6

Pharmacological activities of natural products

DRUGS ACTING ON THE NERVOUS SYSTEM 45
THE HEART, CIRCULATION AND BLOOD 46
ACTION ON THE GASTROINTESTINAL TRACT 48
THE NASAL AND RESPIRATORY SYSTEMS 49
THE LIVER 49
THE URINARY AND REPRODUCTIVE SYSTEMS 49
THE SKIN AND MUCOUS MEMBRANES 49
ACTION ON SUGAR METABOLISM 50
STEROIDS AND ANTI-INFLAMMATORY DRUGS 50
NON-STEROIDAL ANTI-INFLAMMATORY DRUGS 50
TREATMENT OF INFECTIONS 51
TREATMENT OF MALIGNANT DISEASES 51
TREATMENT OF ALLERGIES 51
THE IMMUNE SYSTEM 51
VITAMINS 52

As indicated in Chapter 2, a valid scheme for the study of medicinal plants and their products, and one which emphasizes pharmaceutical use, can be based on pharmacological action. The scheme can be extended to include numerous plants which, although eliciting a pharmacological response, are not, for varied reasons, used as drugs. In the latter category would be placed hundreds of alkaloid- and glycoside-containing plants.

Some major pharmacological groupings involve drugs which act on the nervous systems, heart and blood vessels, lungs, gastrointestinal tract, kidneys, liver, reproductive organs, skin and mucous membranes. Other categories include hormones, vitamins and chemotherapeutic drugs used for the treatment of infections and malignant diseases. Some plants (e.g. *Papaver*, ipecacuanha and liquorice) contain a range of compounds with differing pharmacological properties. Oliver-Bever's classical review (*J. Ethnopharmacol*., 1983, **7**, 1) on West African plants which act on the nervous system well illustrates the problems of constructing a purely pharmacological classification for herbal materials. A system based on clinical usage may be more straightforward for the throughly studied allopathic drugs used in Western medicine but difficulties can arise for plants used in traditional medicine because of the often numerous conditions for which any one drug may be employed. However, this is a very active area of research and the situation for a particular drug becomes clearer as the chemical nature of the active constituents together with their pharmacological properties are elucidated.

DRUGS ACTING ON THE NERVOUS SYSTEM

The nervous system coordinates and regulates the various voluntary and involuntary activities of the body and is conveniently considered under two headings—the central nervous system (CNS) and the autonomic nervous system. The two are interlinked and some drugs which affect the CNS may also produce reactions associated with the autonomic system. In the case of others which act via the autonomic system it is sometimes more convenient to classify them under other headings appropriate to the organs involved; thus, those producing vasoconstriction or vasodilation may appear under the consideration of the circulatory and respiratory systems.

THE CENTRAL NERVOUS SYSTEM

The central nervous system comprises the brain (cerebrum, cerebellum, medulla oblongata) and the spinal cord. It coordinates the voluntary activities of the body and exhibits numerous interactions within the system together with linkages to the autonomic system. Drugs involved with the CNS can be broadly classified according to whether they have a general stimulatory or depressant action with further subdivision regarding specific actions such as anticonvulsant and psychopharmacological activities. Some of the most useful natural drugs of the group are the narcotic (opioid) analgesics; a number of herbal drugs are popular sedatives and others such as the hallucinogenic drugs have important sociological implications. See Table 6.1 for a summary of drugs acting on the central nervous system.

THE AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system supplies the smooth muscle tissues and glands of the body. Its function is complex, involving ganglia situated outside the spinal cord; it is composed of two divisions, the sympathetic (thoracolumbar or adrenergic) division, which arises from the thoracic and lumbar regions; and the parasympathetic

Table 6.1 Drugs acting on the central nervous system.

Drugs affecting mental activity	
Lysergic acid diethylamide	Hallucinogenic. Prepared by partial synthesis from ergot alkaloids or by artificial culture
Mescaline	Hallucinogenic. Obtained from peyote cactus
Cannabis	Hallucinogenic. Active constituents contained in the resin of *Cannabis sativa*
Purine bases (e.g. caffeine, theophylline, theobromine)	Stimulate mental activity; constituents of beverages—coffee, tea, cocoa, kola, maté
Cocaine	One of the earliest drugs used as a mental stimulant. Produces addiction. Contained in the leaves of *Erythroxylum coca*
Ginkgo biloba	Improves short term memory
Ginseng	Improves mental concentration particularly in the elderly
Galanthamine	Promising Amaryllidaceous alkaloid for treatment of Alzheimer's disease
Hops	Sedative, often combined with other herbs
Hypericum	Popular herbal remedy for relief of mild–moderate depression
Passiflora	Treatment of insomnia often in combination
Sage	Revived interest in its use for counteracting memory loss
St John's wort	Antidepressant, may adversely react with some mainstream drugs
Reserpine	Depresses mental activity. Used in psychiatric treatment. Obtained from *Rauwolfia* spp.
Yohimbine	Similar action to reserpine but its antiadrenaline reactions and effect on heart muscle render it of no clinical use. Found in various species of the Apocynaceae
Valerian	Sedative and hypnotic; aids sleeplessness and improves sleep quality
Analeptic drugs (stimulants of the CNS in addition to the mental stimulants indicated above)	
Picrotoxin	Analeptic previously used in the treatment of barbiturate poisoning. Obtained from berries of *Anamirta cocculus*
Lobeline	Obtained from *Lobelia inflata*
Strychnine	Weak analeptic; toxic doses produce spinal convulsions. Obtained from the seeds of *Strychnos* spp.
Camphor	Weak analeptic. Obtained from *Cinnamomum camphora* and by synthesis
Central depressants of motor function	
Tropane alkaloids (e.g. hyoscine, atropine, etc.)	Formerly the only drugs effective in the alleviation of the symptoms of Parkinson's disease. Used in treatment of travel sickness and delirium tremens
Gelsemium root	Rarely employed clinically owing to high toxicity. Galenical preparations occasionally used as antispasmodics
Analgesic drugs	
Morphine	Effective for relief of severe pain. Depressant action on the cough and respiratory centres. The principal alkaloid of opium
Codeine	Although less active than morphine it is a much safer drug for the relief of mild pain and for use as a cough suppressant

(craniosacral or cholinergic) division, originating in the brain and in the sacral region. In general, an increase in activity of the sympathetic system gears the body for immediate action (fight and flight), whereas stimulation of the parasympathetic or vagal system produces effects more associated with those occurring during sleep and with energy conservation. Two important neurotransmitter substances of the autonomic nervous system are acetylcholine and noradrenaline and its derivatives; hence, other substances which either mimic or antagonize the action of either of these will produce a marked physiological response. Drugs acting on the autonomic nervous system are summarized in Table 6.2.

THE HEART, CIRCULATION AND BLOOD

In developed countries, coronary and associated circulatory diseases now constitute the principal cause of human mortality. Not surprisingly, therefore, this is an area of intensive research, not entirely devoted to treatment, but also to the prevention of these diseases. With increased public awareness of the importance of the latter, healthier living focused on diet, supplementary food factors, exercise, etc. has taken on a more important role, not least in the mind of the commercial world where health food stores now supply many dietary supplements and medicinal plant products which overlap the traditional pharmaceutical range.

Many factors affect the complex regulation of the heart and the large group of drugs which is known to possess cardiovascular activity is not confined to action on the heart muscle itself. Thus those drugs possessing antiarrhythmic, antihypertensive, antihyperlipidaemic, vasoconstrictor, vasodilator, blood anticoagulant, and platelet aggregation activities must also be considered in this group. As with other important areas, there is an active search in the plant kingdom for compounds which may also serve as lead compounds for the semi-synthesis of new drugs. For some therapeutic groups, the lack of simple reliable screening techniques is a problem.

In a review (over 390 refs) E. L. Ghisalberti *et al*. (see Further Reading) have listed some 447 species from 109 families having cardiovascular activity, together with a compilation of over 700 secondary plant metabolites having such activity.

Cardioactive glycosides

A considerable number of plants scattered throughout the plant kingdom contain C_{23} or C_{24} steroidal glycosides which exert a slowing and strengthening effect on the failing heart. In Western medicine it is the glycosides of various *Digitalis* species that are extensively employed.

Table 6.2 Drugs acting on the autonomic nervous system.

Acetylcholine-like drugs	
Pilocarpine	From leaves of *Pilocarpus microphyllus*
Arecoline	From seeds of *Areca catechu*
Muscarine	From *Amanita* spp. and other fungi
Physostigmine	A cholinesterase inhibitor from seeds of *Physostigma venenosum*
Antagonists of acetylcholine	
Tropane ester alkaloids (e.g. hyoscine, atropine)	From a number of Solanaceae (e.g. *Duboisia, Atropa, Datura* etc.) They have widespread uses involving their gastrointestinal, bronchial, genito-urinary and ophthalmic effects in addition to the CNS activity (q.v.)
Neuromuscular blocking agents (e.g. tubocurarine)	From leaves and stems of *Chondodrendon tomentosum*
Ganglion blocking agents (e.g. tubocurarine in large doses) (not clinically important)	
Adrenaline-like drugs	
Ephedrine	From stems of *Ephedra* spp.; mainly synthetic
Antagonists of adrenaline	
Ergot alkaloids (e.g. ergotamine)	From sclerotia of *Claviceps* spp.
Noradrenaline depletion	
Reserpine	Has antihypertensive effect resulting from dilation of heart and circulatory vessels
Ophthalmic preparations	
The eye, being under the control of the autonomic nervous system, is affected by some of the drugs mentioned above; these include atropine, hyoscine, physostigmine and pilocarpine	

The pharmacological effectiveness of the cardioactive glycosides is dependent on both the aglycones and the sugar attachments; the inherent activity resides in the aglycones, but the sugars render the compounds more soluble and increase the power of fixation of the glycosides to the heart muscle.

The overall action of the digitalis glycosides is complicated by the number of different effects produced, and their exact mode of action on myocardial muscle in relation to current views on cardiac muscle physiology is still an area of investigation. Digitalis probably acts in competition with K^+ ions for specific receptor enzyme (ATPase) sites in the cell membranes of cardiac muscle and is particularly successful during the depolarization phase of the muscle when there is an influx of Na^+ ions. The clinical effect in cases of congestive heart failure is to increase the force of myocardial contraction (the positive inotropic effect) resulting in a complete emptying of the ventricles. As a result of depression of conduction in the bundle of His, the atrioventricular conduction time is increased, resulting in an extended P–R interval on the electrocardiogram. Arising from their vagus effects, the digitalis glycosides are also used to control supraventricular (atrial) cardiac arrhythmias. The diuretic action of digitalis, important in the treatment of dropsy, arises from the improved circulatory effect. However, following the introduction of safer diuretics in the 1950s, diuretic therapy for heart failure has become much more important and in some cases can replace digitalis treatment.

Among the many other plant genera containing cardioactive glycosides related to those of *Digitalis*, and used similarly, are *Strophanthus*, *Convallaria*, *Nerium*, *Thevetia* and *Erysimum*. For a full account of these drugs see Chapter 23.

Antiarrhythmic drugs

As mentioned above, the cardiac glycosides can be used to control supraventricular (atrial) cardiac arrhythmias. There are a number of other drugs such as the alkaloid quinidine (obtained from various cinchona barks, q.v.) which act on both supraventricular and ventricular arrhythmias. Quinidine is official in most pharmacopoeias as its salts and finds prophylactic use in recurrent paroxysmal dysrhythmias such as atrial fibrillation or flutter. Its therapeutic use for the attempted conversion of atrial fibrillation to sinus rhythm has now been largely replaced by electrical cardioversion.

Other drugs in this category include hawthorn and motherwort.

Antihypertensive drugs

The control of hypertension is an important element in the management of cardiovascular disorders. Primary hypertension, as distinct from other special forms which usually require hospitalization, represents about 90% of all cases ranging from mild conditions with the occasional rise in blood pressure to those with severe unrelieved high pressure. Of the hypotensive plant drugs rauwolfia and its principal alkaloid reserpine together with *Veratrum* extracts were recognized in allopathic medicine in the early 1950s. A number of plants regularly employed by Western herbal practitioners include mistletoe, *Crataegus*, Yarrow, *Tilia* and *Fagopyrum*. In fact, a large number of other herbal drugs, used to treat various conditions, have also been shown to possess antihypertensive activity. In Ayurvedic medicine *Piper betle*, *Jasminum sabac*, *Cardiospermum halicacabum* and *Tribulus terrestris*, used in the treatment of hypertension, have been shown to exhibit a high angiotensin converting enzyme inhibition suggesting a possible mechanism of action for these drugs (B. Somanadhan *et al.*, *J. Ethnopharmacology*, 1999, **65**, 103).

Platelet-activating factor (PAF) antagonists

In the circulatory system thrombi may be caused on the arterial side as a result of the adhesion of blood platelets to one another and to the walls of the vessels. This platelet aggregation is triggered by the platelet activating factor which is released from activated basophils. PAF from the rabbit was characterized in the 1970s as a 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine. A number of prostaglandins and thromboxanes are also involved in the aggregation mechanism and

thromboxane A_2 which is synthesized from arachidonic acid (q.v), is particularly potent. In undamaged vessels thromboxane A_2 is possibly balanced by a prostaglandin e.g. prostacyclin of the arterial intima which has deaggregation properties.

For the secondary prevention of cerebrovascular or cardiovascular disease, aspirin, which irreversibly acetylates the platelet enzyme cyclo-oxygenase has been employed at dosages of 300 mg daily with useful results. A large number of plants have been screened for anti-PAF activity. One of the first natural products so identified was the neolignan kadsurenone obtained from *Piper futokadsura*, a plant long used in Chinese traditional medicine for allergy treatments. Other plants of traditional medicine reported to have anti-PAF activity include species of *Forsythia*, *Arctium*, *Centipeda*, *Tussilago*, *Pyrola*, *Populus* and *Peucedanum*. The active constituents include lignans, sesquiterpenes, coumarins, pyrocatechol and salicyl alcohol.

Extracts of the maidenhair tree, *Ginkgo biloba* have proved especially interesting and are commercially available in Europe for the treatment of various circulatory disorders.

Certain fish oils (e.g. cod-liver, halibut-liver) once employed solely for their vitamin contents together with 'oily fish' body oils have recently received a resurgence in popularity as dietary supplements. One favourable response is that they decrease the ability of platelets to aggregate by virtue of their high eicosapentaenoic acid content; this acid tends to favour the biosynthesis of thromboxane A_3, a weaker stimulator of platelet aggregation than thromboxane A_2.

Drugs acting on blood vessels (Table 6.3)

These drugs are essentially either vasoconstrictor or vasodilator substances but their action may originate in a variety of ways (direct, central, peripheral or reflex). Some of the drugs (e.g. ergot, bronchodilators, diuretics), which are particularly useful in relation to specific systems, are classified elsewhere.

Oral anticoagulants

These compounds inhibit the clotting mechanism of the blood and are of value in arterial thrombosis; they have no effect on platelet aggregation. One group of active drugs constitutes the 4-hydroxy-coumarins which act by antagonizing the effects of vitamin K (see Chapter 31). Warfarin sodium is one of the most widely used drugs. Plants used in herbal medicine which contain coumarin derivatives and possess anti-vitamin K activity include *Melilotus officinalis*, *Galium aparine* and *Lavandula officinalis*.

Other anticoagulants are heparin, which is given by injection, and hirudin, produced by the leech; hirudin, a polypeptide of 65 amino acids, can also be obtained from genetically modified *Saccharomyces*.

Hypolipidaemic drugs

In recent years much prominence has been given to the association of high levels of blood cholesterol and plasma triglycerides with atherosclerosis and ischaemic heart disease. Treatment of hyperlipidaemia is preferably dietary accompanied by other natural regimens. Drug therapy is reserved for the more intractable conditions. Natural products having a beneficial action include nicotinic acid (Chapter 31) and those fish oils containing high quantities of ω-3-marine triglycerides. The latter involve eicosapentaenoic acid and docasahexaenoic acid which, when counting from the methyl end, possess the first double bond at C-3 (see Chapter 19).

The suggested beneficial properties of garlic (*Allium sativum*) for the treatment of various cardiovascular conditions remain a subject of extensive investigation. With reference to hyperlipidaemic patients, the majority of published data supports the hypothesis that garlic lowers serum total cholesterol and improves the lipid profile. There is a tendency towards reduction of low-density lipoprotein and an increase in high-density lipoprotein giving a more favourable HDL:LDL ratio. Similar hypocholesterolaemic properties have been demonstrated for globe artichoke. A reduction in the serum levels of total cholesterol, low-density lipoprotein cholesterol together with a lowered atherogenic index was observed with mild hypercholesterolic patients after a three-month course of psyllium seeds (K. Sagawa *et al.*, *Biol. Pharm. Bull*., 1998, **21**, 184).

ACTION ON THE GASTROINTESTINAL TRACT

The gastrointestinal tract can be divided into three regions—the upper (mouth, stomach and upper portion of the duodenum), the

Table 6.3 Drugs acting on blood vessels.

Peripheral vasoconstrictor drugs	
Ergotamine (tartrate) from *Claviceps purpurea*	Produces a direct constrictor effect in vascular smooth muscle; the reversal of the dilation of cranial vessels leads to its use at the onset of classical migraine attack
Ergotoxine	Similar to ergotamine
Ephedrine (synthetic and from *Ephedra* spp.)	Prolonged action on blood pressure—see also 'Autonomic Nervous System'
Nicotine	Vasoconstrictor effects arise from its action on sympathetic ganglia, and by it promoting release of vasopressin and adrenaline
Central vasoconstrictor drugs	
Most of the drugs (e.g. picrotoxin) which stimulate the central nervous system also stimulate the vasomotor centre in the medulla, producing a rise in blood pressure. Although at one time used as respiratory stimulants, these drugs have been largely replaced by mechanical devices for artificial ventilation of the lungs	
Vasodilator drugs	
Papaverine (an opium alkaloid)	Acts directly on the blood vessels by causing relaxation of smooth muscle. An intravenous injection used for the treatment of pulmonary arterial embolism
Xanthine derivatives (caffeine, theobromine, theophylline)	As papaverine; they also have a central vasoconstrictor action counteracting the peripheral effect. Also diuretic
Ergotamine	Adrenaline antagonist—see 'Autonomic Nervous System'
Reserpine	Vasodilatation is produced by a peripheral and central action (q.v.)
Veratrum alkaloids (from *Veratrum* spp.)	Bradycardia and peripheral vasodilatation by sensitization of cardiac, aortic and carotid sinus baroreceptors

middle (lower half of the duodenum to the ileocolic sphincter) and the lower (caecum, colon and rectum). It is the upper and the lower portions that are most susceptible to disorder and are consequently associated with the greatest number of drugs for their treatment (see Table 6.4).

