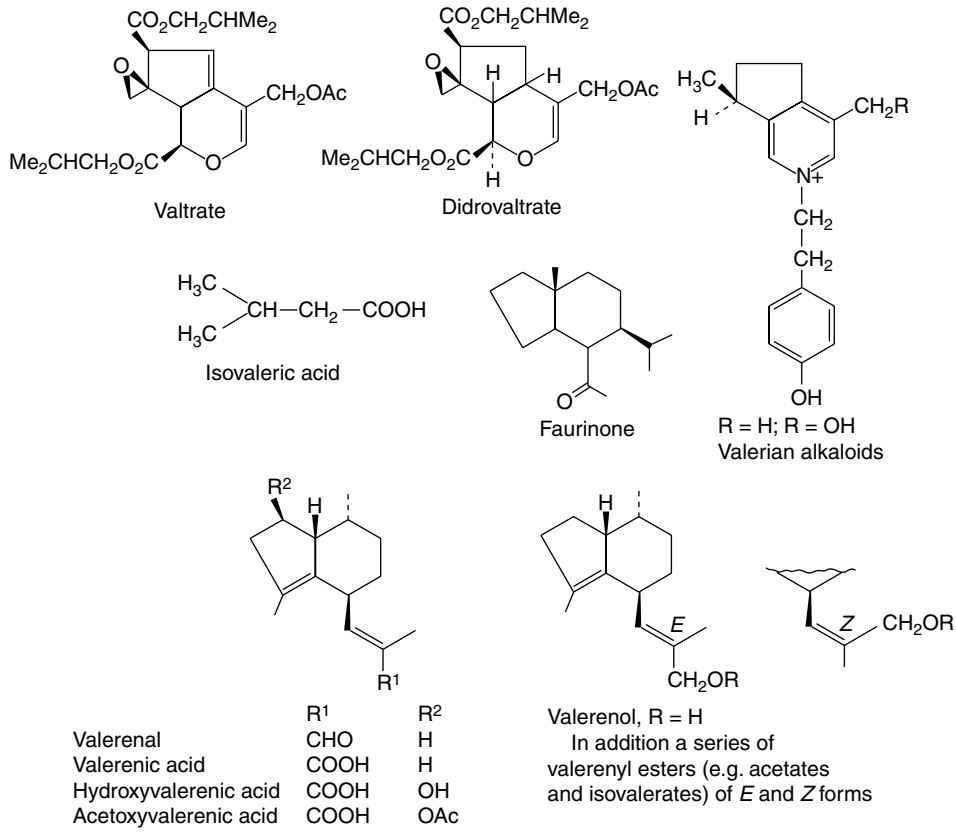
A transverse section of a root shows an epidermis bearing papillae and root hairs, and an exodermis containing globules of oil. The cortex and pith, the latter well-developed in old roots, contain starch. The starch is present mainly in compound grains with two to four components, measuring 3–20 μm diameter.

Constituents. The drug yields about 0.5–1.0% of volatile oil. This contains esters (bornyl isovalerate, bornyl acetate (*c*. 13.0%), bornyl formate, eugenyl isovalerate, isoeugenyl isovalerate), alcohols, eugenol, terpenes and sesquiterpenes (e.g. valerenal, *c*. 12%). The latter comprise various acids, esters, alcohols and a ketone (faurinone) some of which are illustrated in the formulae shown (Fig. 24.2).

Also present in the drug are epoxy-iridoid esters called valepotriates: for example valtrate, didrovaltrate, acevaltrate, and isovaleroyloxyhydroxydidrovaltrate (see formulae).

Valerian also contains alkaloids (0.05–0.1% in the dried root); no structures have been assigned to those (e.g. chatinine and valerine) described in the older literature. Two quaternary alkaloids with a monoterpene structure and which are not identical with those previously isolated have been reported; they are similar to skytanthine and related alkaloids, which occur in widely separated families.

Seasonal variations in the constituents of valerian raised in the Netherlands have been reported. Thus the accumulation of valerenic acid and its derivatives together with valepotriates reached a maximum



	R^1	R^2
Valerenal	CHO	H
Valerenic acid	COOH	H
Hydroxyvalerenic acid	COOH	OH
Acetoxyvalerenic acid	COOH	OAc

Fig. 24.2
Some constituents of valerian.

in February to March whereas the volatile oil remained essentially constant during the period of study. Strains producing 0.9% essential oil and a high content of valerenic acid and derivatives (0.5%) were recognizable (R. Bos *et al*., *Planta Medica*, 1998, **64**, 143). For the clinical significance of such strains see 'Action and uses'.

Thirteen valepotriates have been identified in the suspension root culture of *V. officinalis*; root differentiation promotes production. A new iridoid diester, not present in untransformed roots, has been reported in hairy root cultures of var. *sambucifolia* which also produce various kessane derivatives, tentatively identified as kessyl alcohol and acetate. (F. Grünicher *et al*., *Phytochemistry*, 1995, **38**, 103; **40**, 142).

Quality control. The pharmacopoeia requires a minimum volatile oil content of 0.5% for the whole drug and 0.3% for the cut drug. There is a minimum requirement for sesquiterpenic acids of 0.17% calculated as valerenic acid and maximum values for total ash (12.0%) and ash insoluble in hydrochlaoric acid (5.0%). Stem bases are limited to 5.0%.

Allied drugs. *Indian valerian*, which is official in the *Indian Pharmacopoeia*, consists of the dried rhizome and roots of *Valeriana wallichii*. It is collected in the Himalayas. The drug consists of yellowish-brown rhizomes, 4–8 cm long and up to 1 cm thick, and a very variable amount of roots up to 7 cm long and 1–2 mm thick. The rhizomes are unbranched and somewhat flattened dorsiventrally. The upper surface bears leaf scars and the lower surface roots or root scars. The rhizome breaks with a short fracture, and the horny interior shows a small dark bark, a well-marked cambium, about 12–15 light-coloured xylem bundles and a dark pith and medullary rays. The odour is valerianaceous and the taste bitter and camphoraceous. The drug contains valepotriates and about 0.3–1.0% of volatile oil containing esters of isovalerenic and formic acids.

Centranthus ruber root (Valerianaceae) also contains a number of the valepotriates of valerian.

Japanese valerian or *kesso* is obtained from *Valeriana angustifolia*. It yields as much as 8% of volatile oil, which is, however, not identical with the oil in the European drug.

Action and uses. Valerian is used as a carminative, and as an antispasmodic in hysteria and other nervous disorders. It is often prescribed with bromides or other sedatives. Considerable quantities of valerian are used by the perfumery industry.

Previously, one problem with valerian preparations was their unreliability of action and this undoubtedly arose from both the unstable nature of the active constituents and the genetic variability of the plant material. The situation was not helped by the lack of success in ascetaining the identity of the sedative components. The volatile oil did not appear to account for the entire action of the drug and the alkaloids were also ruled out in this respect. Subsequent characterization and demonstration of activity in the group of compounds termed valepotriates in the late 1960s and early 1970s appeared in part to resolve the situation and interest turned to these compounds. Nevertheless, it had previously been demonstrated that two sesquiterpene components of the oil, valerenic acid and valeranone, were physiologically active. Further, a related species *Nardostachys jatamansi*, which is used in Asia for the treatment of nervous diseases, was shown to contain valeranone but lacked valepotriates. In 1978 the pharmacological properties of valeranone were confirmed and Japanese workers concluded that the sedative properties of Japanese valerian could be ascribed to this group of compounds. When, therefore, reports on the cytotoxicity of valtrate and didrovaltrate appeared in 1981 and 1982 (although no side-effects of oral administration of valerian in man have been reported), attention switched to races and species of valerian, as well as selective preparations of the drug, which lacked these compounds.

Further reading

Houghton PJ (ed), Hardman R (series ed) 1997 Medicinal and aromatic plants—industrial profiles, Vol 1. Valerian—the genus *Valeriana*. Harwood Academic, Netherlands

DEVIL'S CLAW (HARPAGOPHYTUM)

Devil's claw *BP*/*EP* consists of the cut and dried tuberous secondary roots of *Harpagophytum procumbens* D.C. and/or *H. zeyheri* L. Decne. It contains not less than 1.2% harpagoside calculated with reference to the dried drug.

The plant, which derives its name from the characteristic structure of the fruit, is native to Southern and Eastern Africa and is largely obtained from Namibia, with lesser amounts from S. Africa and Botswana. In 2002, at the height of the drug's popularity, exports from S. Africa amounted to some 1018 tonnes of dried tubers, representing millions of plants. To avoid extinction of the plant, a proposal was made to add it to the CITES list but in deference to the effect on the economy of rural areas this was withdrawn and efforts were initiated to develop microprogation techniques to solve the problem. For a full review, see 'Further reading'.

Description. The drug consists of mainly transverse, often fan-shaped slices of the tuberous root with a reddish-brown to dark brown, longitudinally wrinkled, cork. Seen in transverse section the vascular bundles are arranged in radial rows. It is odourless but has a very bitter taste.

Microscopical features of the root include thin-walled yellowish-brown cork cells, thin-walled cells of cortical parenchyma which may contain reddish-brown contents, needles and crystals of calcium oxalate together with the vascular elements. Starch grains are absent.

Constituents. The roots contain iridoid glycosides, flavonoids, various phenolic acids, triterpenes including oleanic and ursolic acids, a quinone (harpagoquinone) and a high concentration of sugars consisting principally of the trisaccharide stachyose (Table 20.1). The principal glycosides are harpagide and its cinnamoyl ester (Fig. 24.1) together with the epoxyiridoid glycoside procumbide. 6-Acetylacteoside and 2,6-diacetylacteoside have been isolated from commercial roots (N. M. Munkombwe, *Phytochemistry*, 2003, **62**, 1231).

The *BP*/*EP* includes a TLC test for identification using a solution of harpagoside as reference and a liquid chromatographic assay with methyl cinnamate as an internal standard. The total ash should not exceed 10.0% and the loss on drying not more than 12.0%.

Action and uses. Devil's claw has a wide reputation for the treatment of rheumatic disease and although the therapeutic contributions of the various constituents have not been unambiguously established, animal tests indicate that the iridoids are involved in the anti-inflammatory and analgesic effects.

Further reading

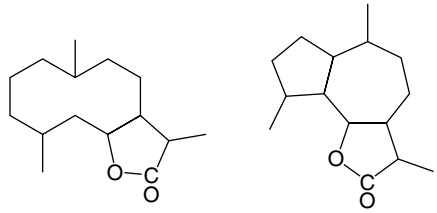
Stewart KM, Cole D 2005 The commercial harvest of devil's claw (*Harpagophytum* spp.) in southern Africa: the devil's in the details. J Ethnopharmacology 100 (1–2): 225–236

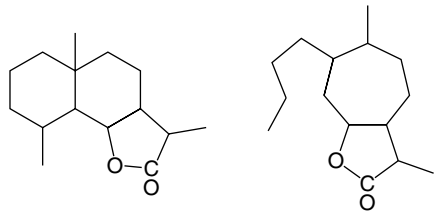
SESQUITERPENES

Sesquiterpenes are biogenetically derived from farnesyl pyrophosphate (Figs 18.18 and 18.19) and in structure may be linear, monocyclic or bicyclic. They constitute a very large group of secondary metabolites, some having been shown to be 'stress compounds' (q.v.) formed as a result of disease or injury. For many years their presence in certain volatile oils and resins has been recognized.

SESQUITERPENE LACTONES

Over 6000 compounds of this group are known and continue to constitute an active area of research. They are particularly characteristic of the Compositae but also occur sporadically in other families. Not only have they proved of interest from chemical and chemotaxonomic viewpoints, but also many possess antitumour, antileukaemic, cytotoxic and antimicrobial activities. They can be responsible for skin allergies in humans and they also act as insect-feeding deterrents.





Chemically, the compounds can be classified according to their carbocyclic skeletons; thus, from the germacranolides can be derived the guaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides, xanthanolides, etc. A structural feature of all these compounds, which appears to be associated with much of the biological activity, is the α,β-unsaturated-γ-lactone. As examples see the entries below on 'Santonica Flowers', 'Feverfew', 'Chicory' and 'Arnica'. Other Compositae which are herbal remedies and contain sesquiterpene lactones are *Taraxacum officinale* (dandelion), *Artemisia absinthium*, *Cichorium* spp., *Bidens* spp. and *Eupatorium* spp.

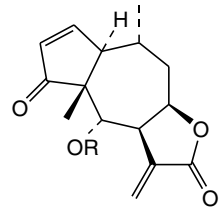
Sesquiterpene lactones of the Umbelliferae are interesting in that the usual skeletal types (germacranolides, guaianolides, etc.) are found but all differ in their stereochemistry from the analogous compounds of the Compositae. It is therefore possible that although the biosynthetic steps in the two families form two parallel series of compounds, the conformation of the *trans*,*trans*-farnesyl diphosphate precursor is different in the two cases.

ARNICA FLOWERS

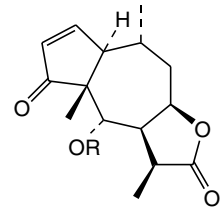
The drug consists of whole or partially broken dried flower-heads of *Arnica montana* L. (Compositae), a perennial herb with a creeping rhizome. The principal producers are the former Yugoslavia, Spain, Italy and Switzerland where it grows on the lower mountain slopes.

Characters. The receptacle, if present, is about 8 mm in diameter and is slightly convex. It bears pits, corresponding to the position of the flowers, in each of which is a stiff bristle. The involucre consists of two rows of dark-green, hairy, lanceolate bracts about 1 cm in length.

The pistillate, ligulate florets are about 3 cm long. Each consists of a yellow corolla having three teeth and seven to twelve veins, a style and stigma, and a pubescent, dark-brown achene 5 to 7 mm long. The latter is pubescent and glandular and is surmounted by a large, white pappus consisting of very characteristic, barbed bristles. The disc florets resemble the ligulate ones but have a tubular corolla and are hermaphrodite. When examined microscopically, numerous spiny pollen grains and the form of the hairs are seen. Odour, slight but agreeable; taste, bitter and acrid.



R = H (helenalin), and various lower fatty acid groups (see text)



R = H (11,13 dihydrohelenalin), and various lower fatty acid groups (see text)

Constituents. The flowers contain volatile oil (0.5–1.0%), a range of methylated flavones and sesquiterpene lactones of the pseudoguaianolide type which include esters involving acetic acid and various C_4 and C_5 acids (e.g. isobutyric, 2-methyl butyric, isovaleric, and tiglic acids). The principal active constituents (antirheumatic, antiarthritic, antihyperlipidaemic, respiratory analeptic) are esters of helenalin and 11,13-dihydrohelenalin. The former is characteristic of Eastern European flowers and the latter of Spanish flowers. Other constituents include diterpenes and pyrrolizidine alkaloids (tussilagine and isotussilagine).

J. A. Douglas *et al.* (*Planta Medica*, 2004, **70**, 166) have studied variations in the sesquiterpene lactone levels throughout the flower-heads; highest levels were recorded for the disc florets (0.872%), lower levels for the ray florets (0.712%) with the flower receptacles (0.354%) and stems (0.028%) the lowest. The total steroidal lactone levels for the drug rose as the flowers matured.

Quality control. The *BP/EP* 2000 tests include thin-layer chromatography to exclude *Calendula officinalis* and a liquid chromatography assay to determine total lactone sesquiterpenes (not less than 0.40% expressed as helenalin tiglate).

Allied Drug. *Arnica rhizome* consists of the dried rhizome and roots of *Arnica montana*. The rhizome is dark brown in colour, about 2 to 10 cm long, and 2 to 6 mm in diameter. It bears numerous wiry roots and cataphyllary leaves. The transverse section shows a yellowish bark containing oleoresin ducts, a ring of wedge-shaped vascular bundles, and a large pith. The constituents are similar to those of the flowers. About 10 per cent of inulin is also present, but starch is absent.

Uses. Arnica has astringent properties; tinctures and infusions of the dried flower-heads and rhizomes have both been long used as a

domestic remedy for the treatment of sprains and bruises. However, neither should be applied to broken skin and treatment should be discontinued should dermatitis develop. In some countries, the use of the drug is subject to legal restrictions.

An arnica gel product was the first 'traditional herbal medicine' to be granted registration in the UK under new regulations introduced by the Medicines and Healthcare products Regulatory Agency (*Pharm. J.*, 2006, **277**, 566).

FEVERFEW

Feverfew, *Tanacetum parthenium* (L.) Schultz Bip. [*Chrysanthemum parthenium* (L.) Bernh.] family Asteraceae/Compositae has a long history as a medicinal plant. It is probably native in S.E. Europe, Asia Minor and the Caucasus but is now established throughout Europe and in N. and S. America, where it is found on roadsides and waste areas.

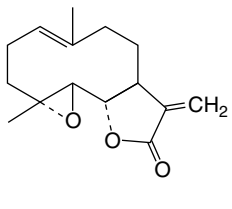
The plant is a strongly aromatic herb with erect, branching, somewhat downy, stems reaching a height of up to 60 cm. The leaves are pinnate with ovate or oblong segments, pinnatifid and toothed; yellowish-green and pubescent to subglabrous. The numerous flowerheads, 12–22 mm in diameter are long-stalked and form broad terminal corymbs. The involucre is hemispherical with pubescent bracts, white ray florets and yellow disc florets. The fruits are achenes.

Features of the powdered drug include portions of leaf epidermis having a striated cuticle and anomocytic stomata, vascular tissue from the stems and veins, numerous large uniseriate covering trichomes, glandular trichomes, portions of the florets and typical Compositae pollen grains.

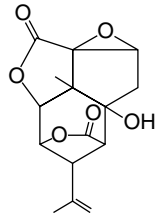
Some commercial samples of the drug may be devoid of flowers.

Constituents. In common with many other Compositae, feverfew is phytochemically characterized by the production of sesquiterpene lactones, which can be classified as indicated above. Germacranolides include parthenolide, 3β-hydroxyparthenolide, costunolide, 3β-hydroxycostunolide and others. Chrysanthemin A (canin) and chrysanthemin B are stereoisomers of the guaianolide group, and magnoliolide and others are eudesmanolides. Other constituents are a small amount (up to about 0.07%) of volatile oil containing monoterpenes and sesquiterpenes, tannins and flavonoids.

Feverfew is standardized on its parthenolide content and the *BP*/*EP* requires a minimum of 0.2% with reference to the dried drug. The assay involves liquid chromatography of a methanolic extract of the drug using parthenolide as reference compound and absorbance measurements at 220 nm. The assay is particularly important as commercial products vary enormously in parthenolide content, some having none at all. This may be due to chemical races known to exist for the species or to confusion in nomenclature, particularly in the US, where the term 'feverfew' may be applied to species other than *T. parthenium*.



Parthenolide



Picrotoxinin

Uses. Feverfew has long been used for the treatment of fever, arthritis, migraine, menstrual problems and other disorders. More recently, it attracted much popular and scientific interest resulting from favourable reports concerning its use for the prophylactic treatment of migraine headaches.

Chicory

Chicory (*Cichorium intybus*, family Compositae) is indigenous to Europe and is now widespread in northern states of the USA, Canada and parts of Asia; it is widely cultivated. The plant prefers calcareous soils and is easily recognized by its bright blue flowers borne on stiffly erect grooved stems with coriaceous dark-green toothed leaves. In Europe and the USA the root is a traditional herbal remedy and in India the seeds and flowers are also used.

As with some other species of the Compositae the dried roots contain a high proportion (up to 58%) of inulin (q.v.) together with sugars. The coumarins chicoriin, esculetin, esculin, umbelliferone and scopoletin (see Table 21.2) are found in the leaves. Chicory roots also contain various sesquiterpene lactones and glycosides (M. Sato *et al.*, *Chem. Pharm. Bull.*, 1988, **36**, 2423); examples from the 13 isolated compounds include cichorioside A (eudesmane type), 8-deoxylactucin (guaiane type) and picriside B (germacrane type). Lactucin and lactucopicrin show antimalarial activity (T. A. Bischoff *et al.*, *J. Ethnopharmacology*, 2004, **95**, 455),

Decoctions of the root are used as a diuretic and to treat liver ailments; the root is also cited as a tonic and laxative. Extracts of the root and root callus culture have been pharmacologically tested, with positive results, for their antihepatotoxic properties. The roasted roots are well-known for their use in coffee mixtures and as a coffee substitute.

The roots of the culinary *Cichorium endivia* (endive) contain the same constituents as those of *C. intybus*.

Fish berries

Fish berries or cocculus indicus consists of the dried fruits of *Anamirta cocculus* (Menispermaceae), a climbing shrub found in south-eastern Asia (particularly the Malabar coast of India) and the East Indies.

As in the other members of the Menispermaceae, the dorsal side of the fruit grows more rapidly than the ventral, with the result that the fruit becomes reniform and the base and apex both lie on the concave side. The pericarp is rough and woody and the cup-shaped seed consists of an oily endosperm surrounding the embryo, which lies with its radicle pointing towards the apex of the fruit. The two cotyledons occupy separate slit-like cavities in the endosperm. The drug has no odour; the pericarp is tasteless, but the seed is intensely bitter.

The seed contains about 1.5% of a bitter, crystalline, highly toxic substance, 'picrotoxin'. This consists of equimolecular proportions of picrotoxinin, $C_{15}H_{16}O_6$, and picrotin, $C_{15}H_{18}O_7$. Picrotoxinin (see formula) is a highly oxygenated sesquiterpene derivative. The seeds also contain about 50% of fat.

Picrotoxin has been official. It is used intravenously in poisoning by barbiturates and other narcotics. Very small quantities of the fruits are sufficient to stupefy fish.

Orris

Orris rhizome is obtained from three species of *Iris* (Iridaceae), namely *I. florentina*, found in northern Italy, *Iris germanica* found in northern Italy, France, central Europe, Morocco and northern India, and *Iris pallida*, found in Italy (Florence and Lucca) and eastern France. The chief varieties in English commerce are known as Florentine and Veronese. Orris is also produced in Morocco.

Orris root has been used in perfumery from Greek and Roman times. The plants are dug up in August and September, and the peeled

rhizomes are dried in the sun for about 5 days either on matting (Florentine) or threaded on cords (Veronese). When dry they are stored for about 3 years in order to develop their full aroma.

Mogadore orris is usually inferior to the European, the rhizome being smaller, darker and less fragrant.

Orris rhizome contains volatile oil which contains irone (see formula below), a substance having an odour of violets. An isomeric substance, ionone, is used as a synthetic violet perfume. Orris also contains starch, calcium oxalate, iridin (a flavone related to rutin), isoflavones, and β-sitosterol and its glycosides.

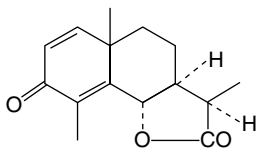
Powdered orris root is used in dusting powders, while the oil is used in perfumery not only for its delicate odour, but also as a fixative for artificial violet perfumes.