Table 6.4 Drugs acting on the gastrointestinal tract.

Bitters At one time these were extensively used in liquid medicaments to stimulate appetite. The bitter constituents stimulate the gustatory nerves in the mouth and give rise to an increase in the psychic secretion of gastric juice. Extracts of the following drugs have been so employed: gentian, quassia, calumba, cinchona (or quinine), nux vomica (or strychnine). Considerable recent research has involved the investigation of a number of these bitter compounds for other possible uses, e.g. the bitters of the Simaroubaceae as antitumour and antimalarial agents

Anticholinergic drugs In this capacity hyoscine and hyoscyamine help disturbances caused by gastric mobility and muscle spasm particularly with some ulcer patients

Emetics Ipecacuanha preparations, on oral administration, have a delayed emetic action produced by irritation of the mucous membranes (see 'Expectorants'). Picrotoxin stimulates the vomiting centre through its general effect on the central nervous system

Antiemetics Ginger has received scientific approval for the prevention of the symptoms of travel sickness. Cannabis affords sickness relief to patients undergoing chemotherapy

Carminatives These are aromatic substances which assist the eructation reflex; their mode of action is obscure. Dill oil is used for the relief of flatulence, especially in babies. Other plants or oils used as carminatives include caraway, fennel, peppermint, thyme, nutmeg, calamus, pimento, ginger, clove, cinnamon, chamomile, matricaria. Chalk is used as an antacid and charcoal as an adsorbent

Ulcer therapy Derivatives of glycyrrhetinic acid (a triterpenoid of liquorice root) prove effective in the treatment of peptic ulcer. Deglycyrrhizinized liquorice has also been employed. Other antiulcer agents include alginic acid, marshmallow and comfrey

Demulcents These soothe and protect the alimentary tract and overlap with some materials used in ulcertherapy. Iceland moss, orris and elm bark may be included here

Laxatives and purgatives Purgatives may be classed according to their mode of action

Agar, psyllium and ispaghula	Hydrophilic colloids which function as bulk-producing laxatives
Bran	An indigestible vegetable fibre which absorbs water and provides bulk
Senna (leaves and fruit)	Contains anthraquinone derivatives which are hydrolysed in the bowel to stimulate Auerbach's plexus in the wall
Cascara, rhubarb, aloes	As senna
Castor oil	Contains glycerides which on hydrolysis yield riconoleic acid, irritant to the small bowel
Podophyllum resin, jalap resin, colocynth	Drastic purgatives, now little used for this purpose. They were often prescribed with belladonna to reduce griping

Rectal and colonic drugs Arachis oil, esculin, hamamelis, pilewort and balsam of Peru are examples of this group; they include those used in suppositories

Antidiarrhoeal drugs Morphine and codeine act by increasing the smooth muscle tone of the bowel and by reducing its mobility. Commonly prescribed with kaolin

THE NASAL AND RESPIRATORY SYSTEMS

A large number of drugs of plant origin are to be found in this group. As infections of the respiratory tract are amongst the most common of illnesses, it is not surprising that there are numerous proprietary preparations for their treatment (Table 6.5).

THE LIVER

The liver, the principal organ of metabolism and excretion, is subject to a number of diseases which may be classed as liver cirrhosis (cell destruction and increase in fibrous tissue), acute or chronic hepatitis (inflammatory disease) and hepatitis (non-inflammatory condition). The most common drug of plant origin used in Western medicine for its antihepatotoxic properties is *Silybum marianum*. In Indian and Oriental medicine many plants are so used. For a discussion of this field of current interest see Chapter 29.

THE URINARY AND REPRODUCTIVE SYSTEMS

A number of plant materials are to be found in this group; the examples given in Table 6.6 are confined to Western usage.

THE SKIN AND MUCOUS MEMBRANES

In addition to acting as covering for the body, the skin performs a number of other physiological functions. Drugs affecting the skin may be of an emollient nature or they may act as absorbents, astringents, irritants or antiseptics (see Table 6.7). A number of substances are easily absorbed through the skin; this fact is utilized in transdermal medication but must also be borne in mind with respect to various poisons.

Table 6.5 Drugs acting on the nasal and respiratory systems.

Aromatic inhalations	Benzoin, cineole, eucalyptus oil, menthol and peppermint, pumilo pine oil, balsam of Tolu, thymol, turpentine, menthol, eucalyptus
Bronchodilators and nasal decongestants	Ephedra, ephedrine, xanthines (theophylline)
Expectorants	Ipecacuanha (in subemetic doses), senega root, liquorice root, squill bulb, tolu balsam, pulmilio pine oil, lobelia, grindelia, angelica root and leaf, storax, cocillana, coltsfoot, sweet violet, bloodroot, balm of Gilead
Antiexpectorants	Codeine, atropine
Cough depressants	Morphine, codeine, noscapine, wild cherry
Demulcents	Marshmallow, verbascum, plantago, Iceland moss, honey

Table 6.6 Drugs acting on the urinary and reproductive systems.

Diuretics	Xanthine derivatives as present in many beverages (tea, coffee, etc.) promote dilation of the renal medullary blood vessels. Digitalis glycosides improve the failing heart thereby increasing renal perfusion and glomerular filtration; hence, Withering's original introduction of digitalis for the treatment of dropsy. There is also a small but finite effect on tubular reabsorption of sodium ions
Diuretics and urinary antiseptics	Buchu, boldo, horsetail, Java tea, bearberry, juniper, copaiba. These include drugs used for the treatment of cystitis and urethritis
Drugs acting on the uterus	Preparations of ergot were traditionally used in childbirth and then largely replaced by the isolated alkaloid ergometrine. Administered as its salts it has a direct stimulant action on the uterine muscle and reduces the incidence of postpartum haemorrhage. Ergotamine acts similarly but is not suitable for obstetric use because of its marked peripheral vasoconstrictor action
	Black haw is a uterine tonic and sedative used for the prevention of miscarriage and for dysmenorrhoea after childbirth
	Hydrastis is employed for menorrhagia and other menstrual disorders
	Agnus castus has been traditionally employed for the treatment of menopausal disorders, premenstrual symptoms, dysmenorrhoea, etc.
Oral contraceptives	Female, see 'Steroids'. Male, gossypol (q.v.)
	Numerous plants have been, and are being, tested for antifertility activity
Male impotence	Papaverine (under careful medical supervision), yohimbine (erectile dysfunction)
Benign prostate hyperplasia (BPH)	A number of phytomedicinals are employed (often as admixtures) to treat the symptoms of BPH. Two metabolites associated with the condition are dihydrotestosterone and oestrogen which require two enzymes (5α-reductase and aromatase) for their synthesis in the body. It has been shown, for some of the drugs used, that they are inhibitors of these two enzymes. The following examples are well-established: *Cucurbita pepo* seeds (pumpkin), *Epilobium angustifolium* and other species, *Prunus africana* (*Pygeum africanum*) bark, *Serenoa repens* fruits (saw palmetto, sabal), *Urtica dioica* and *U. urens* root extracts

ACTION ON SUGAR METABOLISM

Many plants have been used in traditional systems of medicine for the oral treatment of diabetes and it is particularly important that Western practitioners be aware of any patients already taking such medication. Among the plants so used are karela fruit (*Momordica charantia*), cumin fruit, ginseng, *Teucrium oliverianum*, neem (*Azadirachta indica*), onion, *Aloe* spp., Job's tears (*Coix lachryma-jobi*) and *Galega officinalis*. For a discussion of the current position on plant-derived oral hypoglycaemic substances see Chapter 29.

Table 6.7 Drugs used on the skin and mucous membranes.

Emollients and demulcents	These include a number of vehicles used in the preparation of ointments, creams, lotions, etc., and include fixed oils (e.g. olive, arachis, coconut, theobroma), fats (wool-fat, lard), waxes of animal origin (beeswax, spermaceti), gums (acacia, tragacanth) and mucilages (psyllium, elmbark)
Absorbents	Starch, alginates, charcoal
Astringents	Tannins (e.g. Tannic acid), krameria, catechu, galls, *Aspidosperma*, hamamelis, pomegranate rind, kinos
Counter-irritants	Camphor, turpentine, capsicum, aconite, methyl salicylate, mustard seed
Antiseptics	Tars, eucalyptus oil, thyme oil, eugenol, thymol, cajuput
Anti-inflammatory agents	Corticosteroids used locally, matricaria, arnica
Psoriasis and eczema treatment	Comfrey, allantoin, cadeoil, evening primrose oil, chrysarobin, *Lithospermum*, savin, myrrh, grindelia
Wound coverings	Type of wound covering (occlusive, non-occlusive, haemostatic) is important in the healing process. See alginates, cotton, etc.

STEROIDS AND ANTI-INFLAMMATORY DRUGS

Two types of corticosteroidal hormone are the glucocorticoids, which regulate carbohydrate and protein metabolism and which also possess a strong anti-inflammatory action, and the mineralocorticoids, which influence the electrolyte and water balance of the body. The clinical indications for systemic treatment with these drugs are complex, but include use in replacement therapy, Addison's disease, reduction of lymphatic tissues (leukaemias), suppression of lymphopoiesis (lymphomas) and as anti-inflammatory agents (a variety of conditions including rheumatoid arthritis, cerebral oedema and raised intracranial pressure).

These hormones are produced naturally in the adrenal cortex but a wide variety of semi-synthetic drugs of this type is commonly in use. These are synthesized using plant steroids as intermediates; diosgenin and hecogenin being the principal sources. To a lesser extent the steroidal alkaloids of the Solanaceae are employed. There is a large world demand for these compounds, particularly for the synthesis of oral contraceptives, and their distribution in nature and chemistry is considered in more detail in Chapter 23.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

Aspirin, first synthesized in 1853 by Carl Gerhardt, is still one of the most widely-used mild analgesic and non-steroidal anti-inflammatory drugs (NSAID). It had its medicinal origin in the salicylates and glycosides of willow bark, long used for the treatment of rheumatic diseases, gout and painful conditions of all types.

In view of the universal requirement for NSAIDs very many plants have been utilized for the purpose in traditional medicine and in recent years considerable research effort has been expended on their investigation.

Enzymes have been used to detect anti-inflammatory activity of plants—galangin, a flavonoid of *Alpinia officinarum* (Zingiberaceae; galangal rhizome) was found to be a cyclo-oxygenase inhibitor. Lipoxygenase inhibitors are present in *Spilanthes oleracea* (a S. American plant of the Compositae used for the treatment of rheumatic disorders) and also in *Echinacea purpurea* root, also Compositae, which contains a range of compounds including isobutylamides.

In a review of plants exhibiting anti-inflammatory activity Handa *et al*. (1992) cite that species of 96 genera belonging to 56 families are ascribed such activity.

In addition to the wide range of plants involved there is a similar diversity in the chemical nature of the active constituents. Flavonoids constitute one group widely associated with anti-inflammatory activity and are exemplified in the *BHP* (1996) by the monographs on Balm of Gilead Bud, Cimicifuga Rhizome, Equisetum, Jamaica Dogwood, Marigold, Matricaria Flowers, Meadowsweet, Poke Root, Red Clover Flower and Willow Bark. In the case of an infusion of matricaria flowers (*Chamomilla recutita*, German chamomile), used for its anti-inflammatory action in the treatment of acute gastritis, it has been shown by the mouse-ear test that it is the flavonoids and not the volatile oils that are responsible for activity; however, for *Calendula officinalis* the terpenoids were found to be active constituents (see Anon., *Pharm. J*., 1992, **249**, 474). With liquorice root (*Glycyrrhiza glabra*) both the triterpenoid saponin glycyrrhizin and the flavonoids have, among their other pharmacological actions, anti-inflammatory activity.

Colchicine, an alkaloid of *Colchicum autumnale*, is the classical drug for the treatment of acute attack of gout. It may act by reducing the inflammatory response caused by deposits of urate crystals in the joint and by reduction of phagocytosis of the crystals. Its use has been somewhat replaced by allopurinol (inhibition of xanthine oxidase) and by phenylbutazone. Guaiacum resin (q.v) is cited for the treatment of chronic rheumatic conditions and gout; it contains a mixture of lignans. The dried root of *Harpagophytum procumbens* (Devil's claw) (Pedaliaceae), has recently received popular attention for the treatment of painful rheumatic conditions; iridoid glycosides, e.g. harpagoside, are the characteristic constituents. The juice of *Ananas comosus*, the pineapple (Bromeliaceae) contains a mixture of at least five proteolytic enzymes collectively called bromelin or bromelain. In Western medicine the enzyme has been introduced for its ability to dissolve fibrin in conditions of inflammatory oedema.

The action of ginkgolides (C_{20} terpenes from *Ginkgo biloba*) as potent antagonists of platelet activating factor has already been mentioned.

TREATMENT OF INFECTIONS

For natural products in this category, see Table 6.8.

Table 6.8 Drugs used for the treatment of infections.

Antibiotics Many higher plants possess constituents having antibacterial properties; none has been utilized clinically, mainly because of high toxicity. Moulds and streptomyces are the principal sources; see Chapter 30 for a consideration of antibacterial and antiviral drugs

Antimalarials Until the advent of the synthetic antimalarials, quinine, isolated from the bark of various *Cinchona* spp., constituted the most effective agent for the treatment of malaria; it is still used in Third World countries and is of some resurgent importance for combating malarial organisms resistant to other drugs. Artemisinin (Qinghaosa), an unusual sesquiterpene lactone, is the active constituent of an ancient Chinese drug derived from *Artemisia annua*. It is effective against chloroquine resistant strains of *Plasmodium vivax* and *P. falciparum* as well as against cerebral malaria; see Chapter 28

Amoebicides	
Emetine	An alkaloid of ipecacuanha root, used as its hydrochloride or bismuthiodide in the treatment of amoebic dysentery. Complete eradication of the chronic infection is difficult and combined therapy with other drugs is often necessary
Anthelminthics	
Extract of male fern	For tapeworm infections
Santonin	Possesses a powerful action in paralysing round-worms; although once extensively used its high toxicity has led to replacement by piperazine
Oil of Chenopodium	Like santonin; it has also been extensively used in hookworm disease but it gives variable results
Thymol	At one time much used in hookworm treatment

TREATMENT OF MALIGNANT DISEASES

The last 50 years has witnessed a vast search of the plant kingdom for phytochemicals with anticancer activity, and medicaments derived from *Catharanthus*, *Taxus* and *Podophyllum* are among the most effective in current usage. Originally, most research centred on plants of Western origin but since 1986 focus has shifted towards the investigation of tropical and subtropical species. The introduction of new techniques designed to eliminate 'nuisance' compounds (see Chapter 9) has accelerated the process of screening many hundreds of specimens. Clinical trials continue on a number of promising compounds. For a fuller discussion, see Chapter 27.

TREATMENT OF ALLERGIES

A large number of materials give rise to allergic conditions in sensitive individuals. Extracts containing specific allergies are available as diagnostic kits or for desensitization. Examples of plant allergens are grass, flower and tree pollens, dried plants and moulds (see Chapter 39).

THE IMMUNE SYSTEM

Drugs affecting the immune system are termed immunomodulatory or adaptogenic. Some repress the system and are of value in, for example, preventing rejection of transplanted organs and others are stimulatory and can be used to help combat viral infections such as AIDS or assist in the treatment of cancer. Until relatively recently such herbal drugs were largely ignored by Western orthodox medicine, although they have always featured in traditional Chinese and Indian medicine in seeking to achieve homoestasis with regard to

bodily functions. Now, however, ginseng leads the market in herbal sales in Europe and the US and *Echinacea* spp., used by native N. American Indians, ranks around fifth in the US herb market sales and is widely used in Europe, with 800 preparations being quoted as available in Germany. For immunomodulators of Chinese origin see L.-H. Zhang *et al*., *Phytotherapy Research*, 1995, **9**, 315, and for Indian drugs see A. A. Mungantiwar *et al*., *J. Ethnopharmacology*, 1999, **65**, 125.

VITAMINS

These accessory food substances are considered in Chapter 31.

Further reading

Ghisalberti EL, Pennacchio M, Alexander E 1998 A review of drugs having cardiovascular activity (*nearly 400 refs*). Pharmaceutical Biology 36(4): 237–279

Handa SS, Chawla AS, Sharma AK 1992 Plants with anti-inflammatory activity (*a review with 278 references and 34 structural formulae*). Fitoterapia 63, 3

Wagner H (ed) 1999 Immunomodulatory agents from plants. Birkhauser Verlag, Basle

Williamson EM, Okpako DT, Evans FJ (eds) 1996 Pharmacological methods in phytotherapy research, Vol 1. Selection and pharmacological evaluation of plant material. John Wiley, Chichester

Wright CI, Van-Buren L, Kroner CI *et al* 2007 Herbal medicines as diuretics: a review of the scientific evidence. Journal of Ethnopharmacology 114(1): 1–31 (*around 120 references*)

7

Synergy and other interactions in phytomedicines

E. M. Williamson

WHAT IS SYNERGY? 53
MEASURING SYNERGY 54
DEMONSTRATING SYNERGY AND POLYVALENT ACTION IN PHYTOMEDICINES 55
ENHANCEMENT OR REDUCTION OF ABSORPTION OR BIOAVAILABILITY 56
EXAMPLES OF SYNERGY, POLYVALENT ACTION OR ANTAGONISM IN HERBAL MEDICINES 56
NEW TECHNOLOGIES FOR LOOKING AT SYNERGY AND OTHER INTERACTIONS 60
CONCLUSION 60

The term 'synergy' (or synergism, from the Greek *syn-ergo*, meaning working together) refers to the phenomenon where two or more agents act together to produce an effect greater than would have been predicted from a consideration of individual contributions. Synergy is generally assumed to play a part in the effects of phytomedicines, and the use of combinations of herbs is fundamental to the philosophy of Western medical herbalism, traditional Chinese medicine (TCM) and Ayurveda. This attitude to their formulation and use differentiates herbal products from conventional medicines, even those originally obtained from plants. Modern phytomedicines are usually found as whole or semipurified extracts and should, ideally, be standardized for their active constituents, where known, to ensure clinical reproducibility. The likelihood of synergistic interactions is also recognized in reports from the European Pharmacopoeia Commission, where the most common type of extract, exemplified by *Hypericum perforatum*, is described as having 'constituents with known therapeutic or pharmacological activity which are not solely responsible for the overall clinical efficacy of the extract'. To complicate matters further, herbalists use preparations and mixtures that are not necessarily intended to target a particular organ, cell tissue or biochemical system. This kind of application has been described as the 'herbal shotgun' approach, as opposed to the 'silver bullet' method of conventional medicine, to distinguish the multitargeted approach of herbals from the single-target approach of synthetic drugs.