Santonica flowers

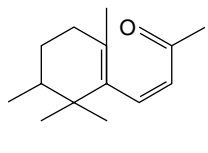
Wormseed consists of the dried unexpanded flower-heads of *Artemisia cina* and other santonin-containing species of *Artemisia* (Compositae). *A. cina* is a small plant abundant in Turkestan, where a factory for the extraction of santonin exists at Chimkent. Santonin is prepared from *Artemisia* species found wild in the Kurran valley in Pakistan, and cultivation in this area has been successfully commenced.

The chief anthelminthic constituent of the drug is the sesquiterpene lactone santonin. It has the structure given below. Wormseed also contains a little volatile oil and a second, crystalline lactone, artemisin, closely related to santonin. The amount of santonin present varies considerably not only in the different species and hybrids, but also at different seasons of the year; Russian workers have reported diurnal variations.

In use wormseed has been replaced by santonin, which is very efficient in its action on roundworms. It has less effect on thread worms and none whatever on *Taenia*.



α-Santonin



β-Irone

Artemisinin

This unusual sesquiterpene lactone possesses an endoperoxide moiety and is a component of the Chinese antimalarial drug Qinghaosu. It has been successful in treating cases of chloroquine-resistant *Plasmodium falciparum* and particularly cerebral malaria. The increased demand for the drug has led to a supply problem, accompanied by a vast increase in price, giving the WHO concern that the African campaign against malaria would be put in jeopardy. Production of the plant source (sweet Annie) is being increased and it is hoped that a new semi-synthetic bioequivalent artemisinin will lower the cost of treatment (see K. Purcell, *HerbalGram*, 2006, **69**, 24).

Artemisinin (Fig. 24.3) occurs in the herb *Artemisia annua* along with smaller amounts of other cadinane-type sesquiterpenes; by 1993 around 16 of these compounds had been isolated from the plant. Various studies on the accumulation of artemisinin during the development of the plant have been reported; some indicate the highest content before flowering, others at full flowering (see J. F. S. Ferreira *et al*., *Planta Med*., 1995, **61**, 167). K.-L. Chan *et al*. (*Phytochemistry*, 1997, **46**, 1209–14) report that artemisinin as isolated from *A. annua* is polymorphic in form. Previously regarded as orthorhombic, the crystals may also be triclinic with the latter possessing a higher dissolution rate.

Callus cultures of *Artemisia annua* have been reported to produce scopoletin and a triglyceride but no artemisinin. However, shoots differentiated from the callus were comparable with the whole plant (G. D. Brown, *J. Nat. Prod*., 1994, **57**, 975). A suggested pathway for the biosynthesis of artemisinin involves the conversion of a germacranolide to a cadinane-type compound (structure p. 264) and thence through a series of intermediates including artemisinic acid and artemisitene, two sesquiterpenes which have also been isolated from the plant. The structure, biosynthesis and functions of artemisinin have been reviewed (90 refs) by S. Bharel *et al*. (*Fitoterapia*, 1996, **67**, 387).

Further reading

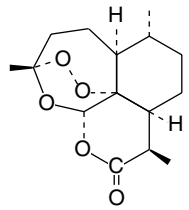
Efferth T 2007 Antiplasmodial and antitumor activity of artemisinin—from bench to bedside. Planta Medica 73(4): 299–309. *See also Chapter 28*

Gossypol

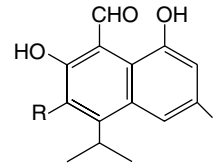
Hemigossypol and related aldehydes together with the dimeric gossypol (Fig. 24.3) are sesquiterpene stress compounds found in the subepidermal glands, immature flower buds and seed kernels of the cotton plant (*Gossypium* spp.). Gossypol was first isolated in 1899, its structure was established in the 1930s and later confirmed by synthesis and spectroscopy.

In addition to having insecticidal and various pharmacological properties, gossypol is of considerable pharmaceutical interest in that in humans it functions as a male antifertility agent. In China it was tested experimentally as a contraceptive with 12 000 men. Work is in progress to reduce possible side-effects and to find alternative systems of delivery. Chinese workers also claim the drug to be active in the therapy of menorrhagia, leiomyoma and endometriosis. Endometrial atrophy occurred in all cases (67 women) and complete recovery of the endometrium was observed within 6 months of the cessation of gossypol treatment.

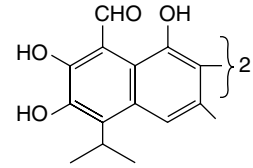
Inspection of the structural formula of gossypol reveals no chiral centre for the molecule. However, it acquires chirality by the restricted rotation of the bond connecting the two naphthyl moieties and so a pair of atropisomers exist. The compound isolated from the cotton plant was racemic, and this was used in the Chinese clinical trial above;



Artemisinin



Hemigossypol, R = OH
6-Methoxyhemigossypol, R = OMe
6-Deoxyhemigossypol, R = H



Gossypol

Fig. 24.3
Cadinane-type sesquiterpenes of *Artemisia* and *Gossypium*.

in 1987 the (−)-isomer was shown to be the pharmacologically active principle. By using modern quantitative enantiomorphic separation techniques Cass *et al*. (*Phytochemistry*, 1991, **30**, 2655) have shown that the gossypol enantiomer ratio appears to be species related. Thus, an excess of (+)-gossypol was found in the seeds of each variety tested of *Gossypium arboreum*, *G. herbaceum* (Asiatic cotton) and *G. hirsutum* (Upland cotton) whereas (−)-gossypol was in excess in each variety of *G. barbadense* (Egyptian, Tanguis or Pima cotton). Concordant findings have also been reported by other workers (J. W. Jaroszewski *et al*., *Planta Med*., 1992, **58**, 454).

DITERPENOIDS

The origin of the C_{20} diterpenoids, involving the mevalonate pathway, was indicated earlier in Fig. 18.2. The group comprises a structurally diverse range involving hundreds of compounds which may be acyclic or possess 1–5 ring systems. They may also be of mixed origin as illustrated by the diterpenoid alkaloids of *Taxus* and *Aconitum*.

Diterpenoids constitute the active constituents of a number of medicinal plants and are of current interest for their potential as future drugs, either, as isolated from the plant, or as modified derivatives. They include such resin acids as (+)- and (−)-pimaric acid, their isomers, and abietic acid of pine resin. Different stereochemical configurations having the same skeletal structure are also seen in the tetracyclic kaurane and *ent*-kaurane groups (Fig. 24.4); the latter includes the sweetening agent stevioside (Chapter 33) and the gibberellins. The cytotoxic activity of a number of natural and synthetic *ent*-kauranes has been studied (S. Rosselli *et al.*, *J. Nat. Prod.*, 2007, **70**, 347).

The gibberellins, first obtained from fungi of the genus *Gibberella* but also found in higher plants, are diterpenoid acids which have a marked effect on growth of seedlings; they are considered in Chapter 12. Phytol, $C_{20}H_{39}OH$, an unsaturated alcohol, is a component of the chlorophyll molecule. Vitamin K_1, an antihaemorrhagic compound, first discovered in plants in 1929, is also a phytol derivative. Vitamin A, a diterpenoid, is referred to below under 'Carotenes'. Furanoditerpenes constitute the bitter principles of calumba root (q.v.). *Teucrium chamaedrys*, wall germander, and *T. scorodonia*, wood sage, family Labiatae, are both used in herbal medicine as diaphoretics and antirheumatics. Besides containing small amounts of volatile oil, flavonoids and tannins, both herbs produce diterpenes of the neoclerodane type. Other diterpenoid derivatives include some of the alkaloids of species of *Aconitum* (q.v.), *Daphne*, *Delphinium*, *Garrya*, *Taxus* and *Tripterygium*. Some diterpenes from *Kalmia latifolia* (Ericaceae) have antifeedant properties with respect to the gypsy moth.

Forskolin (coleonol; Fig. 24.4) a diterpene isolated by Indian workers from *Coleus forskohlii* (Labiatae) is the last compound to be formed in the biogenetic sequence of the polyoxygenated diterpenes. Many chemical races of the plant have been revealed and studies on artificial propagation are in progress as, in India, the species is fast becoming extinct owing to large-scale indiscriminate collection. For a pharmacognostical evaluation of the root, see S. K. Srivastava *et al.*, *Pharm. Biol.*, 2002, **40**, 129. Preparations of *Coleus* species have long been used in Hindu and Ayurvedic traditional medicine particularly for the treatment of heart diseases, abdominal colic, etc. Forskolin has been demonstrated to have hypotensive, spasmolytic, cardiotonic and platelet aggregation inhibitory activity; because of its unique adenylate cyclase stimulant activity it is considered a promising drug for the

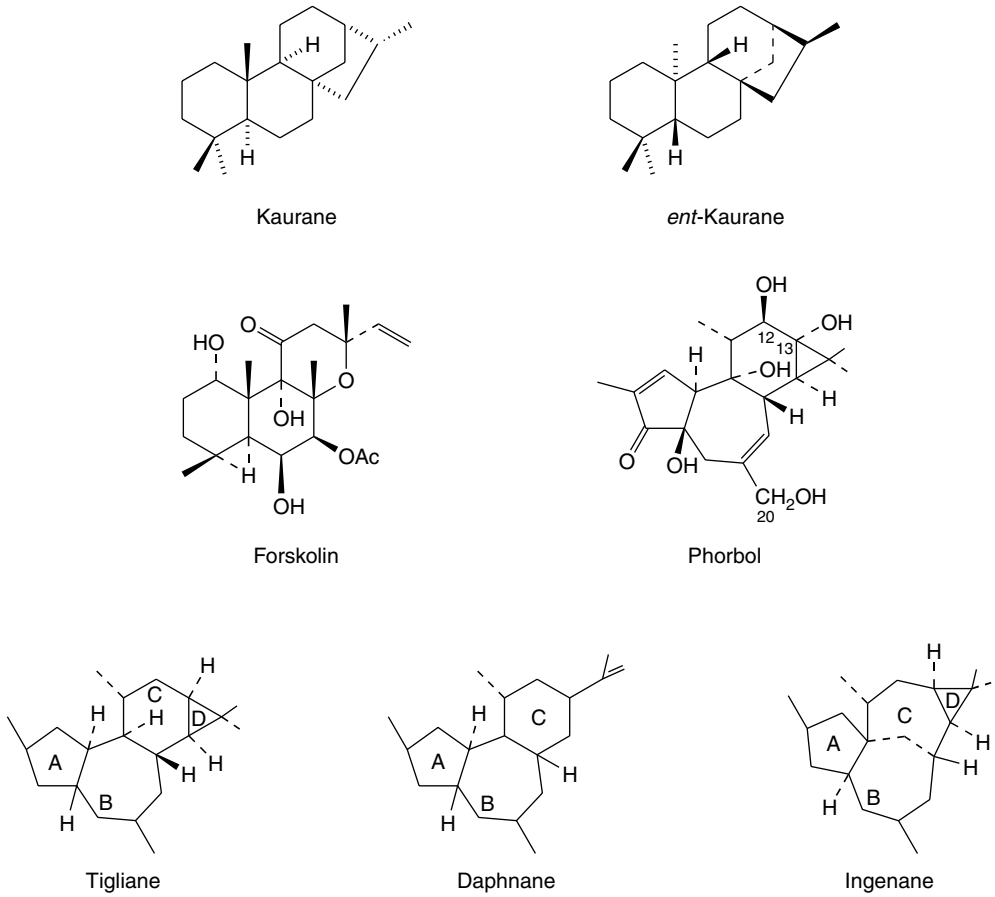


Fig. 24.4
Diterpenoid hydrocarbons and oxygenated compounds (see text).

treatment of glaucoma, congestive cardiomyopathy and asthma (see R. A. Vishwakarma *et al.*, *Planta Med*., 1988, **54**, 471.)

The diterpenes of the Euphorbiaceae, e.g. esters of phorbol (Fig. 24.4), and related compounds of other families not only have medicinal potential but are also proving to be useful pharmacological tools; they are described below.

Other drugs containing diterpenes, described in Chapter 21, are agnus castus (rotundifuran and vitexilactone), Java tea (orthosiphols, etc., isopimarone-type diterpenes) and motherwort (labdane-type diterpenes).

Tiglianes, Daphnanes and Ingenanes. These three related groups of diterpenoid compounds (Fig. 24.4) are found in the Euphorbiaceae (e.g. *Croton tiglium* q.v., *Euphorbia* spp.) and the Thymelaeaceae (e.g. *Daphne*, *Lasiosiphon*, *Pimelea* and *Gnidia* spp.). Biologically, they produce intense inflammation on application to the skin and have both tumour-promoting and antitumour activity. Of particular interest are the esters of phorbol (a tigliane derivative). It is the 12,13-diester, 12-*O*-tetradecanoyl-phorbol-13-acetate, which has been most extensively used in pharmacological investigations although *Croton tiglium* contains some 10 others. As pharmacological tools they are valuable in that they substitute for diacylglycerol in the activation of the phosphorylating enzyme protein kinase C; the shape of the molecules, with their long-chain ester groupings, seems to match the side-chains on the natural second messenger, diacylglycerol. The 12,13,20-triesters of phorbol are termed 'cryptic irritants' because they do not exhibit pro-inflammatory activity on mammalian skin unless the C-20 acyl group is removed by hydrolysis.

Further reading

Baloglu E, Kingston D S 1999 The taxane diterpenoids. Journal of Natural Products 62(10): 1448–1472. *Review with 71 references. 350 Taxane diterpenoids classified with information on plant sources, structures, actions, etc.*

Ginkgo

The leaves of ginkgo are obtained from the dioeceous tree *Ginkgo biloba* (Maidenhair-tree) (Ginkgoaceae), the only extant species of an otherwise fossil family of the pre-Ice Age flora. As the specific name implies the leaves are bilobed, each lobe being triangular in outline with a fine radiating, fan-like venation. The leaf is glabrous, petiolate and has an entire margin. The drupe-like fruits possess a bad-smelling pulp and contain seeds with an edible kernel.

Native to China and Japan but cultivated ornamentally in many temperate regions, the tree has a long medicinal history being recorded as early as 2800 BC in the Chinese literature; traditional Chinese medicine uses mainly seed preparations. It is only relatively recently that the drug has received much attention in the West, where in the USA, for the first 8 months of 1999, ginkgo held its place as top of the herbal mainstream market with retail sales valued at over $100 million (M. Blumenthal, *Herbalgram*, 1999 (No. 47), p. 64). In Europe it was estimated in 1993 to have an annual turnover of about $500 million (O. Sticher, *Planta Medica*, 1993, **59**, 2). Standardized extracts prepared in France and Germany are much used in Europe for the treatment of circulatory diseases resulting from advancing age. The leaves are official in the *BHP* 1996 and the *BP*/*EP*.

Constituents. From among the many groups of compounds isolated from ginkgo it is the diterpene lactones and flavonoids which have been shown to possess therapeutic activity.

Five diterpene lactones (ginkgolides A, B, C, J, M) have been characterized; these have a cage structure involving a tertiary butyl group and six 5-membered rings including a spiro-nonane system, a tetrahydrofuran moiety and three lactonic groups (Fig. 24.5). These compounds are platelet-activating factor (PAF) antagonists (see Chapter 6) and as they do not react with any other known receptor their effect is very specific. Related to the above, and also possessing a tertiary butyl group, is the sesquiterpene bilobalide; no PAF-antagonist activity has been demonstrated for this compound.

Some 33 flavonoids have now been isolated from the leaves and involve mono-, di- and tri-glycosides of kaempferol, quercetin, myricetin and isorhamnetin derivatives. The tree also synthesizes a number of biflavonoids based on amentoflavone; there has been recent interest in these compounds arising from their antilipoperoxidant, antinecrotic and radical-scavenging properties.

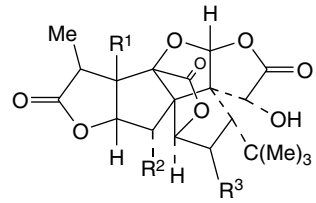
Ginkgolic acids are urushiol-type alkylphenols and occur in quantity in the seed coat and to a much lesser extent in the leaves. They are most noticeably observed in poison ivy (Chapter 39) and are associated with allergic responses, particularly dermatitis. For this reason in 1997 the German Government Commission E limited the ginkgolic acid content of standardized extracts to 5 ppm. Other potentially toxic alkylphenols are the cardanols and cardols (Fig. 24.5). The albumen of the seed also contains neurotoxic 4′-*O*-methylpyridoxine (ginkgotoxin) and this has been shown by A. Arenz *et al*. (*Planta Medica*, 1996, **62**, 548) to be present also in the leaves, but in concentrations too low to exert any significant ill-effects in medicines and foods.

Bioproduction. Various investigators have reported considerable fluctuations of terpene concentration in leaves throughout the year, with a maximum in early autumn. With the flavonoids there is a higher concentration of flavonol glycosides in spring leaves and of biflavones in autumn leaves. The age of the tree is an important factor in determining the terpene content of the leaves; those leaves from young trees (10 yr) are the richest source whereas the content is dramatically lowered in leaves of old trees (100–120 yr).

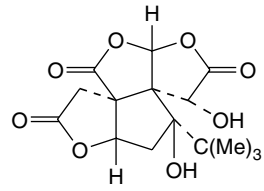
Work by D. J. Carrier *et al*. (*Phytochemistry*, 1998, **48**, 89) has suggested that terpene trilactone (bilobalide + ginkgolides) synthesis might occur in actively growing tissues such as terminal buds. Ginkgolide B can be produced in cultured cells derived from ginkgo leaves and attempts to maximize yields by optimization of the cultural conditions have been reported (M. H. Jeon *et al*., *Plant Cell Reports*, 1995, **14**, 501). Whereas terpene production is low in cell cultures, isolated *in vitro* root cultures accumulate terpenes in concentrations of the same order as those found in the leaves of young trees (J.-P. Balz *et al*., *Planta Medica*, 1999, **65**, 620).

Evaluation. Commercial extracts are usually standardized for flavonoid glycosides and triterpene lactones together with a limit for ginkgolic acid. Although the marketing of pure ginkgolides has not yet proved feasible there are a number of reported laboratory separations and assays utilizing GC-MS, HPLC-MS, HPLC-RI. N. Fuzzati *et al.* describe a new HPLC-UV method, not requiring enrichment procedures, for the quantification of ginkgolic acid in extracts (*Fitoterapia*, 2003, **75**, 247). For the crude drug, the *BP*/*EP* requires a 0.5% content of flavonoids calculated as flavone glycosides; these are assayed by the liquid chromatography of a hydrolysed acetone extract and spectrophotometric measurements at 370 nm.

Uses. Ginkgo has a traditional use as an antiasthmatic, bronchodilator, and for the treatment of chilblains. Extracts of the leaf containing selected constituents are used especially for improving peripheral and cerebral circulation in those elderly with symptoms of loss of short-term memory, hearing and concentration; it is also claimed that vertigo, headaches, anxiety and apathy are alleviated and positive results have been obtained in trials involving the treatment of dementia and Alzheimer's disease (see 'Further reading').

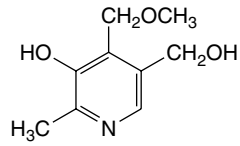


Ginkgolide structures

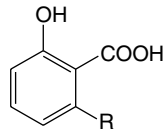


Bilobalide

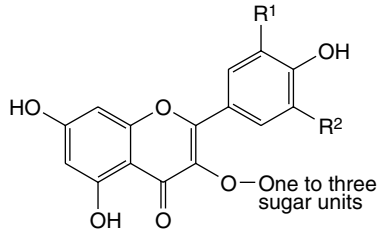
	R1	R2	R3
Ginkgolide A:	OH	H	H
Ginkgolide B:	OH	OH	H
Ginkgolide C:	OH	OH	OH
Ginkgolide J:	OH	H	OH
Ginkgolide M:	H	OH	OH



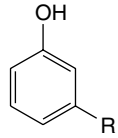
Ginkgotoxin



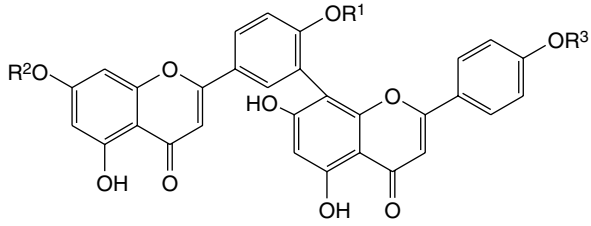
R = C_{13}, C_{15} or C_{17} aliphatic side-chain with 0, 1 or 2 double bonds
Ginkgolic acids



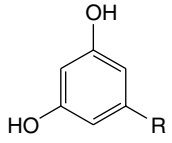
Flavonol structures
Kaempferol derivatives: R^1= OH; R^2= H
Quercetin derivatives: R^1= OH; R^2= H
Myricetin derivatives: R^1= OH; R^2= OH
Isorhamnetin derivatives: R^1= OMe; R^2= H



R = C_{13}, C_{15} or C_{17} aliphatic side-chain with 0 or 1 double bonds
Cardanols



Biflavonoid structures



R = C_{15} aliphatic saturated side-chain a Cardol

	R1	R2	R3
Amentoflavone:	H	H	H
Bilobetin:	Me	H	H
Sequojaflavone:	H	Me	H
Ginkgetin:	Me	Me	H
Isoginkgetin:	Me	H	Me
Sciadopitysin:	Me	Me	Me

Fig. 24.5
Some constituents of *Ginkgo biloba leaves*.

Further reading

Ghisalberti EL 1997 The biological activity of naturally occurring kaurane diterpenes. Fitoterapia 68(4): 303–325. *Review with 180 references*

Singh B, Kaur P, Gopichand, Singh RD, Ahuja PS 2008 Biology and chemistry of *Ginkgo biloba*. Fitoterapia 79(6): 401–418. *Review with 113 references*

Van Beek TA (ed), Hardman R (series ed) 2000 Medicinal and aromatic plants—industrial profiles, Vol 12. *Ginkgo biloba*. Harwood Academic, Amsterdam

24

SESTERTERPENES

This relatively recently recognized family of C_{25} compounds, formed by the addition of a C_5 isopentenyl unit to geranylgeranyl diphosphate, is at the moment of limited medicinal interest. Examples are confined principally to the fungi, some marine organisms (e.g. sponges of the genus *Ircinia*) and insect waxes (e.g. gascardic acid).

TRITERPENOIDS

These C_{30} constituents are abundant in nature, particularly in resins, and may occur as either esters or glycosides. They may be aliphatic (e.g. the squalene found in animals and in the unsaponifiable matter of many oils such as arachis and olive), tetracyclic or pentacyclic. Tetracyclic ones include the limonoids, the sterols found in wool fat and yeast and the cardioactive glycosides. The triterpenoid saponins, most of which are pentacyclic, are discussed elsewhere.

This is again a very active area of research and is regularly reviewed in *Natural Product Reports*.

One group of compounds showing a range of interesting biological activity is the quassinoids. These are degradation and rearrangement products of triterpenes and are described under 'Quassia' below and in Chapter 27.