WHAT IS SYNERGY?

The term 'synergy' is now used very widely, and mainly inaccurately, to describe any kind of positive interaction between drugs. In pharmacology the term has a specific definition, but is often misapplied in practice. Whether an effect can truly be described as synergy, or is merely addition, is rarely established and evidence to prove it conclusively in herbal medicines is sparse. The opposite, antagonism, meaning 'working against', is a reduction in the overall expected effect. Put simply, both antagonism and synergism can be defined in relation to an additivity expectation, which can be calculated from the potency of individual mixture components. Synergism is an effect larger than additive, whereas antagonism is smaller than additive. There are two ways of calculating additivity expectations: dose addition and also independent action.

Interactions can also involve a potentiation of effects. The terms 'synergism', 'additivity' and 'antagonism' are applied to combinations where all components induce the effect of interest, whereas the term 'potentiation' should be applied where one or several 'inactive' compounds enhance or exacerbate the effect of other actives.

Synergy and other interactions can take place between the constituents of a single extract as well as in a mixture of herbs. Medical herbalists have always insisted that better results are obtained with whole plant extracts and combinations of these rather than with isolated compounds. TCM, in particular, uses complicated recipes and it has sometimes been thought that the inclusion of some herbs was unnecessary, but the rationale for such combinations is gaining increasing acceptance. A TCM herbal treatment for eczema was the subject of a clinical trial of 37 young patients (M. P. Sheehan and J. D. Atherton, *Br. J. Dermatol.*, 1992, **126**: 179–184), and investigations were carried out to identify the 'active constituent(s)' of the mixture. However, a programme of pharmacological tests failed to find a single active herb or compound: it was the herbal mixture that was so effective (J. D. Phillipson, reported in *European Phytotelegram*, 1994, **6**: 33–40).

MEASURING SYNERGY

Although the idea of synergy is easy to understand, the measurement of it is more problematic. It is fairly straightforward to identify synergy when one of the agents is inactive and a combination of this with an active agent produces an effect greater than that observed for the active alone (although this is more correctly termed potentiation), but difficulties in measurement arise when more than one (and there might easily be several) are active. Various methods for calculation have been devised over the years, but the following are now thought to be the most useful:

PREDICTION OF EFFECTS

Synergy is deemed present if the total effect of a combination is greater than would be predicted on the basis of expected additive effects of the mixture. Such additivity expectations can be derived from dose addition or independent action. Often, anticipated additivity is calculated by simply adding up the effects of individual mixture components, but this method can produce paradoxical and erroneous results and is therefore deemed unreliable (A. Kortenkamp and R. Altenburger, 1998; see legend for Fig. 7.1). The opposite applies for antagonism, which is observed less than would have been predicted.

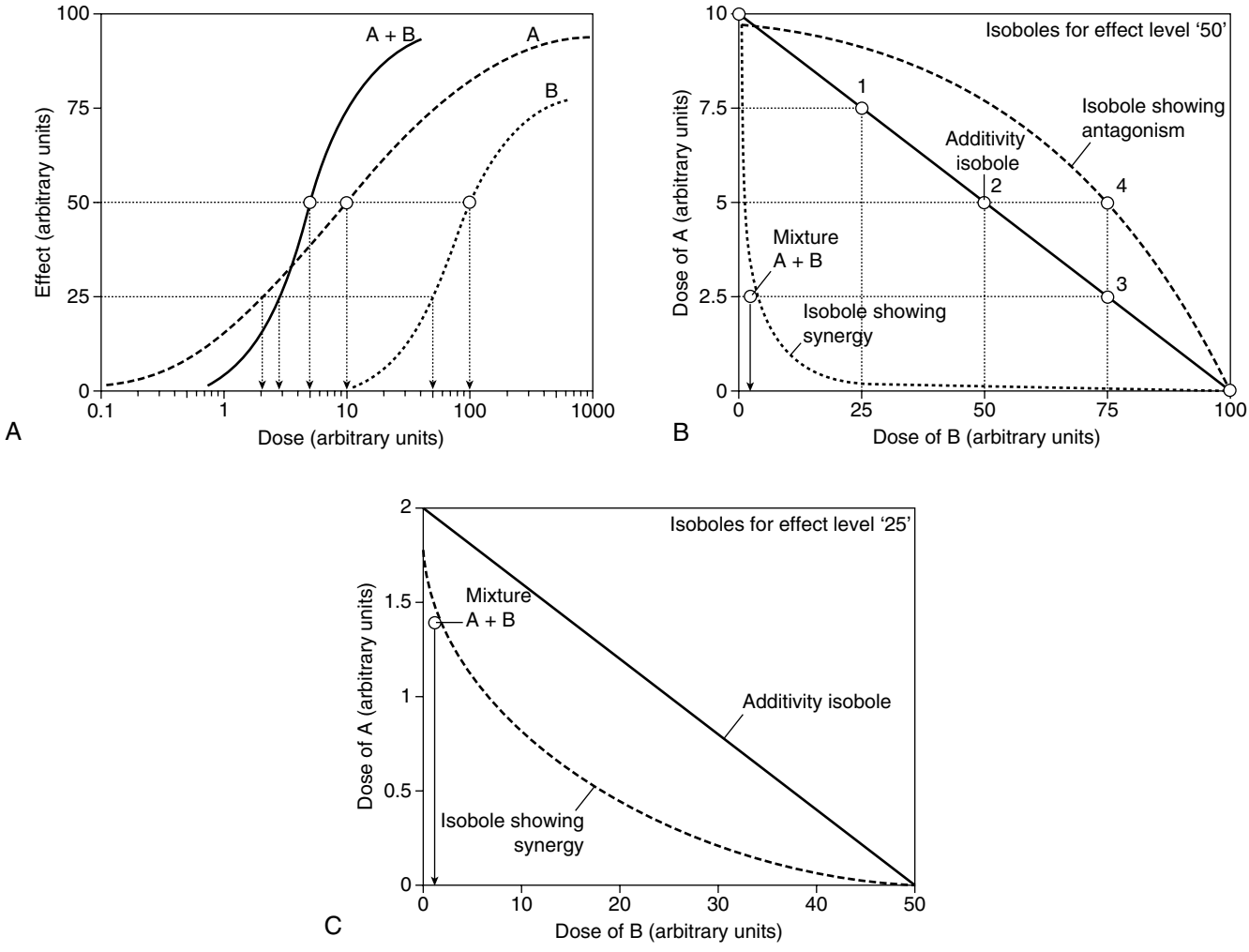


Fig. 7.1
An analysis of combination effects using the isobole method. A, Hypothetical dose–response curves for compounds A and B, and an equimolar mixture of A+B. An effect of 50 is produced by 10 (arbitrary) dose units of A or 100 dose units of B. The combination A+B yields this effect at 5 dose units (2.5 dose units A, 2.5 dose units B). Note that the curves for the individual compounds are dissimilar. B, Diagram showing isoboles for effect level '50' derived from Fig. 7.1A. The solid line (additivity isobole) joining 10 dose units on the A axis and 100 dose units on the B axis describes combinations of A and B that are expected to yield an effect level of '50', if the interaction between A and B is additive. For example, this should be the case with 7.5 dose units A plus 25 dose units B (point 1 on the additivity line), 5 dose units A plus 50 dose units B (point 2) or 2.5 dose units A plus 75 dose units B (point 3). However, the dose–response curves in Fig. 7.1A show that a combination of 2.5 dose units A and 2.5 dose units B is sufficient to produce this effect. Therefore a point below the additivity line is seen, yielding a concave-up isobole. It can be concluded that A and B interact with each other in a way that exacerbates their toxicity (synergism). Conversely, A and B antagonized each other if, e.g., 5 dose units of A plus 75 dose units of B were necessary to produce an effect level of 50 (open square 4). In this case, a point above the additivity line would appear, producing a concave-down isobole. C, Diagram showing isoboles for effect level '25' derived from Fig. 7.1A. (From A. Kortenkamp and R. Altenburger 1998 Synergisms with mixtures of xenoestrogens: a re-evaluation using the methods of isoboles. Science of the Total Environment 221(1): 59–73, with permission.)

THE ISOBOLE METHOD

The isobole method is an application of dose addition. It is unequivocal proof of synergy because it is independent of any knowledge of mechanisms and applies under most conditions. It makes no assumptions about the behaviour of each agent and is applicable to multiple components of up to three constituents, so can be applied to the analysis of effects in herbal mixtures. The isobole method uses graphs constructed to show curves (isoboles) describing combinations of two compounds, A and B, which produce the same specified action – which can be any measurable effect (Fig. 7.1A,B). The axes of the graph (Fig. 7.1B) represent doses of the two compounds on a linear scale. A line joining the iso-effective doses A and B of the single agents predicts the combinations of A and B that will yield the same effect, provided the interaction between A and B is only additive. This is the 'additivity line', in which case, there is no interaction between the two agents and they could be considered to be behaving like dilutions of each other.

For additivity (zero interaction), the relationship can be expressed algebraically by the equation of Berenbaum (*Pharmacol. Rev.*, 1989 **41**, 93–141; see Further reading):

$$d_A / D_A + d_B / D_B = 1$$

However, if synergy occurs, then smaller amounts are needed to produce the effect (i.e. the effect of the combination exceeds expectation) and the equation becomes $d_A / D_A + d_B / D_B < 1$, and the isobole is said to be 'concave-up'. The opposite applies for antagonism, and the equation becomes $d_A / D_A + d_B / D_B > 1$, producing a 'concave-down' isobole. It is actually possible to have synergy at a particular dose combination with antagonism at a different combination, and this would be reflected in the isobole. The position of isoboles varies depending on the effect level chosen for analysis (see Fig. 7.1B,C).

The isobole method can also be applied to mixtures in which only one of the two agents is active; in effect 'potentiation'. In this case, the iso-effective dose of the agent lacking activity can be regarded as being infinitely large, so the additivity isobole runs parallel to the respective dose axis. Synergism will again yield a concave-up isobole and antagonism a concave-down isobole, as shown in Fig. 7.2.

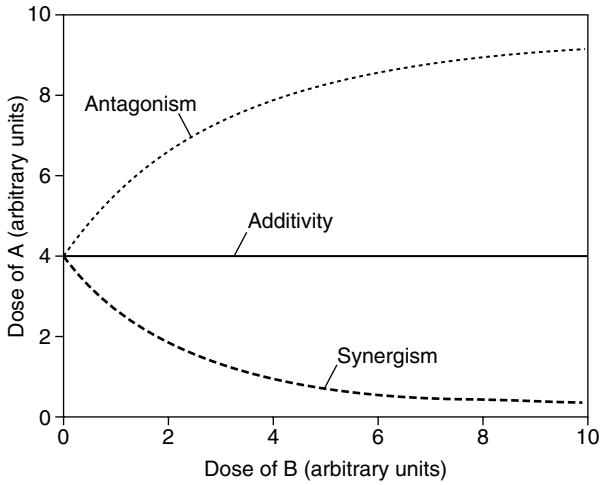


Fig. 7.2
The three types of combination effect for a mixture of an effective agent A and an ineffective compound B. When there is no interaction between A and B, the isobole is a straight line parallel to the dose axis of B (additivity). If there is a synergistic interaction, an isobole deviating towards the B axis is seen: in the presence of B smaller doses of A are sufficient to produce a predetermined effect. When there is antagonism, the isobole deviates away from the B axis. In this case, the presence of B requires higher doses of A to yield the same effect as in the absence of B. (From: A. Kortenkamp and R. Altenburger 1998 Synergisms with mixtures of xenoestrogens: a re-evaluation using the methods of isoboles. Science of the Total Environment 221(1): 59–73, with permission.)

DEMONSTRATING SYNERGY AND POLYVALENT ACTION IN PHYTOMEDICINES

Proving the existence of true synergistic interactions, even within a single herbal extract, is remarkably difficult, and explains why this crucial aspect of herbal medicines is not well documented. To do so requires the extract to be fractionated, tested, recombined and retested in various permutations to see how each is interacting with the others. To complicate matters further, herbalists normally use mixtures of extracts, many of which are traditional combinations that are not necessarily intended to target a single biochemical system or enzyme (S. Y. Mills and K. Bone, *Principles and Practice of Phytotherapy*, Churchill Livingstone, 2000), making evaluation of additive or synergistic effects even more difficult. Simple examples of this practice would be the inclusion of laxative herbs in products used for haemorrhoids, or choleretic herbs in digestive preparations. This is not synergy but a way of approaching treatment from several angles concurrently, and could be described as 'polyvalent action'. This term is used to cover the various effects of multiple active constituents acting in combination, in harmony and possibly in synergy. It therefore overcomes some of the problems of defining the overall effect as synergistic even when it includes antagonism, if that applies to a reduction of undesirable effects. As a preliminary step in looking for synergistic interactions, it is possible to test the effect of individual extracts singly and in combination, which will give an indication of synergy or antagonism although no real evidence as to which compounds are interacting.

In conventional medicine it is now common practice to use several drugs to treat a single complaint, such as in hypertension, psychoses and especially cancer, and this approach applies even more to plant extracts, because combinations are already present within the plant. There might be other sound reasons for not isolating individual components in some herbs such as ginkgo, St John's wort and ginseng, because these are traditionally used as standardized extracts for which there is positive clinical data. In some cases, the active ingredients may not even be fully known, and if synergy is involved, then bioassay-led fractionation (the usual method for identifying actives) would not even be possible (see P. Houghton, *Phytother. Res.*, 2000, **14**(6): 419–423). The identities of the main actives of many important herbs are still under discussion, and it would be unwise to exclude, by overpurification, any constituents that might contribute to efficacy. Even if the phytochemistry of a plant is well documented, the actual contribution of individual components to the overall effect might not have been ascertained. Examples include hawthorn (*Crataegus oxycantha*) as a cardiac tonic, hops (*Humulus lupulus*) as a sedative, black cohosh (*Cimifuga racemosa*) and chasteberry (*Vitex agnus-castus*) as hormone-balancing agents in women, saw palmetto (*Serenoa repens*) as an antiandrogen for prostatic hyperplasia, and devil's claw (*Harpagophytum procumbens*) as an anti-inflammatory agent. In other cases, the actives are unstable, and attempts to remove them from the 'protection' of the herb or whole extract could render them inactive. Here the obvious examples are garlic (*Allium sativum*) and valerian (*Valeriana* spp.). Garlic is often formulated as a product containing the precursor alliin, and the enzyme alliinase, which in solution (i.e. in the stomach) liberates the active allicin and other

unstable, but still active, decomposition products. This is not synergy but, effectively, a drug-delivery system.

Interactions *in vivo* might also occur between combinations that enhance or hinder therapeutic activity by affecting absorption, metabolism or excretion. Some of these can be seen *in vitro*, such as the complexing of plant polyphenols and tannins with many drugs, which could theoretically reduce their effectiveness. This does not seem to be a real problem, otherwise tea drinkers would find many of their prescribed medicines inactive. Other interactions will only be seen clinically, such as the effects of cytochrome P450 enzyme induction, which are only seen after a period of treatment.

ENHANCEMENT OR REDUCTION OF ABSORPTION OR BIOAVAILABILITY

TCM and Ayurvedic formulae often have herbs such as liquorice or pepper included specifically to reduce or increase bioavailability of other ingredients, and this can be considered a form of synergy. Liquorice (*Glycyrrhiza* spp.) features as a synergist in a great many multiherbal preparations in TCM and the reason for its inclusion has not always been apparent. *Glycyrrhiza* extracts are used for their antiulcer, anti-inflammatory and antihepatotoxic properties and the saponin glycyrrhizin (formerly considered to be the 'active' constituent) exhibits its activity in all of these areas. However, so also do the flavones, isoflavones and chalcones, and glycyrrhizin is certainly responsible for the more serious side effects related to its corticosteroid-like activity (such as Cushing's syndrome) associated with ingestion of large amounts of liquorice. A study on the bioavailability of glycyrrhizin, when given on its own or as part of a liquorice-root extract, showed that absorption of glycyrrhizin is lower when taken in the form of an extract (G. Cantelli-Forti *et al.*, *Environ. Health Perspect*., 1994, **102** Suppl. 9: 65–68). Although the sample size was small, when a similar experiment was carried out in rats the results were in agreement. The authors suggest that differences are due to an unspecified interaction taking place during intestinal absorption.

Ayurveda also uses fixed combinations of herbs, and an important ingredient of many recipes, some of which date back to 6000 BC, is '*Trikatu*' (Sanskrit, meaning 'three acrids'). *Trikatu* is a mixture of black pepper, *Piper nigrum;* long pepper, *Piper longum*; and ginger, *Zingiber officinale* and a theory for its use has been proposed that involves enhancement of bioavailability, not only by *Trikatu*, but especially by the alkaloid piperine, which is found in many *Piper* species (R. K. Johri and U. Zutshi, *J. Ethnopharmacol*., 1992, **37**: 85–91). The effects on bioavailability probably result from the fact that piperine is a potent inhibitor of drug metabolism. Piperine also inhibits glucuronidation of epigallocatechin gallate in the small intestine, as well as slowing gastrointestinal transit, which would increase its availability and residence time in the intestine, allowing for greater absorption (J. D. Lambert *et al.*, *J. Nutr.*, 2004, **134**(8): 1948–1952). Another example is the co-administration of piperine and curcumin to humans and rats, which enhanced the bioavailability of curcumin by 2000% and 154%, respectively, due to inhibition of the glucuronidation of curcumin (G. Shoba *et al.*, *Planta Med.*, 1998, **64**(4): 353–356.)

EXAMPLES OF SYNERGY, POLYVALENT ACTION OR ANTAGONISM IN HERBAL MEDICINES

SYNERGISTIC EFFECTS DEMONSTRATED IN SINGLE PLANT EXTRACTS

Synergy between ginkgolides present in *Ginkgo biloba* in a platelet aggregation test

Most ginkgo preparations are standardized for their terpene lactone (ginkgolide) and flavonoid content, and although these groups of compounds have discrete modes of action, it is likely that they work together. Recently, synergy has been demonstrated between ginkgolides A and B using a platelet aggregation assay. This is shown in the graph (Fig. 7.3) of the results obtained from investigating synergy between ginkgolides A and B as antithrombotic agents (Table 7.1). This is clinically significant because it means that if the most effective ratio of

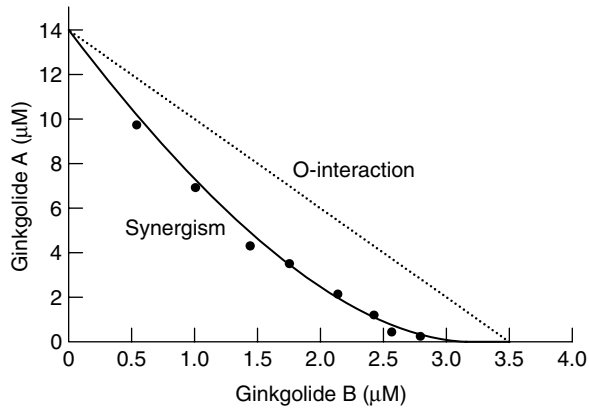


Fig 7.3
Synergy between ginkgolides A and B: the isobole drawn from values in Table 7.1. (From H. Wagner 2006 Multitarget therapy – the future of treatment for more than just functional dyspepsia. Phytomedicine 13: 122–129 with permission.)

Table 7.1 Synergy demonstrated by mixtures of ginkgolides A and B. IC_{50} values of various ginkgolide A + B mixtures, obtained by an in-vitro platelet-aggregation test.

Mixture ratio GA:GB	IC_{50} μg/ml	Concentration ginkgolide A		Concentration ginkgolide B	
		μg/ml	μM	μg/ml	μM
3:1	2.40	1.80	4.41	0.60	1.42
2:1	2.20	1.47	3.60	0.73	1.72
1:1	1.80	0.90	2.21	0.90	2.12
1:2	1.55	0.52	1.27	1.03	2.43
1:3	1.40	0.36	0.88	1.09	2.57
1:10	1.30	0.12	0.29	1.18	2.79

The IC_{50} is the concentration causing a 50% inhibition of the platelet aggregation induced by platelet-activating factor (PAF) (n = 2–9).

ginkgolides is selected, a lower total dose of extract is needed. Ginkgo is used mainly for vascular insufficiency, especially in the brain, where it can result in impairment of cognition, and numerous studies have confirmed its efficacy. The effect can be partly related to the different constituents. The ginkgolides are diterpenes and are known to be platelet-activating factor (PAF) antagonists. Numerous studies have shown that the ginkgolides antagonize many of the effects of PAF, including inflammation, bronchoconstriction, bronchial hyperresponsiveness, platelet aggregation and allergic responses. The ginkgolides might thus contribute to the efficacy of ginkgo in cerebral insufficiency but may also produce some benefit in inflammatory disorders, including asthma. Ginkgo flavones are also anti-inflammatory, the combination being considered additive and possibly synergistic in effect, as well as increasing blood circulation to the brain, and a total ginkgo extract acts as an antioxidant activity in brain preparations. Clinical studies have shown ginkgo to be effective in improving cognitive function as well as the early stages of dementia; the preparation used is a total extract not just the flavonoids. This suggests polyvalent as well as synergistic activity.

Potentiation of the effect of berberine by 5′-methoxyhydnocarpin in *Berberis* extract in prevention of bacterial resistance

Although this has been cited as a clear example of synergy between components of a single plant extract, it is more correctly termed potentiation. The phenomenon is demonstrated by a compound isolated from *Berberis fremontii* on the antimicrobial effects of berberine, another constituent, as shown in Fig. 7.4. Multidrug-resistance pumps (MDRs) protect bacteria from antimicrobials, and berberine is readily extruded by such MDRs. Several *Berberis* species were found also to synthesize an inhibitor of the norA (a membrane-associated efflux protein) MDR pump of a human pathogen *Staphylococcus aureus*. The inhibitor is

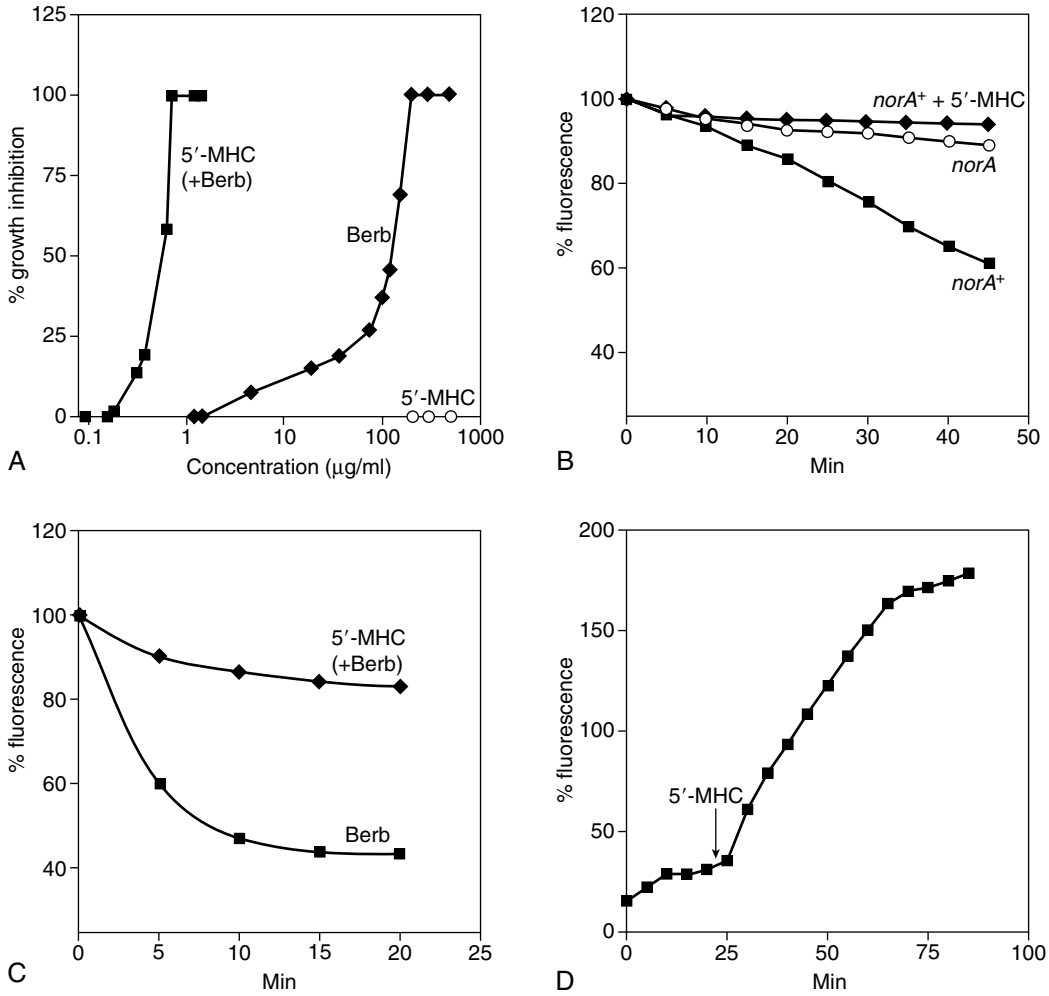


Fig. 7.4
Synergistic action of berberine and 5′-methoxyhydnocarpin (5′-MHC). A, Growth inhibition of *Staphylococcus aureus*. Berberine (Berb) was present at a concentration of 30 μg/ml when combined with 5′-MHC. Measurements were performed in triplicate, and the average values are shown. B, Inhibition of norA transport activity by 5′-MHC. *S. aureus* cells were loaded with ethidium bromide (EtdBr) and washed; the efflux was measured in the presence of 100 mM formate, a respiratory substrate. 5′-MHC was added at a final concentration of 10 μg/ml. C, Cells were loaded with berberine and efflux was measured in the presence of formate. D, Uptake of berberine added at time 0 by cells in the presence of formate. A small increase of fluorescence produced by 5′-MHC alone was subtracted from the plot. (From: F. R. Stermitz, P. Lorenz, J. N. Tawara, L. A. Zenewicz, K. Lewis 2000 Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5′-methoxyhydnocarpin, a multidrug pump inhibitor. Proceedings of the National Academy of Sciences of the USA 97(4): 1433–1437 (with permission). Copyright (2000) National Academy of Sciences, USA.)

5′-methoxyhydnocarpin (5′-MHC), originally found as a minor component of chaulmoogra oil. 5′-MHC had no antimicrobial activity alone but strongly potentiated the action of berberine and other norA substrates against *S. aureus*. MDR-dependent efflux of berberine and ethidium bromide (EtdBr; used for comparison because of its known mechanism of action and similarity in some properties to berberine) from *S. aureus* cells was completely inhibited by 5′-MHC. The level of accumulation of berberine in the cells was greatly increased in the presence of 5′-MHC, indicating that this compound effectively disabled the bacterial resistance mechanism. 5′-MHC has also been found to be present in *B. aquifolia* and *B. repens* suggesting that whole herbal extracts of these plants may have a superior antimicrobial effect to berberine alone where MDRs are involved (F. R. Stermitz *et al.*, *PNAS*, 2000, **97**(4): 1433–1437).

Enhancement of activity of Δ⁹–tetrahydrocannabinol in cannabis extract by other constituents

An example of the action of a known active compound being enhanced by the presence of other (inactive) compounds is shown by an experiment in which the antispastic effects of cannabis extract and isolated Δ⁹-tetrahydrocannabinol (Δ⁹-THC) were compared in an immunogenic model of multiple sclerosis. It can be seen from Fig. 7.5A that a cannabis extract (SCE) has a more rapid effect on relieving muscle spasticity than isolated Δ⁹-THC at matched concentrations. Fig. 7.5B shows that the extract from which the Δ⁹-THC has been removed (Δ⁹-THC-free SCE) has no effect on spasticity, confirming that THC alone is responsible for the effect. The extract had been passed through a high-performance liquid chromatography preparative column, so to ensure that this had no effect on the effect of the extract, it was recombined to give extract TSCE, which had similar properties to SCE in the biological model (J. D. Wilkinson *et al.*, *J. Pharm. Pharmacol.*, 2003, **55**(12): 1687–1694).

ANTAGONISTIC OR OPPOSING EFFECTS DEMONSTRATED IN SINGLE PLANT EXTRACTS

Opposing effects on blood glucose levels of flavonoids in *Pterospartum tridentatum*

The isoflavonoid isoquercitrin and the flavonol sissotrin, both isolated from *Pterospartum tridentatum,* have been shown to have opposing actions on oral glucose tolerance in rats. The overall effect of the aqueous extract of *P. tridentatum* on blood glucose levels of normal rats given an oral glucose challenge was complex, in that it produced an antihyperglycaemic effect during the first 30 minutes, but subsequently blood glucose levels rose above those of control group (Fig. 7.6). This suggested the presence of compounds with different actions on glucose tolerance. An oral glucose tolerance test performed using isolated isoquercitrin and sissotrin, found these compounds to have opposing effects. Isoquercitrin showed a time-dependent antihyperglycaemic activity, by delaying the post-oral glucose load glycaemic peak, in a similar manner to the sodium-dependent glucose transporter inhibitor phloridzin (a flavonoid glucoside found in apples). By contrast, sissotrin produced an opposite effect by impairing glucose tolerance. These results show that the effect

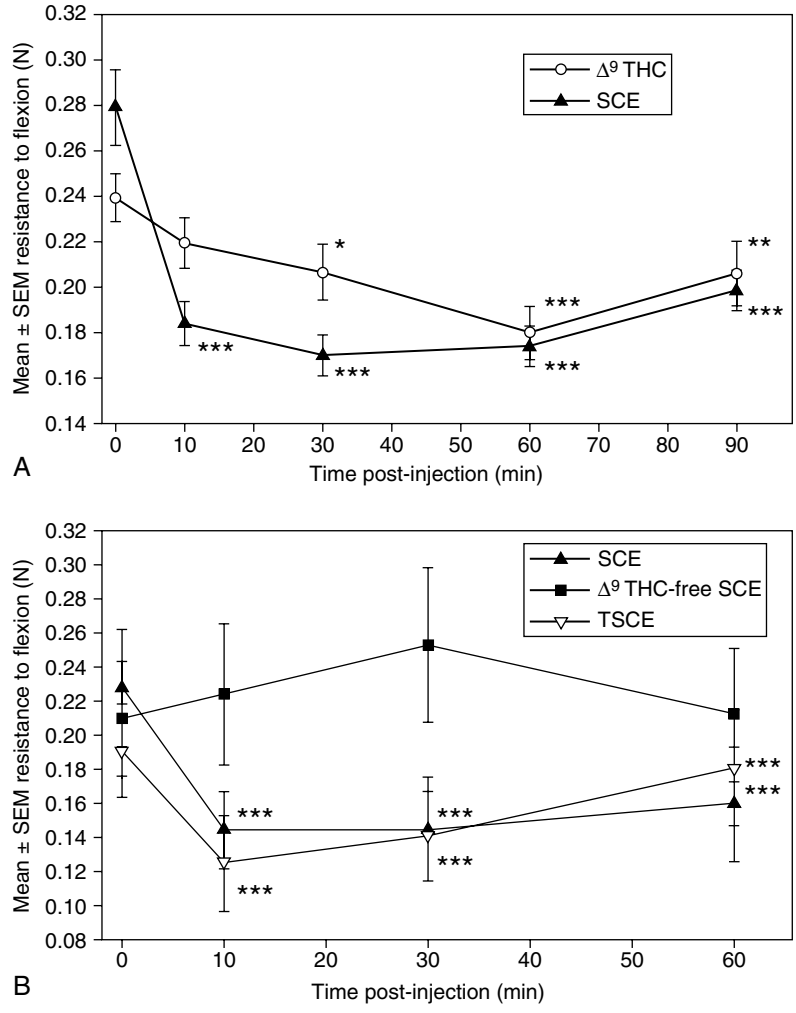


Fig 7.5
Effect of various cannabis extracts compared with isolated Δ⁹-tetrahydrocannabinol (Δ⁹-THC) on spasticity in an in-vivo model of multiple sclerosis. Following the induction of chronic relapsing experimental allergic encephalomyelitis, spasticity of the hind limbs developed. This was measured by the resistance to full flexion of the hind limbs against a strain gauge before and following intravenous administration of: A, 1 mg/kg Δ⁹-THC and, 1 week later, in the same group of animals (n = 8 mice) with 5 mg/kg SCE (a cannabis extract) containing 20% Δ⁹-THC in vehicle; or B, 5 mg/kg SCE, Δ⁹-THC-free SCE and TSCE in the same group of animals (n = 6 mice), separated by at least 48 h. The data points represent mean ± SEM of resistance force (Newtons, N) of 12 individual spastic hind limbs in each experiment. *P < 0.05, **P < 0.01, ***P < 0.001 are significantly different means compared with baseline control of individual experiments. (From J. D. Wilkinson, B. J. Whalley, D. Baker, G. Pryce, S. Gibbons, A. Constanti, E. M. Williamson 2003 Medicinal cannabis: is Δ9THC responsible for all its effects? Journal of Pharmacy and Pharmacology 55(12): 1687–1694, with permission.)

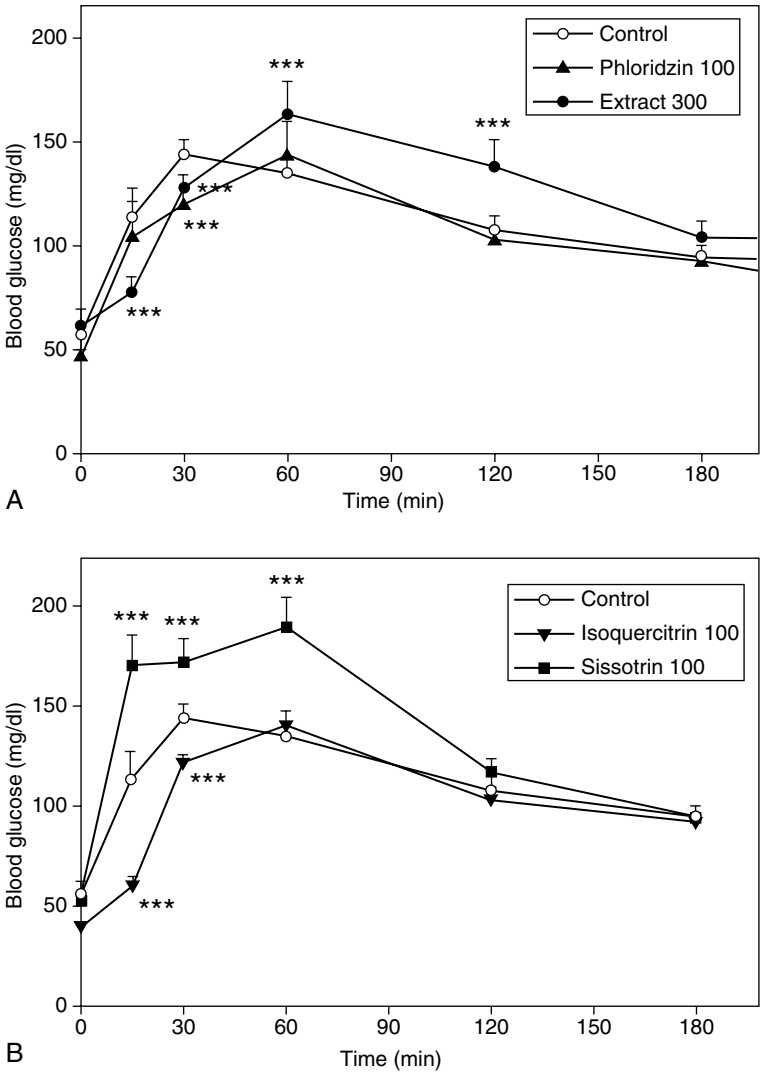


Fig 7.6
Opposing effects on blood glucose levels of flavonoids present in *Pterospartum tridentatum*.
Effect of (A) aqueous extract of *P. tridentatum* (300 mg/kg; n = 5) and phloridzin (100 mg/kg; n = 6); (B) isoquercitrin (100 mg/kg; n = 5) and sissotrin (100 mg/kg; n = 6) on oral glucose tolerance in normal Wistar rats compared with a control group (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P = 0.001$. (From: A. Paulo, S. Martins, P. Branco, T. Dias, C. Borges, A. Rodrigues *et al* 2008 The opposing effects of the flavonoids isoquercitrin and sissotrin, isolated from *Pterospartum tridentatum*, on oral glucose tolerance in rats. Phytotherapy Research, **22**(4), 539–543. © John Wiley & Sons Ltd. Reproduced with permission.)

of the extract on blood glucose may be either antihyperglycaemic or hyperglycaemic, and that it depends on the relative concentrations of isoquercitrin and sissotrin in the extract (A. Paulo *et al.*, *Phytother. Res.*, 2008, **22**(4), 539–543). This type of antagonism shows the importance of chemically characterizing an extract, because the relative flavonoid composition might vary among plant samples of the same species.