Quassia Wood

Quassia (*Jamaica Quassia*) is the stem wood of *Picrasma excelsa* (*Picroena excelsa* or *Aeschrion excelsa*) (Simaroubaceae), which is known in commerce as Jamaica quassia. The tree, 15–20 m high, grows in the West Indies (Jamaica, Guadeloupe, Martinique, Barbados and St Vincent).

Characters. Quassia occurs in logs, chips or raspings. The logs are of variable length and up to 30 cm diameter (those of Surinam quassia never exceed 10 cm diameter). The logs are covered with a dark grey cork which readily separates from the phloem. The wood is at first whitish but becomes yellow on exposure. It frequently shows blackish markings owing to the presence of a fungus. The logs split readily and the commercial chips, which are cut across the grain, break very readily into smaller fragments. The drug has no odour but an intensely bitter taste.

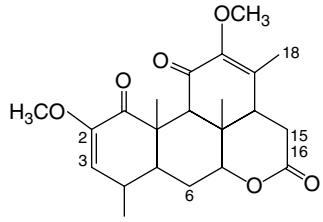
A small piece of quassia wood should be smoothed and the transverse, radial and tangential surfaces examined with a lens.

A transverse section of quassia shows medullary rays, which are mostly two to five cells wide. The xylem is composed of vessels, wood fibres, and wood parenchyma. The vessels are large (up to 200 μm diameter) and occur singly or in groups of 2–11 which often extend from one medullary ray to the next. Single prisms of calcium oxalate, each 6–30 μm long and enclosed in a delicate membrane, occur scattered in the medullary ray cells and wood parenchyma cells. Starch grains are few; mostly simple, spherical and about 5–15 μm, occasionally two-compound.

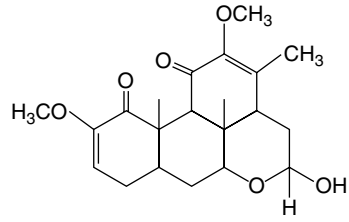
Constituents. Quassia contains the amaroid (terpenoid) compound quassin, an intensely bitter lactone; also neoquassin, 18-hydroxyquassin, and scopoletin.

The quassins have been traditionally estimated by sensory means (*BP*, 1973) but Wagner and colleagues (*Planta Med*., 1980, **38**, 204) described three equally effective methods for the quantitative determination of the individual quassinoids. These involve separation by TLC, HPLC and circular chromatography, followed by absorption measurements.

Quassia wood also contains alkaloids, as illustrated by cathine-6-one.



Quassin



Neoquassin

Allied drugs. Surinam quassia is derived from *Quassia amara* (Simaroubaceae), a shrub growing in the Guianas, northern Brazil and Venezuela. It occurs in smaller billets than those of Jamaica quassia. The medullary rays are only 1 or 2 cells wide but up to 30 cells deep. Calcium oxalate is absent. Cathine-6-one type alkaloids and quassinoids have been isolated from the wood. For a recent phytochemical study see J. A. Dou *et al*., *Int. J. Pharmacognosy*, 1996, **34**, 349.

A number of *Picrasma* species produce similar constituents to the above. Three novel C_{18} quassinoids have been isolated from the leaves of *Samadera madagascariensis* leaves (P. H. Coombes *et al*., *Phytochemistry*, 2005, **66**, 2734).

Uses. Quassia is used as a bitter tonic, as an insecticide, and as an enema for the expulsion of thread worms.

Q. amara wood is used in S. American traditional medicine for its stomachic, antiamoebic, antimalarial and antianaemic activity. The commercial 'quassin' prepared from *Q. amara* contains principally quassin and neoquassin and is widely used to give a bitter taste to beverages. It is also used as an insecticide because of its antifeedant properties.

Other quassinoids. Various parts of a number of plants of the family Simaroubaceae have been used in traditional medicine for the treatment of a variety of diseases including cancer, amoebic dysentery and malaria, and research has established that it is the quassinoid (simaroubolide) content of these plants that is responsible for this activity. Such compounds, and in some instances their glycosides, have also been shown to have antileukaemic, antiviral, anti-inflammatory, and (for insects) antifeedant properties. Recent studies are discussed in Chapter 28.

BLACK HOREHOUND

The dried aerial parts of *Ballota nigra* L. family Labiatae collected during the flowering period are included in the *BP*/*EP* and *BHP* 1996. Dispersed throughout Europe, N. Africa, western Asia, the USA and Australia, the plant is common to roadsides, hedges, etc. and is often regarded as a weed.

Characters of this species, many common to other Labiatae, are the erect square stems, often reddish-brown in the lower parts and up to 100 cm in height; leaves arranged oppositely, 2–5 cm in length, petiolate, rounded to ovate in outline, margin coarsely toothed, surfaces rugose and covered with whitish hairs, venation particularly prominent on the lower surface, depressed on the upper; flowers numerous in whorls in the axils of bracts, calyx about 1 cm in length ten-nerved

with five teeth; corollas purple or more rarely white and two-lipped; fruits consist of four small, three-sided achenes. The plant has an unpleasant odour.

Features of the powdered drug include numerous jointed uniseriate trichomes, various glandular trichomes, predominantly anisocytic but some anomocytic stomata on the lower leaf surface, portions of corolla with a papillose epidermis, pollen grains 25–30 μm in diameter with a smooth exine.

Constituents. The constituents of black horehound have been extensively studied during the last 35 years, commencing principally with the work of G. Savona and colleagues in 1976, who characterized the diterpenoid content. Marrubiin, recognized for many years as a constituent of white horehound, is present in very small amounts, its derivates ballotinone, ballonigrine, 7α-acetoxymarrubiin, ballotenol and 13-hydroxyballonigrinolide form the major representatives of the group.

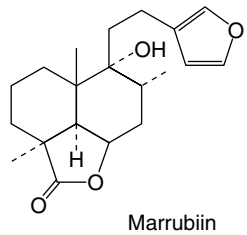
Flavonoids include derivatives of luteolin and apigenin.

The *BP*/*EP* states a minimum requirement of 1.5% of total *ortho*-dihydroxycinnamic derivatives for the dried drug expressed as acteoside (verbascoside) (for formula see 'Mullein'); this also includes apiosyl, xylosyl and arabinosyl derivatives of verbascoside.

WHITE HOREHOUND

White horehound consists of the dried leaves and flowering tops of *Marrubium vulgare* L., family Labiatae. It is described in the *BP*/*EP*, *BHP* and the *Complete German Commission E* monographs. The plant is common throughout Europe, including the UK, having become naturalized in many places. Small amounts are obtained commercially for medicinal purposes from S.E. Europe, Morocco, Italy and France. Its principal use is as an expectorant and antispamodic in the treatment of bronchitis and whooping cough; it also possesses choleretic properties.

The active principals appear to be diterpenes. Marrubiin is one such, which, on the opening of its lactone ring, gives marrubinic acid, to which is ascribed the choleretic property of the drug. Related compounds present to a lesser extent are the diterpene alcohols marrubenol and vulgarol. Other constituents are: ubiquitous flavonoids including vitexin, apigenin and luteolin together with their glycosides; the alkaloids betonicine and stachydrine; and a small amount of volatile oil (0.06%) giving the drug its pleasant smell.

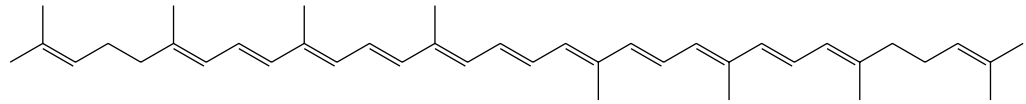


Marrubiin

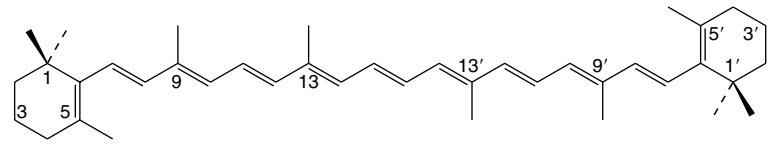
The *BP*/*EP* gives a TLC test for the drug and requires a minimum content of 0.7% marrubiin determined by liquid chromatography using a solution of marrubiin in methanol as a reference solution with absorption measurements at 217 nm.

TETRATERPENES—CAROTENOIDS

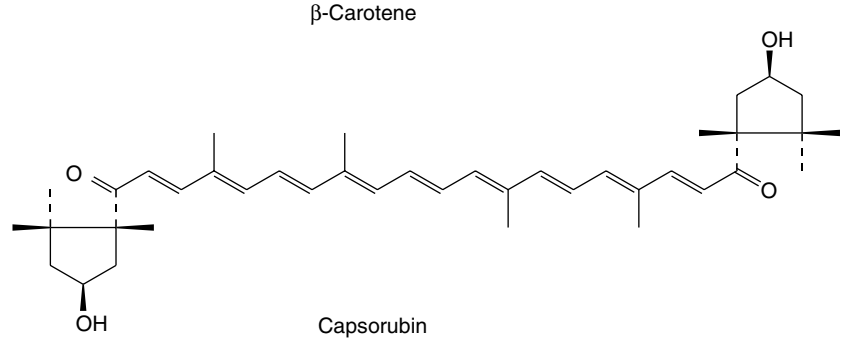
Important among these compounds are the C_{40} yellow or orange-red carotenoid pigments of which about 500 have been reported. As indicated in Chapter 18 they are formed by the tail to tail union of two molecules of the C_{20} geranylgeranyl diphosphate to give an acylic intermediate with a *cis*-configuration of the central double bond. By a change of configuration of the latter to *trans* and further desaturation of the isoprenoid chain, lycopene, the all-*trans* pigment of the ripe tomato fruit is formed. The various carotenes and derivatives can be envisaged by cyclization of one or both ends of the lycopene molecule (Fig. 24.6); all are all-*trans*. *Cis*-isomers, usually present in extracted carotenoid preparations, are probably artefacts.



Lycopene



β-Carotene



Capsorubin

Fig. 24.6
Structures of carotenoids.

Table 24.1 Examples of oxygenated carotenoids.

Carotenoid	Formula	Occurrence
Bixin	$C_{25}H_{30}O_4$	Annatto
Capsanthin	$C_{40}H_{56}O_3$	*Capsicum* spp.
Capsorubin	$C_{40}H_{60}O_4$	*Capsicum* spp.
Crocetin	$C_{20}H_{24}O_4$	Saffron
Crocin	$C_{44}H_{64}O_{24}$	Saffron
Fucoxanthin	$C_{40}H_{60}O_6$	Brown algae
Lutein	$C_{40}H_{56}O_2$	*Tagetes erecta*

In association with chlorophyll, carotenes participate in photosynthesis, but also occur in other non-photosynthetic plant organs such as the carrot and in fungi and bacteria. 'Carotene', a mixture of all the carotenes but with β-carotene predominating, was isolated from carrots as early as 1831. Between 1913 and 1915 a fat-soluble growth factor, vitamin A, was recognized to be present in materials such as butter and cod-liver oil and was subsequently shown to be a diterpenoid produced in the livers of animals by enzymic hydrolysis from β-carotene. There are many derivatives of the carotene molecule; some are oxygenated (Table 24.1), some have allenic and acetylenic bonds while others are formed by the loss of a portion of one end of the molecule as with β-citraurin, the characteristic apocarotenoid of citrus fruits. The striking pigments of the red peppers, capsanthin and capsorubin illustrate a contraction of either one (capsanthin) or two (capsorubin) of the usual cyclohexene end groups to a cyclopentane ring.