MULTIPLE PHARMACOLOGICAL EFFECTS DEMONSTRATED IN A SINGLE PLANT

Synergy and antagonism between compounds and fractions in Psyllium (ispaghula) husk

Psyllium husk, also known as ispaghula, is equally acceptable in traditional and modern medicine; it is also considered to be effective for use in both constipation and diarrhoea, which are two opposite disease states of the gut. The general perception is that its laxative effect is achieved mainly through its fibre content, which may be true, but what it makes more effective in chronic constipation than other fibre-containing remedies is not clear. However, evidence is now accumulating to suggest that it also contains constituents with gut-stimulatory properties, mediated partly through cholinergic activation, which is likely to supplement the laxative effect (A. H. Gilani *et al.*, *Phytother. Res.*, 1998, **12**(S1): S63–S65). Interestingly, it also contains gut-inhibitory constituents, which not only are likely to offset the side effects associated with cholinergic components, but also provide a scientific explanation for the traditional use of ispaghula in diarrhoea (A. H. Gilani *et al.*, *Naunyn-Schmied. Arch. Pharmacol.*, 1998, **358**(S1): 40–73). In addition to gut-stimulatory and gut-inhibitory constituents, ispaghula also contains antiamoebic constituents, explaining its traditional use in amoebic dysentery (V. Zaman *et al.*, *Phytother. Res.*, 2002, **16**: 78–79), and thus demonstrating multiple effects, some supporting and some opposing a particular activity, in one medicinal plant.

SYNERGISTIC EFFECTS SHOWN BETWEEN TWO DIFFERENT PLANT EXTRACTS

Effect of soya and tea extracts on prostate tumour growth and angiogenesis in mice

A combination of a soya phytochemical concentrate (SPC) with tea extracts synergistically inhibited tumour growth and reduced serum concentrations of testosterone and dihydrotestosterone in a mouse model of androgen-sensitive human prostate cancer (Fig. 7.7). The inhibition of tumour progression was also associated with reduced tumour-cell proliferation and angiogenesis. SPC and black tea alone significantly reduced final tumour weights and, although green tea did

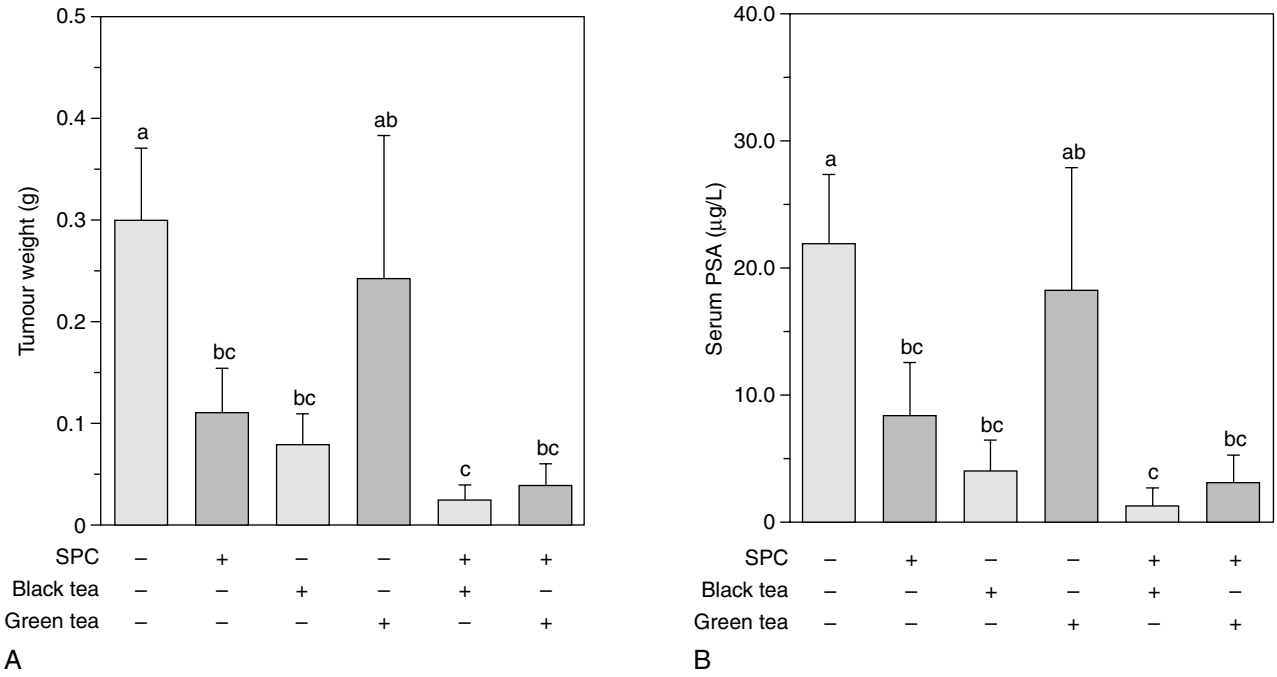


Fig. 7.7
Combined effects of soy phytochemicals and tea on final tumour weight and serum prostate-specific antigen (PSA) levels.
Effects of soy phytochemicals and tea combinations on final tumour weight (A) and serum prostate-specific antigen levels (B) in severe combined immune deficient (SCID) mice bearing LNCaP human prostate cancer cells. Compared with the control (Fig. 7.7A), all treatments other than green tea alone significantly reduced the final tumour weight. The combined effects of the soya phytochemical concentrate (SPC)/black tea combination (93%) and the SPC/green tea combination (88%) on final tumour weight reduction were greater than the expected additive effects (91% and 70%, respectively), suggesting that the combination of SPC with either black tea or green tea synergistically inhibited final tumour weight. In parallel, serum levels of PSA, a marker that is secreted by LNCaP cells and reflects tumour size, in mice in the experimental groups other than the green tea group were reduced (Fig. 7.7B), compared with the control. Comparisons of expected and observed values suggest that SPC combined with black tea or green tea synergistically reduced serum PSA concentration. Values are means ± SEM, n = 14–16. Means without a common letter differ, $P < 0.05$. (From: J.-R. Zhou, L. Yu, Y. Zhong, G. L. Blackburn 2003 Soy phytochemicals and tea bioactive components synergistically inhibit androgen-sensitive human prostate tumors in mice. Journal of Nutrition 133(2): 516–521, with permission.)

not reduce final tumour weight, it tended to elevate serum dihydrotestosterone concentrations. This study is significant because it supports the idea that the chemopreventive properties of the Asian diet might result from interactions between several components. In Asia, where the intake of soy products and tea consumption are very high, aggressive prostate cancer is significantly less prevalent than in other parts of the world.

NEW TECHNOLOGIES FOR LOOKING AT SYNERGY AND OTHER INTERACTIONS

One outcome of the recent development of informatics tools is the advancement of systems biology, which has the potential to revolutionize natural product research and scientific-based herbal medicine. The integration of data into systems biology can enable the understanding of living systems from a holistic perspective and facilitate the study of multitarget approaches. Evidence obtained from the new '-transcriptomic' technologies (genomics, proteomics and metabolomics) can hopefully support the identification of synergy and polyvalent pharmacological activities, as complex gene expression analysis by microarray can detect differences in cellular responses to drug combinations versus single agents (M. H. Cheok *et al.*, *Nat. Genet.*, 2003, **34**: 85–90). It could further demonstrate whether drug combinations can lead to the activation of entirely different genes to those activated by individual agents. Thus, the mode of action of a combination can be, based on the gene expression, entirely different from the mode of action of the single agents contained in it. Although it is questionable whether the discriminating genes for treatment are the same as those responsible for the main action of the single agent, the method is still suitable for the discrimination of different treatments (see Further reading for reviews on the use of metabolomics and systems biology in research in phytomedicines).

CONCLUSION

There can be no doubt that most herbs rely for their effects on a variety of constituents, and the idea of synergy within and between them is also gaining acceptance. Whether they are acting in a truly synergistic way or by additive effects is not well documented, but it is important for both developing methods of standardization as well as furthering our knowledge of mechanisms of drug action. Clinical evaluation is also more difficult without knowing the extent to which synergy occurs within the herbal preparation, and it should be further investigated for all these reasons. In the meantime, evidence is accumulating to show that synergism does occur in extracts and mixtures, and that there is benefit in using whole extracts. However, it is still vital to ensure that extracts are standardized for the active principles known at the time and that any known synergistic interactions are taken into account. If done properly, this should lead to improved products with increased efficacy at lower doses and correspondingly reduced toxicity. Synergistic

principles apply to all forms of drug treatment, not only those that are plant based, and although this is a well-known concept in phytotherapy, it is relatively new to other forms of conventional medicine.

Acknowledgement

The author wishes to thank Prof. Andreas Kortenkamp, University of London School of Pharmacy, for his expert advice on the different methods for the measurement of synergy.

Further reading

Synergy

Berenbaum MC 1989 What is synergy? Pharmacological Reviews 41: 93–141

Duke JA, Bogenschutz-Godwin MJ 1999 The synergy principle in plants, pathogens, insects, herbivores and humans. In: Kaufmann PB *et al* (eds) Natural products and plants. CRC Press, New York, pp. 183–205

Gilani AH, Atta-ur-Rahman 2005 Trends in ethnopharmacology. Journal of Ethnopharmacology 100: 43–49

Kortenkamp A, Altenburger R 1998 Synergisms with mixtures of xenoestrogens – a re-evaluation using the method of isoboles. Science of the Total Environment 221: 59–73

Wagner H 2001 Trends and challenges in phytomedicine: research in the new millennium. In: Yaniv Z, Bachrach U (eds) Handbook of medicinal plants. Haworth Medical Press, Binghamtown, NY, pp. 3–28

Williamson EM 2001 Synergy and other interactions in phytomedicines. Phytomedicine 8(5): 401–409

Metabolomics and systems biology

Ulrich-Merzenich G, Zeitler H, Jobs D, Panek D, Vetter H, Wagner H 2007 Application of the '-Omic-' technologies in phytomedicine. Phytomedicine 14: 70–82

Verpoorte R, Choi YH, Kim HK 2005 Ethnopharmacology and systems biology: a perfect holistic match. Journal of Ethnopharmacology 100: 53–56

Wang M, Lamers R-JAN, Korthout HAAJ, Nesselrooij JH, Witkamp RF, van der Heijden R *et al* 2005 Metabolomics in the context of systems biology: bridging traditional Chinese medicine and molecular pharmacology. Phytotherapy Research 19(3): 173–182

8

Traditional plant medicines as a source of new drugs

P. J. Houghton

DEFINITION 62

HISTORICAL DIMENSION 62

THE PROCESS OF MODERN DRUG DISCOVERY USING ETHNOPHARMACOLOGY 69

SOME MODERN EXAMPLES OF DRUG DISCOVERY BASED ON THE ETHNOPHARMACOLOGICAL APPROACH 71

THE VALUE OF THE ETHNOPHARMACOLOGICAL APPROACH 71

PROBLEMS WITH THE ETHNOPHARMACOLOGICAL APPROACH 73

CONCLUSION 74

DEFINITION

The scientific study of traditional plant medicines can be considered as a major part of ethnopharmacology, a term that was only introduced in 1967 but which describes an approach to the discovery of single biologically active molecules that has been used ever since the first compounds were isolated from plant material. Ethnopharmacology can be defined as the scientific study of materials used by ethnic and cultural groups as 'medicines'; in most instances, this is synonymous with the study of traditional medicines. These are usually the flowering plants, and so in most cases ethnopharmacology can be considered as a branch of ethnobotany: the study of the uses of plants by ethnic groups. However, it should be noted that some cultures, e.g. traditional Chinese medicine (TCM), also make use of animal and mineral matter, and so ethnopharmacology would also encompass the study of these.

Some discussion has taken place concerning the boundaries of what is meant by 'ethnic'. Some would include all ethnically based systems outside Western scientific medicine but the balance of opinion probably rests on a more restrictive definition, which includes only those bodies of knowledge that are restricted to a group that has lived in a locality for a long period of time but that does not have a sophisticated theoretical framework, formal education and a documented written history. This more rigid definition excludes the medical systems that have developed over thousands of years in cultures based in China and the Indian subcontinent, and emphasizes small ethnic groups where there is a threat of the rapid loss of knowledge due to globalization, loss of habitat migration and other factors that might lead to loss of cultural distinctiveness.

Some ethnographers would distinguish between folklore and ethnopharmacology, claiming that the former is common knowledge in the population as a whole, largely concerning remedies for minor conditions, based on relatively innocuous material. Ethnopharmacology is more concerned with the knowledge of a few specialists who are regarded by the society as able to correctly diagnose and treat disease states, generally using more potent products. In many situations, these specialists are also linked with the religious practices of the society.

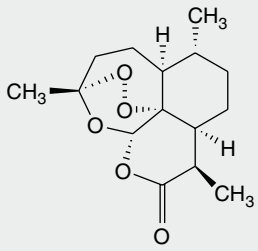
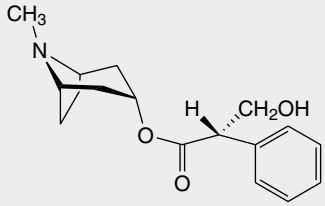
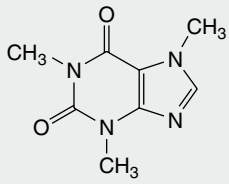
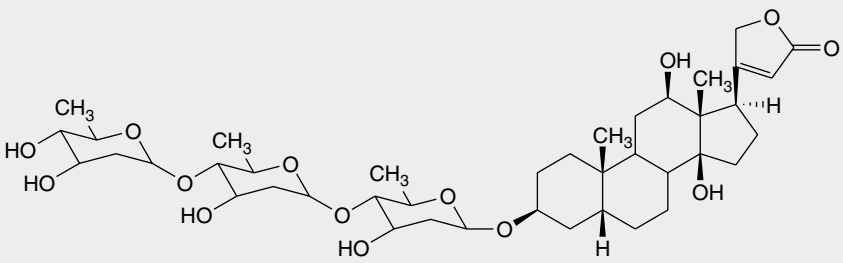
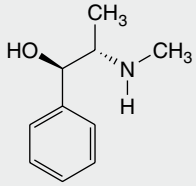
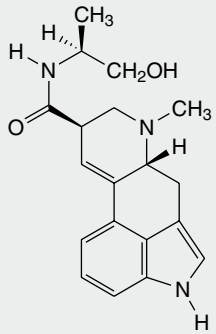
It should also be noted that the discovery of new drugs might derive from a wider use of plants than for strictly medical purposes alone. Thus materials used as poisons, in pest control, in agriculture, as cosmetics, in fermentation processes and for religious purposes might also yield active substances that can be exploited as leads for drug development. It can thus be seen that ethnopharmacology is a very interdisciplinary subject and any thorough investigation will probably need the input of a variety of specialists, such as anthropologists, botanists, chemists and pharmacologists.

HISTORICAL DIMENSION

The isolation of some of the opium alkaloids in the early nineteenth century was a key event in the development of modern pharmacy. It showed that isolated compounds had much the same activity as the existing ethnopharmacological material and so paved the way for current orthodox Western medicine, which uses pure compounds for treatment. Since then, a vast amount of money has been spent on the synthesis of novel compounds but also on the isolation of molecules from natural sources and their development into medicines. The contribution of traditional plant medicines to this process has been significant and some notable examples are shown in Table 8.1.

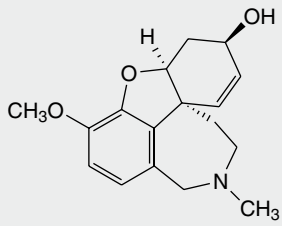
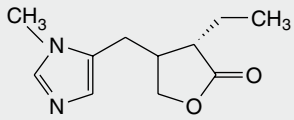
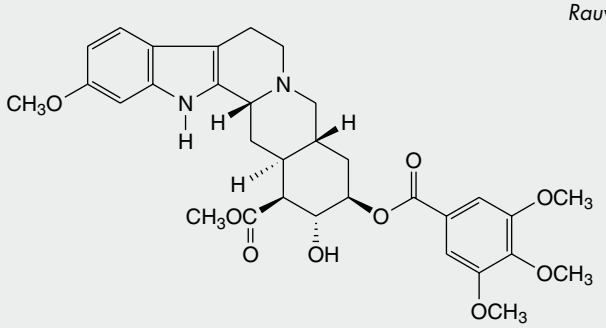
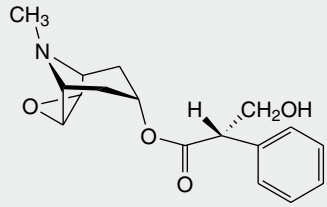
It should also be remembered that the active molecules isolated from traditional medicinal plants might not only provide valuable drugs but

Table 8.1 Some common drugs originating from traditional medicinal plants.

Pharmaceutical	Structure	Plant source	Traditional use
Artemisinin (antimalarial)		Sweet wormwood *Artemisia annua*	In China for treating fever
Atropine (cholinergic blocker – used for dilating pupils and as gastrointestinal sedative)		Deadly nightshade *Atropa belladonna* and other Solanaceous drugs	Hallucinogenic brews in Europe, pupil enlargement in eye
Caffeine (CNS stimulant, diuretic)		Coffee *Coffea arabica*, Tea *Thea sinensis*	Stimulant drink or paste in Ethiopia (coffee), drink in China
Digoxin (anticardiac arrhythmia)		Foxgloves *Digitalis* spp.	*D. purpurea* leaves used in England and Wales for oedema due to congestive heart failure
Ephedrine (bronchial relaxant)		*Ephedra sinica* and other spp.	In China for respiratory complaints.
Ergometrine (contraction of uterus – used in childbirth)		Ergot *Claviceps purpurea*	In central Europe to aid childbirth

(Continued)

Table 8.1 Some common drugs originating from traditional medicinal plants. (Cont'd)

Pharmaceutical	Structure	Plant source	Traditional use
Galantamine (cholinesterase inhibitor, used to treat symptoms of Alzheimer's disease)		Snowdrop *Galanthus* spp.	In Balkans for muscle weakness
Pilocarpine (used to dilate pupils)		Jaborandi leaves *Pilocarpus jaborandi*	In Brazil to induce sweating
Reserpine (hypotensive, tranquillizer)		*Rauvolfia serpentina*	Extensively in Ayurvedic medicine (India) for mental illness
Scopolamine (Hyoscine) (CNS and gastrointestinal sedative)		Mandrake *Mandragora officinalis* and as for atropine	Preoperative sedative and analgesic, hallucinogenic preparations in many parts of the world

are also valuable as 'lead molecules', which might be modified chemically or serve as a template for the design of synthetic molecules incorporating the pharmacophore responsible for the activity. Examples of drugs having this origin are shown in Table 8.2.

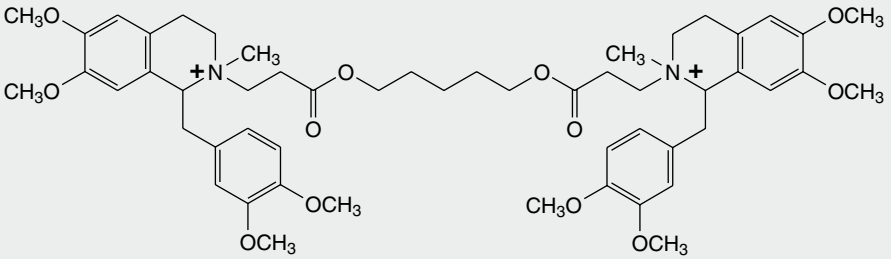
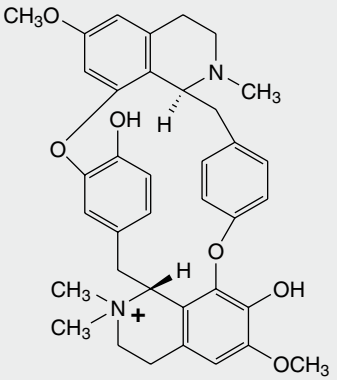
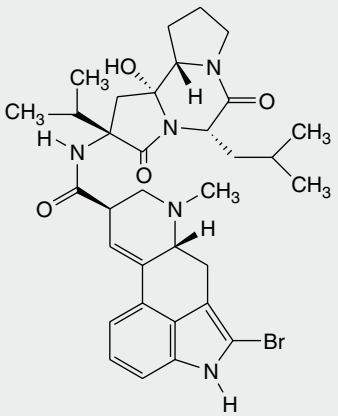
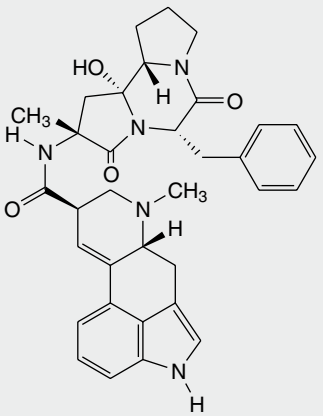
Although the term 'drug discovery' is generally used to refer to the isolation of molecules with activity, it should also be remembered that there is increasing interest and recognition that a 'drug treatment' might consist of a mixture of compounds. This has always been the case for plant extracts (and most other natural substances), which contain several 'active ingredients'. It should be noted that such extracts, usually based on a reputed traditional use somewhere in the world, are being introduced and increasingly used as a complementary therapeutic approach in the West. A selection of common ones, together with their ethnopharmacological roots, is shown in Table 8.3.

Scientific interest in ethnopharmacology has increased over the last few years and this is reflected in the formation of the International Society for Ethnopharmacology in 1990 and the European Society for Ethnopharmacology at about the same time. Both of these groups hold regular meetings. Several scientific journals also publish papers on this topic, notably the *Journal of Ethnopharmacology*, founded in 1979. The 100th volume of this journal, published in 2005, contains many useful 'state of the art' reviews on various aspects of ethnopharmacology.

This scientific interest is reflected by wider Western society, with its fascination with a much wider range of aspects of other cultures (e.g. dress, music, food, philosophy, as well as medicines) and this has been catalysed by large population migrations to the West and the relative ease of exposure to exotic cultures, which has been facilitated by large-scale international travel.

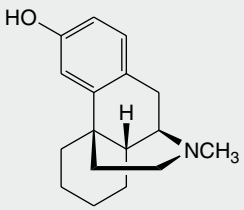
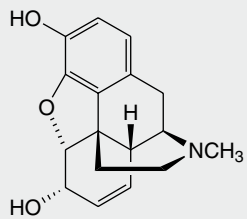
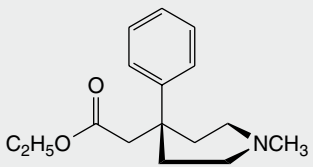
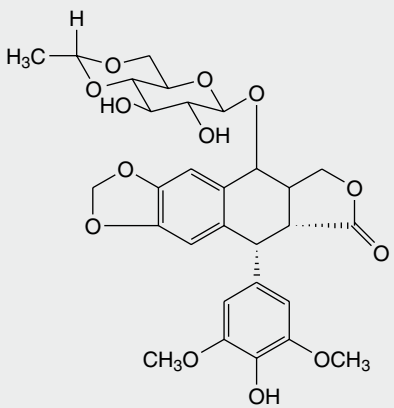
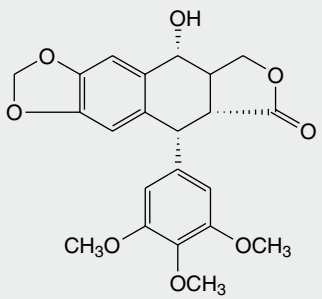
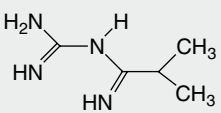
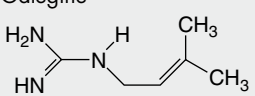
Although primarily concerned with human aspects, there has been a recent upsurge of interest in veterinary ethnopharmacology, i.e. methods and materials used to treat animals, particularly those important to the local economy as providers of food, transport and fibres. Other expansions from a strict definition of ethnopharmacology as being the study of medical practices include aspects of plants and other materials used in the diet, those used for ritualistic purposes, for poisons of various types, as cosmetics and as adjuncts to social gatherings. The increasingly blurred distinction between food and medicine, which has become a notable feature in 'Western' society, is a situation that has always been the case in other medical systems, such as Ayurveda and TCM, and it is now widely recognized that particular plants comprise part of the regular diet as much as for health maintenance as for

Table 8.2 Some important drugs developed from molecules found in traditional medicinal plants.

Drug	Structure	Template molecule, structure	Plant source and traditional use	Clinical use of drug
Atracurium (and other muscle relaxants)		Tubocurarine 	*Chondodendron tomentosum* Paralysing dart poison from Guyana, north Brazil	Muscle relaxant during anaesthesia
Bromocryptine (also cabergoline, methysergide)		Ergotamine 	*Claviceps purpurea* Used in central Europe to aid childbirth	Treatment of Parkinson's disease

(Continued)

Table 8.2 Some important drugs developed from molecules found in traditional medicinal plants. (Cont'd)

Drug	Structure	Template molecule, structure	Plant source and traditional use	Clinical use of drug
Dextromethorphan		Morphine 	Papaver somniferum Analgesic, soporific from Mediterranean region	Cough suppressant Analgesic
Pethidine				
Etoposide		Podophyllotoxin 	*Podophyllum peltatum* Used as purgative and wart treatment by native North Americans	Anticancer
Metformin		Galegine 	*Galega officinalis* Used to treat diabetes in Europe	Antidiabetic in type 2 diabetes

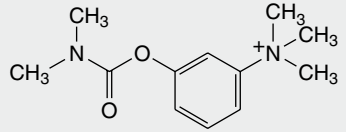
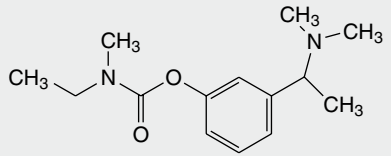
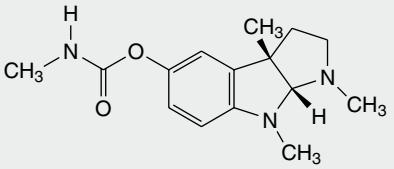
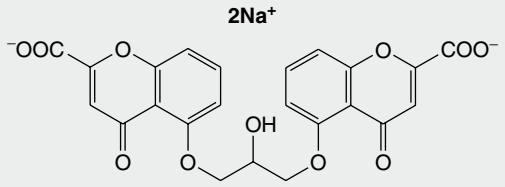
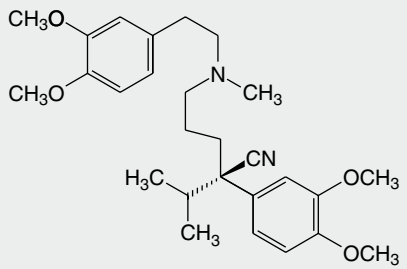
Neostigmine		Physostigmine	*Physostigma venenosum*	Treatment of myasthenia gravis
Rivastigmine			Ordeal poison from West Africa	Treatment of early symptoms of Alzheimer's disease
Sodium cromoglycate		Khellin	*Ammi visnaga* Treatment of bronchial complaints in Egypt	Antiasthmatic
Verapamil		Papaverine	*Papaver somniferum* Analgesic, soporific from Mediterranean region	Angina and anticardiac arrhythmia

Table 8.3 Ethnopharmacological origins (other then Europe) of some common herbal 'medicines'.

Herbal 'medicine' and botanical source	Current use	Geographical source	Traditional use (if different)
African prune bark *Pygeum africanum*	To reduce benign prostatic hyperplasia	Central African highlands	
Ashwagandha *Withania somniferum* roots	To enhance memory, general tonic	India	'Rasayana' general tonic
Black cohosh *Cimicifuga racemosa* roots	Depression associated with menstrual cycle and menopause	North America	Arthritis, neuralgia, menstruation disorders
Cat's claw *Uncaria tomentosa. U. guianensis* roots, stem bark, leaves	Rheumatism	Amazon area of South America	Antirheumatic and to treat infections and tumours
Dan shen, Chinese sage *Salvia miltiorrhiza* roots	Cardiovascular and cerebrovascular disease including ischaemic stroke	China	Cardiovascular diseases and cognitive decline
Devil's claw *Harpagophytum procumbens* fruit	Rheumatism	South-west Africa (Kalahari desert)	Purgative and for treating ulcers and boils
Dong quai *Angelica sinensis* root	Menopausal symptoms	China	Irregular menstruation, blood deficiency
Echinacea *Echinacea angustifolia, E. purpurea, E. pallida* roots and aerial parts	Common cold and other respiratory infections	North America	Anti-infective and to treat snakebite
Eleutherococcus, Siberian ginseng *Eleutherococcus senticosus* roots	Relief of fatigue, general health	Siberia	To help cope with stress
Ginkgo *Ginkgo biloba* leaves	To reduce CNS effects of ageing	China	For bronchitis
Ginseng *Panax ginseng* root	Relief of fatigue, general health	China, Korea	
Golden seal *Hydrastis canadensis* roots	Catarrh, appetite and digestion stimulant	North America	Gastrointestinal and skin disorders
Gotu Kola, hydrocotyle *Centella asiatica* herb	Wound healing and skin conditions	India	Rheumatism and skin conditions
Guarana *Paullinia cupana* seed kernels	Tonic	Northern Amazon forest	Stimulant drink
Hoodia *Hoodia gordonii* stems	Obesity	Southern Africa	Used to prevent hunger
Karela *Momordica charantia*	Antidiabetic	India and Southeast Asia	
Kava *Piper methysticum* roots	Anxiolytic and tranquillizer	Tonga, Fiji	Social drink to aid relaxation, treatment for skin conditions
Lapacho, Pau d'Arco *Tabebuia avelladanae* (and other *Tabebuia* spp.) inner bark	Stimulation of immune system to prevent infections	Tropical South America	General tonic
Maca *Lepidium meyenii* tuber hypocotyl	Erectile dysfunction, menopausal symptoms	High Andes of South America	Aphrodisiac
Saw palmetto *Serenoa repens* fruits	To reduce benign prostatic hyperplasia	South-east USA	

their macronutritional properties. Attention has also been focused on the ways in which the role of a substance can change through time or as it is transferred from one culture to another. Thus, coffee was thought of as primarily medicinal when it was first introduced into northwest Europe in the seventeenth century, but quite rapidly became a beverage. It is also of interest that cultural restraints might minimize abuse of a substance in its indigenous context but that, when these restraints are removed as the plant begins to be used in another part of the world or society, it becomes a problem to that society. An example of this situation is seen with the abuse of kava-kava in Australia by aboriginal peoples, who do not have the framework of ritualistic use of these roots in the Pacific islands of Fiji and Tonga, where it originates.

Several recent surveys have shown that using ethnopharmacology as a basis of selecting species for screening results in a significant increase in the 'hit rate' for the discovery of novel active compounds compared with random collection of samples. It should be noted that several 'classical' drugs stated to have derived from ethnopharmacological investigations, e.g. several shown in Table 8.1, arise from plants known as poisons rather than those with a more 'gentle' action, which comprise the bulk of many herbal medicine species. The latter group often relies on a mixture of compounds with a mixture of activities, where synergism and polyvalence might be occurring, and where the isolation of one 'active constituent' is much less likely.

With a very large number of living organisms still awaiting scientific investigation (about 90% of the estimated 250,000 species of flowering plants, probably the most studied part of the biosphere), ethnopharmacology appears to offer a reasonable selective strategy to be considered in deciding which organisms to study. An interesting overview of some ethnopharmacologically based molecules and the problems involved in their gaining regulatory status was published recently (T. W. Corson and C. M. Crews, *Cell*, 2007, **130**: 769–774).

THE PROCESS OF MODERN DRUG DISCOVERY USING ETHNOPHARMACOLOGY

The discovery process is composed of several stages. The first stage must be the reported use of a naturally occurring material for some purpose that can be related to a medical use. Consideration of the cultural practices associated with the material is important in deciding possible bases of the reputed activity. If there is an indication of a genuine effect, then the material needs to be identified and characterized according to scientific nomenclature. It can then be collected for experimental studies, usually comprising tests for relevant biological activity linked with isolation and determination of the structure of any chemicals present that might be responsible. The 'active' compounds are usually discovered by several cycles of fractionation of the extract linked with testing for the activity of each fraction, until pure compounds are isolated from the active fractions, a process known as bioassay-guided fractionation. These compounds, once their activity is proven and their molecular structure ascertained, serve as the leads for the development of clinically useful products. These various stages are discussed in detail below.

INFORMATION SOURCES

The most reliable type of information arises from in-depth studies carried out by field workers, living in the particular community of a particular ethnic group, on the use of the local plants and other materials. This usually comprises frequent communication with the local population, preferably in their own language. In should be noted, however, that an extensive knowledge of traditional medicines might reside with only a few people and a focus on this group would yield greater results. However, many such people are often reluctant to give away knowledge, which is regarded as 'protected' in some way, and this is exacerbated by concern that such knowledge could be exploited by drug companies, with little or no return to the original possessors of this knowledge.

Although the in-depth approach is most valuable, the fact is that most of the drugs that have been developed have arisen from less rigorous observations as a by-product of conquest or colonization. Thus, the more enlightened members of the Spanish *conquistadores* of Central and South America noted the practices of the various native American groups; and members of the British and French colonial administrations, together with non-governmental groups such as Christian missionaries, catalogued the uses of plants in Africa.

Most of these observations cannot now be checked in any way at first-hand, because the authors are long dead, but their records, books and other documents have been left as sources of information. This also applies to cultures that have left some type of written record, so that information on materials used in medicine in ancient Egypt, Babylonia, India and China is available. A recent paper (E. J. Buenz *et al*., *Trends in Pharmacological Sciences*, 2004 **25**: 494–498) describes some recent advances in electronic scanning of ancient texts that make information retrieval much easier, although the difficulties of linguistics and identifying the plants mentioned are not minimized as obstacles in such research.

Before such knowledge can be investigated scientifically, the information provided will often need clarification and translation into scientific terms. Of particular importance is the correct identification of the species used, which can be very difficult due to a lack of, or poor quality, illustrations as well as language difficulties. However, data on the part used, time of collection, methods of preparation, formulation and application are also necessary as they all affect the nature and amount of any biologically active compounds. Any restrictions on use due to time of year may be important, as they can indicate low levels (leading to inefficacy) or high levels (with concomitant risk of toxicity) of active compounds. Similarly, any types of individual excluded from treatment might indicate groups at risk due to age, gender or occupation.

Definition of the disease state in Western medical terms might also not be easy if the information is derived from a culture in which concepts of disease cause and symptoms are very different. In many records, the condition treated is described by a symptom that might be due to a number of disease states, e.g. a headache might be due to stress, tiredness, migraine attack or a brain tumour. Conversely, a particular disease state might be characterized by a number of symptoms, all of which have to be addressed when searching for possible leads to treating that illness. Thus, as an example, when searching an inventory of plants with a view to selecting those used for diabetes, those used for treating excess urination, weakness and ulcers should be considered, especially if diabetes is not recognized as a distinct illness diagnosed by sweet-tasting urine. Unfortunately, these factors are often ignored in current research, when a statement that 'plant species X is used to treat illness Y by people living in Z' is considered to provide adequate ethnopharmacological information. Such vague statements do not take into account all the possible sources of variation of biological activity that must be considered before any investigation proceeds.

SCIENTIFIC INVESTIGATION

Extraction

The extract used for testing should approximate as closely as possible that obtained by the traditional process used. Much research that has been published on the chemistry and activity of medicinal species is not very relevant to the traditional uses because it has concentrated on extracts made with non-polar solvents, such as ether or chloroform, whereas polar solvents are most commonly used. In many cases these will be simple extractions with hot water but a variety of other solvents can be used, as well as various additives or treatment of the material before use (see Table 8.4). In most instances, however, it is likely that fairly polar compounds will be extracted, although the solubility of less polar substances might be elevated considerably due to solubilizing compounds, e.g. saponins, also being present. Thus, an aqueous extract of the antimalarial plant, *Artemisia annua,* contains appreciable levels of the major antimalarial sesquiterpene artemisinin, which on its own has very low solubility in water.

Tests for activity

In most instances of modern drug discovery carried out by industrial and academic research groups, a particular bioassay, or series of in-vitro bioassays, designed on the basis of the biochemistry or molecular biology of the disease, is used to test extracts. In these situations, ethnopharmacology has little relevance to the tests used except that it provides a number of screening samples selected on the basis of their traditional use for the disease in question. However, this approach is valuable in selecting plants for further investigation from a list of those with a local reputation of treating serious diseases and this has been applied in screening programmes to detect antimalarial, antituberculosis and antidiabetic activity. These are not necessarily aimed at providing new lead compounds for 'conventional' drugs but have the goal of providing a scientific basis for the more effective use of extracts and mixtures. The Global Initiative for Traditional Systems (GIFTS)

Table 8.4 Effect of pretreatment and extraction processes on plant constituents.