In addition to the pro-vitamin A activity of β-carotene, the carotenoids have more recently come to be recognized as essential for human health not only as antioxidants but also for specific functions such as normal vision and actions favouring the immune system. Thus, in 1997 J. T. Landrum and colleagues (*Exper. Eye Res.*, **65**, 57) established that the lutein of the 'macula lutea' of the retina of the eye is chemically identical to that giving the colour of marigold flowers (*Tagetes erecta*, Compositae). This accords with the use of lutein in nutritional supplements as preventatives of age-related macular degeneration and for other health benefits. Importantly, it has been demonstrated (P. Molnar *et al.*, *Carotenoid Sci.*, 2006, **10**, 1) that the natural lutein ester, as extracted from marigold, remains virtually stable at gastric pH and body temperature, in contrast to lutein (unesterified), which is degraded by over 60% with the concurrent formation of anhydroluteins and 3′-epilutein. This implies that, for a given dose, relatively more of the ester will reach the intestinal absorption sites than will free lutein.

In 1835 Marquart observed that certain yellow flowers (e.g. buttercup), when treated with strong sulphuric acid, gave dark blue, green or violet colours. This reaction is characteristic of carotenoids and serves as a means of distinguishing them from other natural pigments such as the anthocyanins. The test is best carried out by stratifying an ether or chloroform solution of the carotenoid with 85% sulphuric acid, when a blue colour is formed at the junction of the two layers. Most carotenoids give a blue colour with antimony trichloride in chloroform (Carr–Price test), or a dark-blue colour with concentrated hydrochloric acid containing a little phenol. These tests have been adapted for use as TLC reagents for the identification of relevant crude drugs.

The considerable commercial demand for carotenes has encouraged the development of biotechnological methods for their production. These include mass-culture production from algae, yeast, fungi and recombinant DNA systems. Immobilized enzyme systems for carotenoid production have been studied.

For a concise and informative article (75 refs) on the biological properties of carotenoids, see N. L. Krinsky *Pure Appl. Chem.*, 1994, **66**, 1003. See also this book, Chapter 32: The plant nutraceuticals.

POLYTERPENOIDS

Polyterpenes are composed of many isoprene units. Common examples, both having macromolecules of molecular weight over 100 000, are found in indiarubber and gutta-percha. Doubtless the rubber-like substances of many other plants have a similar composition. Chemically pure rubber is *cis*-1,4-polyisoprene $(C_5H_8)_n$, although in the natural state other materials are present; its occurrence is confined to the dicotyledons, and the one important commercial source is *Hevea brasiliensis*. Gutta-percha (see below) is *trans*-1,4-polyisoprene, and chicle, obtained from *Manilkara sapota*, contains a mixture of low molecular weight *cis*- and *trans*-polyisoprenes. No biological function for polyisoprenes has yet been discovered.

Rubber

A number of species of the families Euphorbiaceae, Apocynaceae, Moraceae, Asclepiadaceae and others produce a latex either in specialized cells or in anastomosing canals (Chapter 42), from which rubber can be prepared.

In Malaysia, *Hevea brasiliensis* (Euphorbiaceae) is cultivated for commercial use. Tapping is carried out mainly by women in the early morning when the internal latex pressure is highest. Trees are tapped by making an overlapping spiral groove, initially 1.5 m above the ground, with a knife called a *jebong*. The exuded latex is collected in cups placed at the lower end of the groove. After about 11 years the spiral has reached ground level. Following an initial cleaning of the latex in vats it is coagulated and bleached by treatment with formic acid and a bleaching agent. The latex emerges as blocks which are then passed through a mill 30 times to give thin layers ready for further processing.

Rubber consists of linear chains of about 1500 to 60 000 C_5-isoprenoid units linked by *cis* double bonds (Fig. 18.20C). Compounds initiating the biosynthesis of rubber in *H. brasiliensis* have been characterized (Y. Tanaka *et al.*, *Phytochemistry*, 1996, **41**, 1501) and possible mechanisms controlling the molecular weights of rubber produced investigated (J. Tangpakdee *et al.*, *Phytochemistry*, 1996, **42**, 353).

Gutta-percha

Gutta-percha is purified, coagulated latex obtained from trees of the genera *Palaquium* and *Payena* (Sapotaceae), which are found both wild and cultivated in Malaysia and Indonesia. The method of collection resembles that used for rubber but the latex flows less readily. Depletion of these natural sources has led to the use of *Parthenium argentatum* (Compositae) for limited production. Gutta-percha differs from rubber in being almost incapable of vulcanization, and in that it becomes plastic when heated to about 45–60°C. Gutta-percha contains a white, polymerized hydrocarbon gutta, composed of C_5-units linked by *trans* double bonds (Fig. 18.20B); it has fewer units than rubber.

Gutta-percha was used in the form of chloroformic solution as a means of applying drugs to the skin, as gutta-percha tissue for covering moist dressings, and in the manufacture of surgical instruments. The *USP/NF* (1995) directs that it should be preserved under water in well-closed containers protected from light.

Chicle

Chicle is a polyisoprenoid consisting of a mixture of *cis*- and *trans*-C_5 isoprenoids obtained from *Manilkara zapota* (Sapotaceae), the sapodilla plum. It was the base used for the original chewing-gum.

25

Cyanogenetic glycosides, glucosinolate compounds, cysteine derivatives and miscellaneous glycosides

In addition to the important groups of glycosides discussed in previous chapters, there are a number of other groups of some medicinal interest. Two of these, the cyanogenetic glycosides and the glucosinolate compounds, are characteristic of certain groups of plants and have similarities in their biosynthetic origins.

CYANOGENETIC GLYCOSIDES

The poisonous properties of the roots of *Manihot utilissima* (cassava) have long been known to primitive tribes; they use it as an important foodstuff, having first found methods to remove its poison. In 1830 the cyanogenetic glycoside manihotoxin was isolated from it, and in the same year amygdalin was obtained from bitter almonds, linamarin from linseed and phaseolunatin from a bean, *Phaseolus lunatus*. These yield prussic acid on hydrolysis and were the first discovered cyanogenetic or cyanophoric glycosides. Over 2000 plant species involving about 110 families are estimated to be cyanogenetic. Professor Lindley, a teacher of pharmaceutical students in London, realized as early as 1830 that the presence or absence of HCN was of taxonomic importance and used it as a character for separating the subfamilies of the Rosaceae. At the species level the presence or absence of prussic acid may denote varieties or different chemical races of the same species (e.g. *Prunus amygdalus* yields both bitter and sweet almonds). Interest in cyanogenetic principles as chemotaxonomic characters continues to receive much attention, as does the general biochemistry of cyanide in plants and microorganisms.

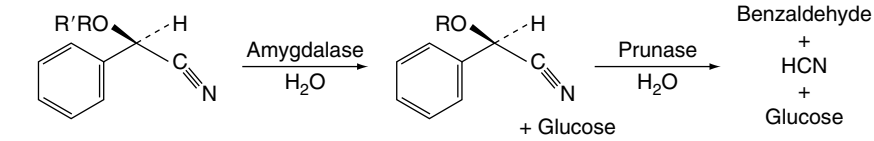
Many of these glucosides, but not all, are derived from the nitrile of mandelic acid. Although they contain nitrogen their structure is that of *O*-and not *N*-glycosides. The sugar portion of the molecule may be a monosaccharide or a disaccharide such as gentiobiose or vicianose. If a disaccharide, enzymes present in the plant may bring about hydrolysis in two stages, as in the case of amygdalin (amygdaloside), Fig. 25.1.

Table 25.1 gives some well-known cyanogenetic glycosides isolated from various sources between 1830 and 1907.

Tests

To test for a cyanogenetic glycoside qualitatively the material is well broken and placed in a small flask with sufficient water to moisten. In the neck of the flask a suitably impregnated strip of filter-paper is suspended by means of a cork. The paper may be treated in either of the following ways to give a colour reaction with free hydrocyanic acid. Either sodium picrate (yellow), which is converted to sodium isopurpurate (brick-red), or a freshly prepared solution of guaiacum resin in absolute alcohol which is allowed to dry on the paper and treated with very dilute copper sulphate solution. The latter test-paper turns blue with prussic acid. If the enzymes usually present in the material have not been destroyed or inactivated, the hydrolysis takes place within about an hour when the flask is kept in a warm place. More rapid hydrolysis will result if a little dilute sulphuric acid is added and the flask gently heated. The depth of colour produced with sodium picrate paper can be used for semiquantitative evaluations.

For materials containing a fairly high percentage of cyanogenetic glycosides (e.g. bitter almonds) the amount may be determined quantitatively by placing the plant in a flask with water and tartaric acid and passing steam through until all the hydrocyanic acid has distilled into a receiver. The distillate is then adjusted to a definite volume and aliquots titrated with standard silver nitrate solution. More sensitive methods including the direct determination of individual glycosides by GLC of their TMS derivatives are now available.



Amygdalin (where RR′ = gentiobiose) Prunasin (where R = glucose)

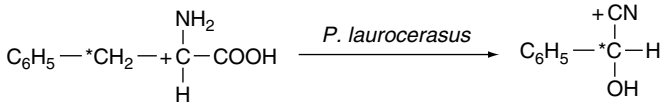
Fig. 25.1
Hydrolysis of amygdalin.

Table 25.1 Some cyanogenetic glycosides and their sources.

Glycoside	Source	Family	Constitution
Amygdalin	*Prunus amygdalus*	Rosaceae	D(–)-Mandelonitrile-gentiobioside
Linamarin	*Linum usitatissimum*	Linaceae	Acetone-cyanohydrin-glucoside
Prulaurasin	*Prunus laurocerasus*	Rosaceae	DL-Mandelonitrile-D-glucoside
Manihotoxin	*Manihot utilissima*	Euphorbiaceae	Identical with linamarin (q.v.)
Dhurrin	*Sorghum vulgare*	Gramineae	β-Glucoside of *p*-hydroxymandelonitrile
Sambunigrin	*Sambucus nigra*	Caprifoliaceae	L(+)-Mandelonitrile-D-glucoside
Vicianin	*Vicia angustifolia*	Leguminosae	Mandelonitrile-vicianoside
Phaseolunatin	*Phaseolus lunatus*	Leguminosae	Identical with linamarin (q.v.)
Prunasin	*Prunus serotina*	Rosaceae	D(–)-Mandelonitrile-D-glucoside

Biogenesis

The aglycones of cyanogenetic glycosides are derived solely from nitrogen intermediates. The biosynthesis of prulaurasin (DL-mandelonitrile glucoside) has been studied in the leaves of *Prunus laurocerasus*. Phenyl[3-^{14}C]alanine, phenyl[2-^{14}C]alanine and phenyl[1-^{14}C]alanine were fed to the leaves and the hydrolytic products of the isolated glycosides were examined. The three labelled precursors gave, respectively, active benzaldehyde and inactive hydrocyanic acid; inactive benzaldehyde and active hydrocyanic acid; and inactive benzaldehyde and active hydrocyanic acid; and inactive hydrolytic products consistent with the following incorporation:



Similarly, phenyl[2-^{14}C]alanine fed to *P. amygdalus* gives amygdalin with most activity in the carbon atom of the nitrile. Experiments with doubly labelled amino acids have shown that the nitrile nitrogen of the cyanogen is derived from the nitrogen atom of the amino acid. Similar results have been obtained with dhurrin isolated from sorghum seedlings fed with labelled tyrosine. More recent work has sought to determine the nature of the intermediates involved in the above conversions and, for prunasin and linamarin, the participation of oximes and nitriles has been demonstrated (Fig. 25.2).