Treatment	Effect	Equivalent scientific process	Example from traditional medicines
Before extraction			
Roasting	Destruction of unwanted components	Heating at regulated temperature	Removal of toxic proteins from seeds of *Cassia occidentalis* (West Africa)
Soaking in water	Hydrolysis of glycosides	Boiling	Removal of cyanogenic glycosides from cassava
Storage	Enzymatic activity leading to formation of desired compounds or removal of unwanted compounds	Incubation with purified enzymes under standardized conditions	Formation of vanilla flavour
			Removal of toxic anthrones from cascara
Extraction process			
Water + ashes	Alkaline medium favours extraction of phenols and acids, slows release of alkaloids	Use of ammonia or dilute alkali	Traditional 'quid' used for coca 'chewing'. Used in 'paan', shredded Betel nut (*Areca catechu* seeds) wrapped in leaf of *Piper betel*
Water + acidic fruit juice	Favours extraction of alkaloids	Use of dilute acids	
Local alcoholic beverages	Extraction of less polar compounds	Dilute alcohol extraction	Many traditional tinctures
Animal fats or plant oils	Lipophilic compounds	Petrol or chloroform extraction	*Hypericum perforatum* oil extract for treating burns

of Health is one example. It arose from academia and cooperates with the Tropical Disease Research programme of the World Health Organization (WHO) in finding new antimalarials in a collaborative network of research, government and community organizations named RITAM, the Research Initiative on Traditional Antimalarial Methods.

In spite of the common use of in-vitro bioassays, ethnopharmacological research can adopt a different approach when a particular biological effect of the traditional medicine or poison has been noted but the causes are not known. This is often the case when historical data are consulted and ailments are described in terms of their symptoms rather than underlying causes (see above). The biological effect might be essentially toxicological, e.g. use of poisoned arrows, and so it is important to seek to ascertain the basis of the toxic effect.

The best type of test to verify a reputed activity (and any toxicity) is a well-designed clinical trial, but this does not lend itself to bioassay-guided fractionation! It has been argued that long-term use of a material in traditional medicine is a good indicator of therapeutic efficacy but many are cautious about making such claims, preferring the suggestion that a long history of use is more an indicator of lack of obvious toxicity. In-vivo animal models of disease states are the next-best approach but expense and ethical considerations preclude this type of experiment in many countries, particularly for a fractionation process.

Most test systems for biological activity therefore utilize in-vitro systems using animal tissue, cultured cells, cloned receptors or enzyme systems. Many tests have been developed in recent years, and these offer the opportunity to carry out large numbers of tests using small amounts of material in a short time and are, therefore, well-suited to bioassay-guided fractionation. To be of most value, the range of tests chosen should be closely related to the possible underlying causes of the disease, e.g. tests for the efficacy of a preparation for an inflammatory disease such as arthritis should encompass key mechanisms associated with the formation of the various mediators involved, such as the lipoxygenases and cyclooxygenases involved in eicosanoid synthesis, histamine antagonists, secondary messenger systems such as the cytokine NFκB, as well as more general oxidation processes involving free radical damage. The advantages and disadvantages of in-vitro testing have been summarized in a paper presented at a recent meeting of the International Society for Ethnopharmacology (Houghton *et al*., *J. Ethnopharmacol*., 2007 **110**: 391–400).

It should be noted that biological testing for the traditional use might reveal a different, but nevertheless interesting, activity. Such was the case in the discovery of the anticancer compounds from *Catharanthus roseus*, a plant originally investigated because of its reputation in Jamaica as a treatment for diabetes. Deaths of animals treated with the extract were traced to dramatic reductions in leucocyte count, and it was from this that the application to treatment of leukaemic cancers was made.

Chemical examination

Chemical examination should be linked with tests for biological activity and it is probably only a happy accident of history that the many alkaloidal drugs were developed from traditional medicines, without the need for bioassay-guided fractionation, because the alkaloids were present in fairly high amounts and they were relatively easy to obtain in a purified state. For many other traditional medicines, where activity is not due to alkaloids, it has been much more difficult to separate the actives from all the other compounds. Chemotaxonomic considerations can often provide a reasoned guess to the nature of the active components and thus a short cut to their isolation. Thus, insecticidal or anti-inflammatory activity noted in a member of the Asteraceae could be ascribed to the sesquiterpene lactones that are present in many members of this family.

The presence of common classes of naturally occurring compounds can be screened by the use of appropriate chromogenic reagents after separation using thin-layer chromatography or by more sophisticated techniques, such as gas chromatography or liquid chromatography

linked with mass spectrometry. These techniques are also valuable in dereplication, the process by which known active compounds present in the extract are detected, and so time is not wasted in a long bioassay-guided process that culminates in the 'discovery' of a well-known compound.

SOME MODERN EXAMPLES OF DRUG DISCOVERY BASED ON THE ETHNOPHARMACOLOGICAL APPROACH

SINGLE COMPOUNDS

Although the list of existing drugs introduced as a result of ethnopharmacological leads is impressive, some newer interesting examples of compounds arising from this approach have appeared in recent years, including galantamine and artemisinin (see Table 8.1). Others, which have attracted considerable research interest, are described below.

Prostratin

An ethnobotanical survey of Samoa carried out by the American Paul Cox in the late 1980s revealed the traditional use of a hot-water infusion of the stem wood of *Homalanthus nutans* for the viral disease yellow fever. Work carried out in conjunction with the National Cancer Institute in USA resulted in the isolation of a phorbol named prostratin, which was found to be effective against the killing of human host cells by HIV. Prostratin appeared to stimulate protein kinase C, which was a novel mode of action for an anti-HIV drug. Although extensive trials were commenced, prostratin has since been dropped from the anti-HIV drug development programme due to some toxic effects.

Flavopiridol

The bark of the Indian tree *Dysoxylum malabaricum* (Meliaceae) was traditionally used to treat arthritis and investigations into the related *D. binectariferum* provided rohitukine, a chromone alkaloid that exhibited anti-inflammatory activity in laboratory animals. Studies into the mechanism of action found that it was different from many other natural anti-inflammatory compounds in that it inhibited tyrosine kinase. A series of analogous compounds was developed to optimize the effect and the flavonoid derivative flavopiridol was shown to have superior activity. As numerous oncogenes encode for the production of protein with such kinase activity, inhibition might decrease tumour growth and this was found to be the case with flavopiridol, which is now in advanced stages of clinical testing as an anticancer agent.

Huperzine A

A tea from *Huperzia serrata,* a club moss, was a traditional drink for elderly people in several areas of China. Over the last twenty years, huperzine A, an alkaloid with cholinesterase inhibitory properties, has been isolated and shown to have significant beneficial effects on memory in patients. As memory appears to be impaired in patients with low levels of acetylcholine (ACh) in the brain, inhibition of the enzyme that degrades ACh will have the net effect of raising ACh levels and thereby improving memory. Huperzine A is in clinical use in China for treatment of elderly patients showing loss of memory and clinical trials in other countries are being planned.

PURIFIED AND STANDARDIZED EXTRACTS

There has been a renaissance of interest in using mixtures of compounds rather than single chemical entities in orthodox therapy (see Schmidt *et al*., *Nature Chemical Biology*, 2007 **3**: 360–366); this has always been the approach in herbal systems of medicine. The acceptance of such products is still in its infancy as regards Western regulatory procedures but is at an advanced stage in some of the emerging economies such as India and China. Even in Western countries such as USA and UK, funds are being released by government agencies, non-profit-making funding organizations and industry for clinical trials of characterized and standardized extracts, practically all of them having an ethnopharmacological basis and many are from Asia. Some of the interesting products being investigated are listed in Table 8.5 but it should be noted that, in some cases, the compounds thought to be most active are also being investigated for use as single chemical drugs or as lead compounds for development of the same.

THE VALUE OF THE ETHNOPHARMACOLOGICAL APPROACH

EFFICACY AND SAFETY

The argument most often used to support the ethnopharmacological approach to drug discovery is the fact that a plant material has been used for generations in a particular culture. Lack of technological sophistication is not synonymous with a lack of appreciation of efficacy or safety and so it is likely that there would be no serious adverse effects associated with the regular use of the material. In many cases it has been shown that potential harm is minimized through a selected method of preparation of the material, by its administration being restricted to trained personnel (most commonly the 'medicine men (or women)' of the community), or by its being used in special ways, particularly by the addition of other materials that might decrease the toxicity by countering unwanted pharmacological effects or by altering the bioavailability or metabolism of the material. The addition of alkaline ash to coca leaves in the traditional method of 'chewing' them is a good example of this. The high pH favours the less water-soluble form of cocaine, so affecting its release into the saliva and uptake into the bloodstream, and possibly reducing the addictive potential.

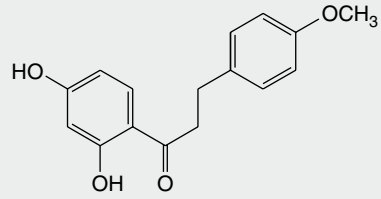
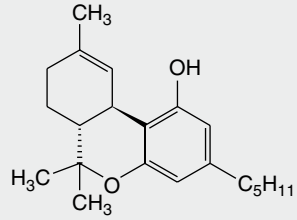
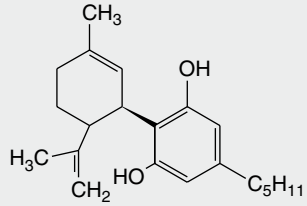
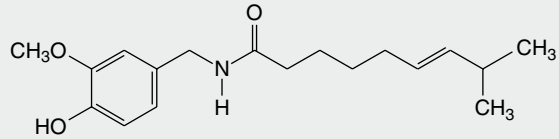
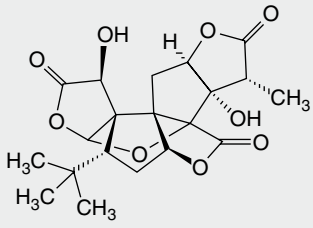
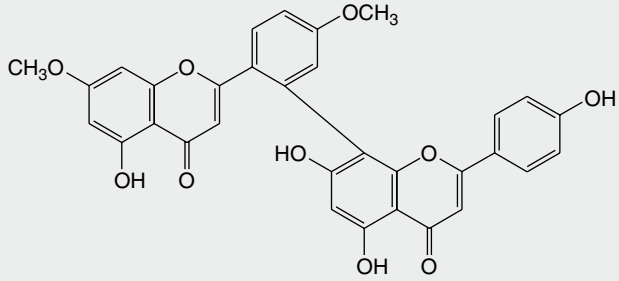
ECONOMIC AND SOCIOPOLITICAL CONSIDERATIONS

Although not closely related to the process of 'drug discovery' by the industrially developed countries, the scientific validation of a local remedy from a developing country can encourage its use and introduction into therapy in its original habitat, or its growth and adoption as therapy in areas with similar growing conditions. Cultivation and production of extracts from such plants might be a substitute for more expensive Western drugs and medicines, especially in countries where healthcare resources are stretched.

A notable example of this is Plantas do Nordeste, a collaboration between the Royal Botanic Gardens Kew, UK, and a consortium of scientists and agriculturists in the impoverished region of north-eastern Brazil. The project has evaluated some of the local medicinal plants and promoted the cultivation of them across the region as an aspect of healthcare, together with the training of personnel in their use.

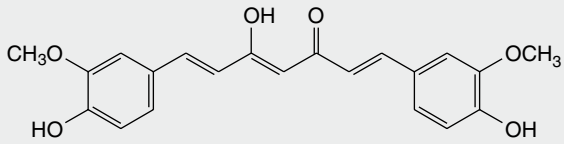
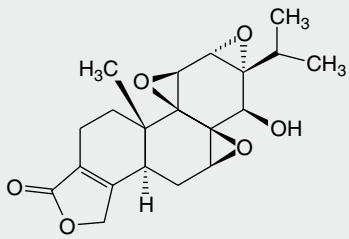
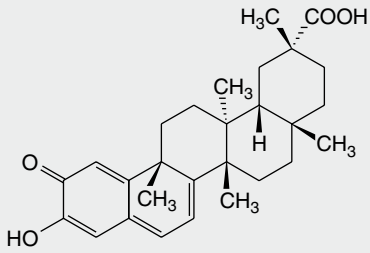
The possible validation of their traditional remedies by the scientific ethnopharmacological approach is also valuable in helping small ethnic groups retain or recover their sense of identity and value, especially when threatened by globalization and cultural imperialism by more politically or commercially powerful groups.

Table 8.5 Standardized herbal extracts under clinical investigation.

Botanical source (commercial name of extract)	Ethnopharmacological link	Clinical effect under investigation	Major active ingredients
Artemisia dracunculus Russian tarragon	Used as antidiabetic plant in Russia	Insulin resistance as a cause of diabetes	2,4-Dihydroxy-4-methoxydihydrochalcone 
Cannabis sativa Indian hemp (Sativex®)	Used as an anaesthetic and analgesic in central Asia for many centuries	As analgesic and antispasmodic in multiple sclerosis	Tetrahydrocannabinol  Cannabidiol 
Capsicum annuum Chilli pepper	Used by Incas and Aztecs in America for coughs and bronchitis	As analgesic in arthritis and neuralgia	Capsaicin 
Coix lachryma-jobi Iijen, Job's tears (Kanglaite)	Used in TCM for various purposes	As adjuvant in anticancer chemotherapy	Not specified, may be fatty-acid derivatives
Ginkgo biloba Ginkgo	Used in TCM for asthma and bronchitis	As cognitive enhancer in old age and Alzheimer's disease	Ginkgolides, e.g.  Biflavonoids, e.g. 

(*Continued*)

Table 8.5 Standardized herbal extracts under clinical investigation. (Cont'd)

Botanical source (commercial name of extract)	Ethnopharmacological link	Clinical effect under investigation	Major active ingredients
Curcuma longa Haldi, Turmeric	Used in India as an anti-inflammatory in skin and gastrointestinal tract	Psoriasis, wound healing, anticancer, Alzheimer's disease	Curcumin 
Tripterygium wilfordii Lei gong teng, Thunder God Vine	Used in TCM for inflammatory diseases	Rheumatoid arthritis	Triptolide  Celastrol 

TCM, traditional Chinese medicine.

PROBLEMS WITH THE ETHNOPHARMACOLOGICAL APPROACH

RELIABILITY OF INFORMATION

Some of the difficulties of obtaining accurate and reliable information have been discussed above. It should also be noted that the use of a plant might not be due to rational observation or experimental evidence, but instead be based on a subjective consideration. Probably the most notorious example of an irrational approach is the 'doctrine of signatures' whereby some sensory aspect of the plant was thought to indicate its medicinal usefulness, e.g. a plant with yellow juice might be considered for treating jaundice, one with heart-shaped leaves for cardiac complaints, etc. Although, in some cases, relevant activity has been found for plants identified in this way, these are usually the exceptions rather than the rule.

BIOLOGICAL VARIATION

On a scientific level, the chief concerns are the difficulty of knowing the chemical composition of a plant or its extract due to its complexity and also due to the variation caused by genetic or environmental factors. This complicates elucidation of the active compounds and also makes the activity of different batches less reliable. Indigenous knowledge often has subtle ways of distinguishing 'best' plant species or varieties and only thorough investigation will uncover the criteria used.

LOSS OF SPECIES

It is estimated that 25% of the flowering plant species of the world might become extinct within the first 25 years of the twenty-first century. Climate change, increasing population making demands on land and resources, as well as commercial exploitation of the environment all play a part in this and result in a loss of habitats. It is not unusual to record the presence of an interesting species in one locality only to return a few months later to find that the whole area has been cleared for some commercial activity. Although many ethnic groups in all parts of the world are affected, and most have a rich fund of knowledge about the medicinal uses of local organisms, it is probably those small groups in the centres of biodiversity, especially tropical rainforests, who are most vulnerable to irretrievable loss of species. Although the imminent extinction or increasing rarity of species are often promoted as a loss of potential new drugs for the developed world, it should be remembered that the local inhabitants could lose many of their basic affordable or available medicines.

In light of this, many medicinally important species have been subjected to some form of cultivation and, in several places, gardens of medicinal plants, formerly collected from the wild, are now being cultivated to provide local healers with species that they formerly collected from the wild.

LOSS OF KNOWLEDGE

The current opportunity to exploit ethnopharmacology might not last long. Whereas much concern has been quite rightly expressed over the

disappearance of the world's biodiversity and habitats, less attention has been paid to the threat to the immense wealth of knowledge about the medicinal uses of the local flora that exists amongst threatened ethnic groups. The irretrievable loss of large amounts of this knowledge is possible, due not only to the extinction of plant species due to climate change, urban expansion and destruction of habitat, but also to the breakdown in traditional societal structures of the transmission of such knowledge. The threat is especially acute in cultures in which information is oral rather than written, and is exacerbated by urban drift, particularly of young people, neglect of local materials, adoption of Western globalized products and health structures, and by war, famine and other causes of migration and consequent disruption of traditional society.

THE NEED FOR DEREPLICATION

Extracts from plants can show an interesting activity and, conventionally, this is followed by laborious, prolonged, bioassay-guided fractionation to isolate the compounds responsible. However, such considerable effort might result in the isolation of known compounds, or the activity might be found to be due to substances, such as polyphenols, which have a general inhibitory activity on many enzymes. This problem might be overcome by the use of dereplication procedures, whereby suspected known active compound types are removed by fractionation and purification procedures. Alternatively, active extracts and fractions can be examined by techniques, such as chromatography linked to mass spectrometry, which can afford substantial amounts of information about each component and data from the peaks obtained can be screened against a data library of known compounds.

INTELLECTUAL PROPERTY RIGHTS (IPR) ISSUES

The commercial aspects of the ethnopharmacological approach have aroused much controversy in recent years with regard to the intellectual property rights of the groups having the knowledge. Several international agreements, particularly the Rio Declaration and Convention of Biological Diversity (CBD) of 1992, have concentrated on sharing with the source countries the benefits and profits that might arise from the development of new drugs based on ethnopharmacological leads. The effect that such measures might have on their patent rights and returns from investment have been considered closely by pharmaceutical companies and some have decided not to take the risks involved. Some other companies, however, have been willing to sign agreements aimed at sharing profits directly or making substantial payments to countries in exchange for access to their flora for testing purposes.