For a report of a lecture on the biosynthesis, compartmentation and catabolism of cyanogenetic glycosides including amygdalin, linamarin and lotaustralin see E. E. Conn, *Planta Med*., 1991, **57** (Suppl. Issue No 1), SI. Nahrstedt (*Proc. Phytochem. Soc. Europe*, 1992, **33**, 249) reviewed (84 refs) progress concerning the biology of cyanogenetic glycosides.

A review (107 refs) asking 'Why are so many plants cyanogenetic?' (D. A. Jones, *Phytochemistry*, 1998, **47**, 155) illustrates the continuing interest in these plants, an interest which is, however, largely non-pharmaceutical.

Wild cherry bark

Wild cherry bark (*Wild Black Cherry* or *Virginia Prune Bark; Prunus Serotina*) is the dried bark of *Prunus serotina* (Rosaceae). The plant is a shrub or tree widely distributed in Canada and the USA, extending from Ontario to Florida and westward to Dakota and Texas. Commercial supplies are obtained from Virginia, North Carolina and Tennessee. The most esteemed bark is collected in the autumn, at which time it is most active. After careful drying it should be kept in airtight containers.

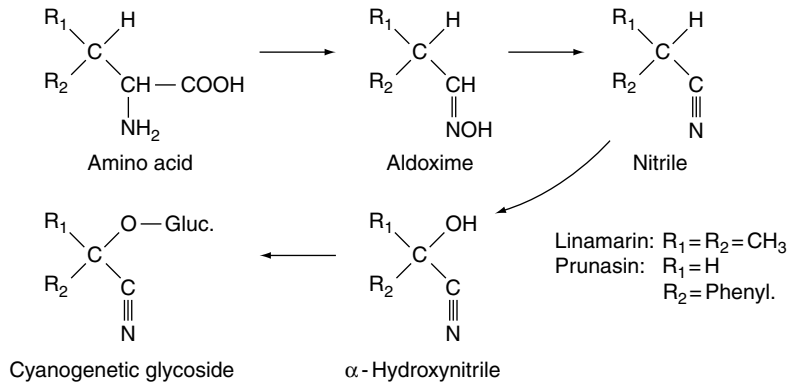


Fig. 25.2
Biosynthetic pathway for cyanogenetic glycosides.

History. The drug was introduced into American medicine about 1787 and appeared in the *USP* in 1820. It first attracted notice in Britain about 1863.

Macroscopical characters. The drug usually occurs in curved or channelled pieces up to 10 cm long, 5 cm wide and 0.3–4.0 mm thick (Fig. 25.3). Much larger pieces of trunk bark, up to 8 mm thick, may be found but the *BP* (1980) maximum thickness is 4.0 mm and is known commercially as 'Thin Natural Wild Cherry Bark'. The branch bark, if unrossed, is covered with a thin, glossy, easily exfoliating, reddish-brown to brownish-black cork, which bears very conspicuous whitish lenticels. In the rossed bark pale buff-coloured lenticel scars are seen and the outer surface is somewhat rough, some of the cortex having been removed and the phloem exposed. The inner surface is reddish-brown and has a striated and reticulately furrowed appearance, which is caused by the distribution of the phloem and medullary rays. Patches of wood sometimes adhere to the inner surface. The drug breaks with a short, granular fracture. When slightly moist it has an odour of benzaldehyde. Taste is astringent and bitter.

Features of the microscopy (Fig. 25.3) are numerous groups of sclereids, prismatic and cluster crystals of calcium oxalate, cork cells with brown contents, and starch granules.

Constituents. The bark contains prunasin (see above) and the enzyme prunase. Samples on hydrolysis yield glucose, benzaldehyde and about 0.07–0.16% of hydrocyanic acid, Also present are benzoic acid, trimethylgallic acid, *p*-coumaric acid, some tannin and a resin which gives scopoletin on hydrolysis. Modern methods of analysis have allowed detection, for the first time, of amygdalin in the leaves of several *Prunus* spp. including *P. serotina* and a cultivar of *P. virginiana* (F. S. Santamour, *Phytochemistry*, 1998, **47**, 1537).

Uses. Wild cherry bark in the form of a syrup or tincture is mainly used in cough preparations, to which it gives mild sedative properties and a pleasant taste. It was regarded as particularly useful for irritable and persistent coughs.

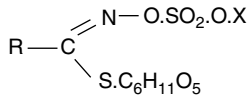
Cherry-laurel leaves

Cherry-laurel leaves are obtained from *Prunus laurocerasus* (Rosaceae), an evergreen shrub common in Europe. They were formerly official in the fresh state.

The leaves have little odour when entire, but when crushed an odour of benzaldehyde is soon apparent and a positive test for cyanogenetic glycoside is obtained. The cyanide content of small young leaves is reported as 5%, rapidly dropping to about 0.4–1.0% as leaf-size increases. For the structure and hydrolysis of the glucoside prulaurasin, see Table 25.1.

GLUCOSINOLATE COMPOUNDS

Over a century ago sinigrin and sinalbin were isolated in crystalline form from black and white mustards. These and similar glycosides have since been isolated from many plants, particularly those used as condiments (e.g. horseradish) or in folk medicine; they have the general structure:



In the above formulae, R represents CH_2=$CHCH_2$ in sinigrin and *p*-$HOC_6H_4CH_2$ in sinalbin; in sinigrin the X represents an atom of

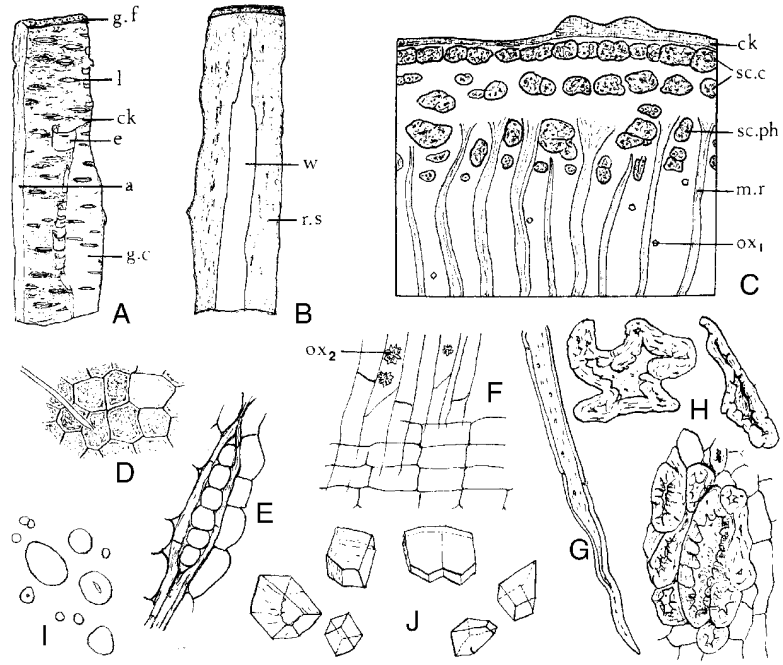


Fig. 25.3
Wild cherry bark. A, Outer surface of bark; B, inner surface (both × 0.5); C, distribution of tissues in TS (× 25). D–J, fragments of powder (all × 200): D, cork cells in surface view with associated fungal hypha; E, medullary ray in TLS; F, medullary ray in RLS with associated parenchyma; G, portion of fibre of the pericycle; H, sclereids; I, starch; J, prismatic crystals of calcium oxalate. a, Obliquely cut edge of bark; ck, cork; e, exfoliating cork; g.c, greenish cortex; g.f, granular fracture; l, lenticel; m.r, medullary ray; ox_1, ox_2, prismatic and cluster crystals respectively of calcium oxalate; r.s, reticulately marked inner surface; sc.c, sclereid groups of cortex; sc.ph, sclereid groups of secondary phloem; w, adhering wood.

potassium but can take the form of a more complex cation—for example, sinapine ($C_{16}H_{25}O_6N$), in sinalbin. A suggestion made in 1961 to rationalize the nomenclature of this enlarging group appears to have found acceptance. This is that the anion of the formula be designated a glucosinolate; thus, sinalbin becomes sinapine, 4-hydroxybenzylglucosinolate. Many such glycosides, with a variety of side-chains, including indolyl, are now known; all contain the β-D-1-glucopyranosyl residue. They have been found only in dicotyledonous plants and are particularly abundant in the families Cruciferae, Capparidaceae and Resedaceae with sporadic occurrences in the Euphorbiaceae, Tovariaceae, Moringaceae, Tropaeolaceae and Caricaceae. The enzyme myrosinase has a similar wide distribution. With the Cruciferae it has been shown that the mustard oil glycosides significantly increase the non-specific resistance of the plants to microorganisms which disrupt plant cells; they do not appear to affect the resistance of cruciferous plants to club root infections. Many glucosinolates have an antithyroid and goitre-inducing effect in man.

Biosynthesis

Biosynthesis of the glucosinolates of the relevant Cruciferae takes place principally in the fruit wall with subsequent translocation to the seed. However it has been shown for oilseed rape (*Sinapis alba*) that the necessary enzymes for the biosynthesis of *p*-hydroxybenzylglucosinolate (derived from tryosine) are present in the seed where a limited synthesis does occur (L. Du and B. A. Halkier, *Phytochemistry*, 1998, **48**, 1145).

The earlier feeding experiments with those members of the Cruciferae which produce mustard oil glucosides showed that suitable amino acids are converted to thioglucosides by the plant. Doubly labelled (^{14}C, ^{15}N) amino acids afforded glucosides with ^{14}C:^{15}N ratios consistent with direct incorporation (Fig. 25.4).

This means that all intermediates in this conversion are nitrogenous compounds giving a similar situation to that found in the biosynthesis of cyanogenetic glycosides (see above). Following the work on cyanogenetic compounds, it was then demonstrated (1967) by different groups of workers that appropriate aldoximes were effective precursors of these compounds in flax (linamarin), *Cochlearia officinalis* (glucoputranjivan), *Lepidium sativum* (benzylglucosinolate) and *Tropaeolum majus* (benzylglucosinolate).

With sinigrin, the thioglucoside found in horseradish leaves and in black mustard seeds, the most effective precursor of the carbon chain appears to be homomethionine rather than allylglycine which inspection of the sinigrin structure might suggest. Homomethionine arises by chain lengthening of methionine with acetate by a mechanism analogous to the formation of leucine from valine (see Fig. 18.16). Although the sulphur atom on the thioglucoside moiety may be introduced by feeding with methionine, Matsuo (1968) showed the sulphur of DL-[^{35}S]cysteine to be a more efficient precursor. The sulphur of the bisulphite portion of the molecule is more readily introduced from inorganic sources. Some incorporations consistent with the envisaged pathway for sinigrin are illustrated in Fig. 25.4.

Mustard seed

Black or brown mustard (*Sinapsis*) is the dried ripe seed of *Brassica nigra* or of *B. juncea* (Cruciferae) and their varieties. The former species is cultivated in Europe and the USA, while *B. juncea* is grown in India and the former USSR.

Characters. The seeds are globular and 1–1.6 mm diameter. The testa is dark reddish-brown to yellow and minutely pitted. The cells of the outer epidermis of the testa contain mucilage. The embryo is oily and greenish-yellow or yellow in colour; it consists of two cotyledons folded along their midribs to enclose the radicle. Powdered mustard acquires a much brighter yellow colour on treatment with alkali.

Constituents. Black mustard seeds contain sinigrin and myrosin and yield after maceration with water 0.7–1.3% of volatile oil. The latter contains over 90% of allylisothiocyanate. The seeds also contain about 27% of fixed oil, 30% of proteins, mucilage and traces of sinapine hydrogen sulphate (cf. white mustard).

Allied drug. White mustard, the seeds of *Sinapsis alba*, are globular and 1.5–2.5 mm diameter. The testa is yellowish and almost smooth, and contains mucilage in its outer epidermal cells. The kernel is oily and the cotyledons are folded as in black mustard. On treatment with water the powder develops a pungent taste but the pungent odour of the black variety is absent. With alkali the powder acquires a bright yellow colour.

White mustard seeds contain the glucoside sinalbin and myrosin. In the presence of moisture decomposition takes place with the formation of isothiocyanate, sinapine hydrogen sulphate and glucose. The isothiocyanate is an oily liquid with a pungent taste and rubefacient properties but, owing to its slight volatility, it lacks the pungent odour of allylisothiocyanate. Sinapine hydrogen sulphate, which is also found in black mustard, is the salt of an unstable alkaloid. The seeds also contain about 30% of fixed oil, 25% of proteins and mucilage.

Uses. The mustards have been traditionally used, particularly in the form of plasters, as rubefacients and counterirritants. In large doses they have an emetic action. Both varieties are used as condiments.

CYSTEINE DERIVATIVES

Derivatives of the amino acid cysteine occur as sulphoxides in the genus *Allium* and are responsible for the lachrymatory factor of onions, garlic etc. Variations in the structure of these compounds are found in different species, thus S-(trans-propen-1-yl)-cysteine

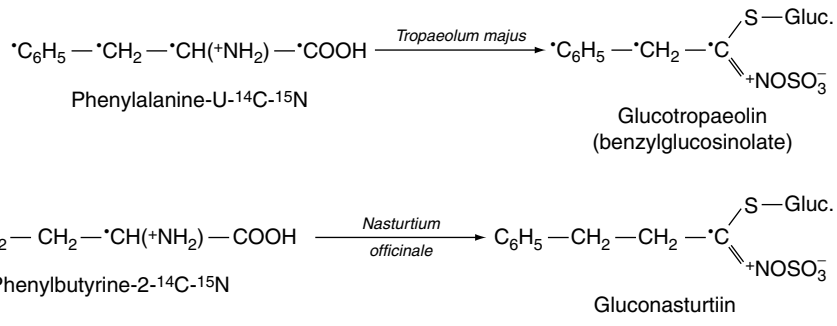


Fig. 25.4
Incorporation of doubly labelled amino acids by cruciferous species.

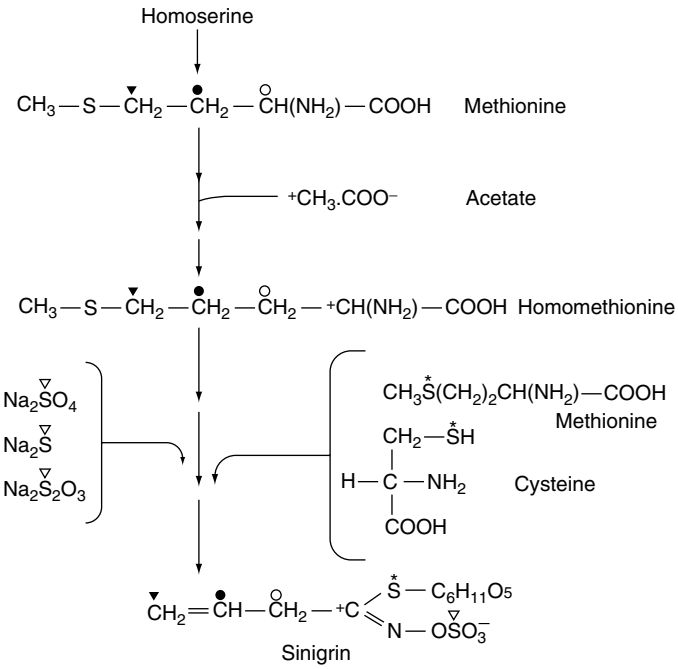


Fig. 25.5
Biosynthesis of sinigrin.

sulphoxide is the active component of the onion and the S-allyl derivative of garlic. As well as its considerable culinary interest, the latter also has medicinal usage.

GARLIC

Garlic bulbs (*Allium sativum* L., family Liliaceae) and the separated cloves need little description, being universally available for culinary purposes. Powdered garlic is prepared from bulbs, cut, freeze-dried or dried at a temperature no greater than 65°C. Conditions are important in order to maintain a uniform product but, even so, powders from different commercial sources may vary in their constituents and the *BHP* cites different standards for two major producers, Egypt and China.

A microscopical examination of the powder shows much parenchymatous tissue accompanied by groups of spiral or annular vessels.

In the plant itself the principal constituent of interest is the sulphur compound alliin. When the bulb is chopped or crushed alliin is brought into contact with the enzyme allinase, normally stored in separate cells, and under moist conditions alliin is rapidly transformed via allylsulphenic acid to allicin the main component of the commercial powder. Under dry conditions both alliin and allicin are relatively stable but the latter when formed is readily converted during processing of the powder to other sulphur compounds (Fig. 25.6) including diallyldisulphide, diallyltrisulphide and other linear and cyclic sulphur compounds. These reactions produce the characteristic garlic odour.

The *BP*/*EP* requires a minimum allicin content of 0.45% calculated with reference to the dried drug. Liquid chromatography is used for the assay employing butyl parahydroxybenzoate as an internal standard.

Votalile oil distilled from the drug contains the above sulphur compounds (no alliin or allicin), and various terpenes.

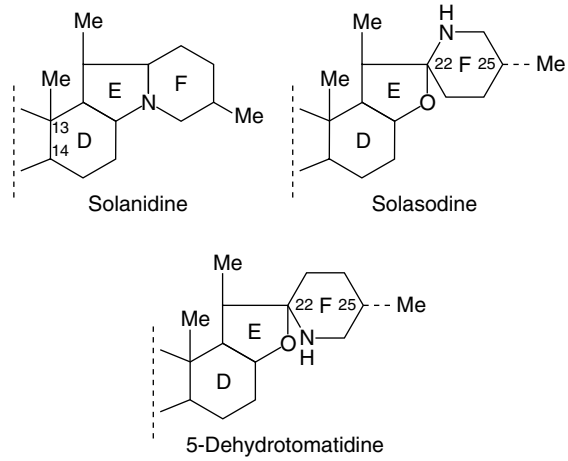
The pharmacological activities and reputed beneficial effects of garlic are numerous and for details and references (138) the reader is referred to J. Barnes *et al*., *Herbal Medicines*, 3rd edn. 2007, Pharmaceutical Press, London.

MISCELLANEOUS GLYCOSIDES

Attention is drawn to the following types of glycoside, some of which have been mentioned under other headings.

Steroidal alkaloidal glycosides

These are particularly abundant in the families Solanaceae and Liliaceae. Like saponins, they have haemolytic properties. Examples are α-solanin (potato, *Solanum tuberosum*), soladulcin (bitter-sweet, *S. dulcamara*), *tomatin* (tomato, *Lycopersicon esculentum*) and rubijervine (*Veratrum* spp.). The sugar components, one to four in number, are attached in the 3-position and may be glucose, galactose, rhamnose or xylose. The formulae (as shown below), in which part of the steroidal structure is omitted, illustrate three variations in the E and F ring systems of the aglycones. Solasodine and 5-dehydrotomatidine are stereoisomeric spirosolanes and the configuration of the nitrogen atom is apparently always linked to that at C-25.



Thus, solasodine, the nitrogen analogue of diosgenin (q.v.), is Δ^5,22β,25α-spirosolen-3β-ol and 5-dehydrotomatidine is Δ^5,22α, 25β-spirosolen-3β-ol (see also 'Chemical Races', Chapter 14 and 'Saponins', Chapter 23).

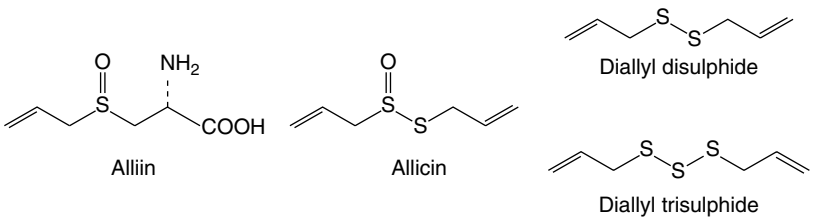


Fig. 25.6
Constituents of garlic.

Glycosidal resins

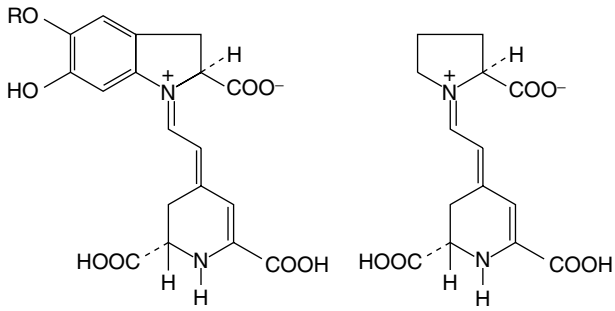
The complex resins of the Convolvulaceae such as those found in jalap and scammony (q.v.) are glycosidal; they yield on hydrolysis sugars such as glucose, rhamnose and fucose together with normal fatty acids and the hydroxyl derivatives.

Glycosidal bitter principles

While many glycosides have a bitter taste, certain of them were described as 'bitter principles' long before their chemical nature was elucidated. These compounds include gentiopicrin or gentiopicroside of gentian root (q.v.); picrocrocin or picrocroside of saffron (q.v.); and cucurbitacins of the Cucurbitaceae (e.g. colocynth, q.v.).

Betalains

For many years a group of plant pigments, associated with the order Centrospermae and containing nitrogen, had been known. These compounds were termed 'nitrogenous anthocyanins'. Following the initial isolation in crystalline form of one such compound in 1957, the structures of two groups of pigments have now been determined; these are the betacyanins and betaxanthins, the former being red-violet in colour and the latter yellow. These names were derived from a combination of *Beta vulgaris* (the red beet) and the anthocyanin and anthoxanthin pigments to which they were thought to be related. That these new compounds contained nitrogen was confirmed, but they are not flavonoid derivatives (see structures below). Betanin, on hydrolysis, gives the aglycone betanidin; indicaxanthin, although not a glycoside, is included here for completeness. Betalains are also responsible for the bright colorations of the flowers and fruits of the Cactaceae. In this case the sugar moiety of betanin may be substituted at C-2 and C-6 by malonyl, apiosyl and feruloyl groups. For a report on betalains from Christmas cactus see N. Kobayashi *et al*., *Phytochemistry*, 2000, **54**, 419. Muscaaurin and muscapurpurin are betalain pigments of the fly agaric, *Amanita muscaria*. *Opuntia dillenii* fruits have been suggested as an industrial source of betanins. Chemotaxonomically these compounds are of considerable interest and are of importance as food colourants (Chapter 33).



Further reading

Strach D, Vigt T, Schlieman W 2003 Recent advances in betalain research. Phytochemistry 62(3): 247–269

Antibiotic glycosides

Certain antibiotics are of glycosidal nature. Streptomycin, for example (see Table 30.1) is formed from the genin streptidin (a nitrogen-containing cyclohexane derivative) to which is attached the disaccharide streptobiosamine. The latter is constituted from one molecule of the rare methylpentose streptose and one molecule of *N*-methylglucosamine.

Nucleosides or nucleic acids

These substances, which are of the highest biological importance, have three components: a sugar unit (either ribose or 2-desoxyribose), a purine or pyrimidine base or bases (e.g. adenine, guanine and cytosine) and phosphoric acid. These are *N*-glycosides. When conjugated with proteins (q.v.) they form nucleoproteins.