CONCLUSION

The study of plants used in traditional medicine has received new impetus with the introduction of a wide variety of small-scale bioassay methods and improved methods for fractionation, isolation and characterization of compounds. In some cases, individual compounds responsible for the reputed activity have been isolated and used as lead molecules. However, it is also apparent that, in many cases, the observed effects seen in patients or animals are due to a mixture of constituents, perhaps each contributing a different biological effect to the overall activity. Thus it is likely that not only will traditional medicines continue to provide new molecules for drug discovery, but that they might also form the basis for the wider acceptance of crude extracts, in a standardized form, as another type of medicine in orthodox practice.

Further reading

Balick MJ, Cox PA 1997 Plants, people and culture: the science of ethnobotany. Freeman, London

Chadwick DJ, Marsh J (eds) 1994 Ethnobotany and the search for new drugs. Ciba Foundation Symposium 185, Wiley, Chichester, UK

Heinrich M, Barnes J, Gibbons S, Williamson EM 2004 Traditional systems of herbal medicine. In: Fundamentals of pharmacognosy and phytotherapy. Churchill Livingstone, Edinburgh, pp. 169–183

Journal of Ethnopharmacology 2005 Perspectives of ethnopharmacology. 100: issues 1–2

Lewis WH 2003 Ethnobotany in new drug discovery – a review. Economic Botany 57: 126–134

Phillipson JD, Anderson LA 1989 Ethnopharmacology and Western medicine. Journal of Ethnopharmacology 25: 61

Pieroni A, Price LL 2006 Eating and healing: traditional food as medicine. Haworth Medical Press, Binghamtown, NY

Prendergast HDV, Etkin NL, Harris Houghton PJ 1998 Plants for food and medicine. Royal Botanic Gardens, Kew, UK

Proceedings of the First International Congress of Ethnopharmacology, Strasbourg, 1990. Journal of Ethnopharmacology 1992 32: 1–235

Proceedings of the Second International Congress of Ethnopharmacology, Uppsala, 1992. Journal of Ethnopharmacology 1993 38: 89–225

9

Discovering new lead compounds in pharmaceutical research and development

*M. J. O'Neill and J. A. Lewis**

BIOLOGICAL ASSAYS AND HIGH-THROUGHPUT SCREENING *75*

SAMPLE AVAILABILITY FOR HIGH-THROUGHPUT SCREENING *76*

SELECTING SAMPLES FOR SCREENING *76*

PROCESS FOR IDENTIFICATION OF PLANTS FOR TARGETED SETS *78*

SAMPLE PREPARATION *78*

DE-REPLICATION AND ISOLATION OF ACTIVE COMPONENTS *79*

AN EXAMPLE OF THE SUCCESS OF HIGH-THROUGHPUT SCREENING OF PLANTS FOR NEW LEAD COMPOUNDS *79*

*Disclaimer: the information in this chapter represents the authors' personal views. In no way does it represent the views of GlaxoSmithKline.

Traditional remedies invariably involve crude plant extracts containing multiple chemical constituents, which vary in potency from highly active (e.g. *Digitalis* leaf) to very weak (e.g. cinnamon bark). By contrast, orthodox medicine relies heavily on single (or a very small number of) chemically well-characterized active ingredients exhibiting selective activities at, in many cases, well-characterized biological targets. These medicines are generally very potent and many exhibit fairly narrow windows between an effective and a toxic dose. Orthodox medicines are formulated into doses that are carefully standardized for bioavailability.

Compounds derived from higher plants continue to feature among the most widely used orthodox medicines we have today (*Martindale*, see Further reading). These include analgesic agents (e.g. morphine, codeine and the non-steroidal anti-inflammatory drugs based originally on the structure of salicin), antimalarial treatments (e.g. quinine), antitumour drugs (e.g. vincristine and taxol) and asthma therapies (e.g. cromoglycate). Other plant-derived compounds are currently being evaluated in pharmaceutical development, an example of which is artemisinin, an extract of the sweet wormwood plant (*Artemisia annua*), which is being assessed in combination with chlorproguanil and dapsone as a new antimalarial treatment.

In some cases, natural materials continue to be the only viable commercial source of the active compound. For example, GlaxoSmithKline harvests up to 10 000 metric tons dry weight of poppy capsule per year to provide a source of opiate alkaloids.

'High-throughput screening' (HTS) is a major strategy for the discovery of new lead chemicals in the pharmaceutical industry. HTS uses miniaturized assay formats, usually microtitre plates in which, for example, 384 or 1536 different samples can be assayed, in volumes of less than 50 and 5 µl, respectively in one run. Using sophisticated automation equipment, typically, hundreds of thousands of samples are screened against each biological target of interest every day: the final numbers for each usually being dictated by the overall cost of the assay, which can vary from < 1 p per well to > 20 p per well. Screening collection sizes range from 400 000 to over 4 million.

HTS is often portrayed, by people who know little about it, as an activity requiring very little intellectual input. The reality is that HTS is a complex process that demands an understanding of the role of specific biological targets in disease progression; the development of bioassays capable of discovering modulators of the target; the design, miniaturization and automation of bioassays (which are automation friendly); an understanding of the macro- and micro-structure of the biological target so that the sample selection strategy is optimized; the engineering of custom-built robots capable of storage, retrieval and bioassay of millions of samples per annum and the development of software systems that can enable scientists involved to make sense of the mass of data that emerges.

BIOLOGICAL ASSAYS AND HIGH-THROUGHPUT SCREENING

Ideal biological assays for screening are those that enable identification of compounds acting on specific biological targets, involve a minimum number of reagent addition steps, perform reliably and predictably, are easily amenable to miniaturization and automation, and involve low-cost ingredients and detection technology. Biochemical targets of interest in pharmaceutical lead discovery range from enzymes to receptors (nuclear and transmembrane) to ion channels and, in the case of infectious disease, to whole microorganism cells.

An example of a biological assay that has the characteristics needed in a good screen is the squalene synthase enzyme assay, which was

developed to look for inhibitors of squalene synthesis, a potential target for the identification of novel cholesterol lowering agents (Tait, 1992). Using either [1-^{14}C] isopentenyl diphosphate as a precursor for squalene or [2-^{14}C] farnesyl diphosphate as a direct substrate of squalene synthase, the production of radiolabelled squalene is determined after adsorption of assay mixtures onto silica gel thin-layer chromatography sheets and selective elution of the diphosphate precursors into a solution of sodium dodecyl sulfate at alkaline pH. The use of [2-^{14}C] farnesyl diphosphate, and of an endogenous oxygen consumption system (ascorbate/ascorbate oxidase) to prevent further metabolism of squalene, allows the method to be applied as a dedicated assay for squalene synthase activity. The assay can be readily operated in microtitre plate format, which allows 96 or 384 samples to be screened per plate. It can be deployed either in a quantitative, low-throughput mode or in a qualitative, high-throughput mode, which has proved to be resistant to interference by compounds other than selective inhibitors.

The endogenous neuropeptide bradykinin (BK) is implicated in the mediation of various types of pain in the mammalian CNS. Antagonism of bradykinin to its receptors is a potential target for the development of new analgesic agents. An assay has been devised to detect compounds that antagonize binding of radiolabelled bradykinin to BKII receptors expressed in Chinese hamster ovary (CHO) cells (Sampson *et al.*, 2000). Compounds under test are added to the wells of microtitre plates to which CHO cells have adhered. After incubation with radiolabelled bradykinin, the excess labelled ligand is removed by washing. The plates are then counted in a scintillation counter so as to assess binding of labelled bradykinin to the receptors expressed on the surfaces of the cells. This particular screen suffers interference from compounds that possess cytotoxicity through a variety of mechanisms. It is therefore essential to run follow-up control assays against other cell types to distinguish false positives.

In the infectious disease arena, it is still common to run high-throughput, whole-cell antifungal or antibacterial assays to detect samples that inhibit growth of the designated strain, e.g. *Candida albicans*, *Staphylococcus aureus*. Optical density or colour changes using a redox indicator are the most frequently used assay technologies. Assays in this therapeutic area may be mechanism based. For example, a *C. albicans* cell-free translation system using polyurethane as a synthetic template, has been established to search for compounds that inhibit fungal protein synthesis (Kinsman *et al.*, 1998).

Screening plant extracts for antitumour activity involves assays against a wide variety of cancer cell lines and mechanism-based *in vitro* targets, which have been documented extensively (Pezzuto, 1997). Among the most frequently used mechanism-based assays are those assessing activity against the biological targets of existing antitumour drugs, such as topoisomerases I and II, collagenase, tubulin binding and stabilization, endocrine hormone synthesis and androgen and oestrogen receptor binding. Mechanism-based assays often require sophisticated or expensive reagents: an assay for activity of DNA ligase I involves incubating plant extracts with recombinant human DNA ligase I cDNA and its radiolabelled substrate, and measuring uptake 5′-^{32}P labelled phosphomonoesters into alkaline-phosphatase-resistant diesters (Tan *et al.*, 1996).

SAMPLE AVAILABILITY FOR HIGH-THROUGHPUT SCREENING

During the 1980s and early 1990s, natural product samples were the mainstay of HTS programmes within the pharmaceutical industry, due at least in part to the lack of availability of large numbers of synthetically derived chemicals. Over recent years, this situation has changed dramatically. The highly competitive arena of drug discovery provides pharmaceutical companies with a clear incentive to be first to discover and patent new lead molecules. Thus, a range of technologies has evolved to facilitate ever-increasing numbers of samples to be rapidly generated and evaluated.

Most large pharmaceutical houses have built up a compound bank containing hundreds of thousands of chemical compounds, which reflect the chemistries of earlier medicines developed by the company. Chemical diversity in these collections can be supplemented by acquisition of new compound types from the growing ranks of specialist compound vendors. Computational modelling techniques are utilized to generate sets of specific interest for given biological targets. Methods are available for electronically filtering out 'undesirable' compounds and techniques such as pharmacophore analysis, two- or three-dimensional structure searching or chemical clustering can be used to derive sets of the required size. Ready access to these compound collections is facilitated by the use of robotic storage and retrieval facilities, which can present the samples in formats appropriate for HTS bioassays.

Combinatorial chemistry techniques are widely applied in the drug-discovery process, especially for generating large numbers of compounds for lead discovery and in the optimization of lead compounds. Using robotic systems, tens of thousands of compounds can be synthesized from a small number of reagents in a few days. To date, however, it appears that the most successful of these compound libraries, in terms of yielding interesting bioactive molecules, have utilized focused chemistry based on structural knowledge of the biological target and the pharmacophoric features required to affect it.

To supplement the chemical diversity of the compound banks and the chemically focused combinatorial libraries, a number of pharmaceutical companies continue to screen natural extracts. Historically, large collections of microbial organisms (notably fungi and filamentous bacteria) were built up from a diversity of environmental niches and emphasis was placed on the development of a range of fermentation conditions capable of eliciting the microbes to produce a variety of secondary metabolites. The extracts generated for HTS from this source can be reproduced on demand, should further studies on bioactivity of interest be required. In particular, industry found microbial fermentations to be a prolific source of antibiotics. More recently, from the same source, valuable immunosuppressant drugs and lipid-lowering agents have been added to the medicine chest.

Plant samples also feature in the HTS programmes of a small number of pharmaceutical discovery organizations. The feasibility of using plants in a drug-discovery programme depends on ensuring that effective procurement strategies are in place to source both the primary material and additional supplies should these be required.

SELECTING SAMPLES FOR SCREENING

Advances in screening technology have increased the throughput capacity of an average HTS from tens of thousands of samples to hundreds of thousands of samples over the last decade. Even so, the availability of so many samples for HTS means that choices might need to be made about the most appropriate sub-set of samples for each particular target. The sample selection strategy may then be 'diversity-based', i.e. samples are chosen to represent as wide a spectrum of chemical diversity as possible, or 'focused', i.e. the samples represent specific chemical types only.

Both strategies are likely to play a role in a pharmaceutical company's methodology. Diversity screening may yield an unexpected interaction between a compound and a biological target, although the question of what constitutes 'representative' chemical diversity

in the vast area of potential chemical space remains unanswered. Focused screening requires a large amount of prior information about a target, and this might not always be available. A combination of both approaches may be adopted. Computational methodologies for hit identification are continuously being developed. For example, compound databases enabling three-dimensional chemical structure searching are often used. If there are known ligands for a target, these can be used to construct a pharmacophore, which can then be utilized to search further chemical databases and select molecules with desired features. Chemical clustering can be used to derive sets of the required size. In the case of combinatorial chemistry-derived libraries, targeted sets can be generated with desired chemical properties, by using appropriately selected chemical building blocks. Natural products offer a potentially infinite source of chemical diversity unmatched by synthetic or combinatorially derived compound collections (Strohl, 2000), thus making them a desirable tool for diversity based screening. If a focused strategy is adopted, however, it is necessary to develop different techniques for natural product sample selection in order that the most appropriate samples are accessed for relevant targets.

The United Nations Convention on Biological Diversity (CBD) of 1992 has, to date, 191 parties, including 168 signatories (see its website http://www.cbd.int and the references therein). The key objectives of the Convention are to ensure the conservation of biological diversity, the sustainable use of natural resources and to implement fair and equitable sharing of benefits. Within the framework of the Convention are the concepts of the sovereignty of states over genetic resources and their obligation to facilitate access. The contracting parties are expected to establish measures for benefit sharing in the event of commercial utilization. This involves collaboration between the collector, the source country and the commercial partner. It is now normal practice to draw up a legal agreement to cover these issues and many companies have issued policy statements relating to this area.

As an example, a statement on GlaxoSmithKline's website (http://www.gsk.com) describes how a pharmaceutical company addresses such issues. The policy recognizes the importance of matters considered at Rio and subsequent meetings of the Congress of the Parties and goes on to state that GlaxoSmithKline will collaborate only with organizations that can demonstrate both the expertise and the authority to supply natural materials. Only relatively small quantities of plant material are collected, from sustainable sources. GlaxoSmithKline supports the CBD's role in providing a framework for the conservation of biological diversity and the sustainable use of its components and the CBD objective 'to provide fair and equitable sharing of the benefits arising from the use of genetic resources'. GlaxoSmithKline further supports the approach laid down in the CBD and in the Bonn Guidelines of leaving it to national governments to determine the conditions under which access to genetic resources should be given and for the parties concerned mutually to agree on the benefits to be shared. Agreements will cover such matters as the permitted use of the resources and the nature and timing of any benefits that are to be shared. This approach allows national governments the flexibility to determine what rules will best serve their national interests and allows the stakeholders involved to reach agreement appropriate to each particular case.

STRATEGIES FOR THE SELECTION OF PLANT MATERIAL FOR HIGH-THROUGHPUT SCREENING

Before a decision is made on what natural materials will be evaluated in a given screen, it is essential to gather some information on whether a target is indeed appropriate for input of natural product extracts. For example, if the biological target is very highly tractable, if there are significant time constraints and cost of goods issues, and if data suggest it is likely to be relatively straightforward to obtain synthetically derived, small-molecule lead compounds then it might be inappropriate to screen natural products against that target. However, if a target is of a class where it is difficult to find small molecule hits, e.g. involving protein–protein interactions, or if there is a strong precedent or rationale for natural-product-derived actives, then natural product input should be considered. The latter may be exemplified by, for example, the antimicrobial area, where the track record of drug discovery from microbial sources is beyond dispute. The same rationale would apply to the superb track record of plant species in yielding analgesic medicines.

If the target appears to be suitable for natural product input, the sample selection for the screen needs to be considered. Various strategies can be adopted, depending on the extent of the available natural materials collections and on the capacity and 'robustness' of the target itself.

Some companies have access to large and diverse natural materials collections. Such collections are likely to include samples acquired to add diversity to the potential collection (and inherent in the desire for taxonomic diversity is the assumption that this will be reflected in chemical diversity of extracts subsequently generated). Most collections will also include samples particularly selected for various reasons, e.g. a microbial producer of a given compound or a plant used ethnomedically for a given condition. These large collections probably still only reflect a fraction of the world's potential biodiversity. It has been estimated that only around 70 000 fungal species are known, out of an estimated 1 500 000 (see the UN CBD website http://www.cbd.int and the references therein). Further, it has been estimated that only about 1% of microbial biodiversity actually comprises 'culturable' organisms (Amman *et al*., 1995). Thus, a huge number of strains may not be amenable to conventional isolation and cultivation methodologies, and many groups are now working on applying cloning techniques to harness the potential chemical diversity of these organisms.

A diversity-based approach requires acquisition of pre-selected taxonomic groups. The strategy may utilize the assumption that taxonomic diversity will inherently be reflected in the chemical diversity of the extracts subsequently prepared and screened. Various techniques can be employed to analyse the taxonomic spread of a plant collection and then make efforts to fill gaps so that the collection more completely reflects available diversity.

A more focused approach depends on having prior knowledge about selected samples, which might suggest that they contain particular chemical classes of interest or that they possess desirable biological properties. This strategy can be considered under two headings 'chemical targeting' and 'biological targeting'.

Chemical targeting

This utilizes natural materials as sources of specific compounds of interest to a particular disease area, or as sources of chemical classes deemed to have suitable pharmacophores. In this way, chemical types that are under-represented in an existing sample collection can be identified. Plant-derived chemicals can provide an effective means of filling any 'gaps', thereby enhancing the overall diversity of available chemistry. It may not always be necessary to expend resource taking this process to full isolation and structure elucidation. In some cases, a set of plants can be selected on the basis that they are reported to produce a general chemical class of interest and appropriate crude or semi-purified extracts can be prepared in order to enrich the extract with the desired chemical types.

Biological targeting. This adopts what may be thought of as a disease-driven process. Plant samples can be selected for biological evaluation using some type of information associated with them that suggests their relevance for evaluation in a given therapeutic target. Perhaps the most striking observations available are ethnobotanical reports of traditional medicinal uses of plants. A number of orthodox medicines available commercially today were discovered by following leads provided from indigenous knowledge (Cox, 1994).

PROCESS FOR IDENTIFICATION OF PLANTS FOR TARGETED SETS

Various approaches are adopted in assimilating the information needed to select plants of particular relevance for a given disease target. Some research groups rely on developing a network of ethnobotanists, who work closely with indigenous colleagues and traditional doctors in various countries. The outcome of this approach is a low number of plant samples identified for evaluation in the laboratory and a great deal of information on their use. Some pharmaceutical companies prefer only to use information that is already in the public domain. Various journals and books hold a significant amount of information relating to the ethnobotanic uses of various plants; for example, there are many publications describing the properties of plants used in Chinese Traditional Medicine (e.g. Chang and But, 1986). Perhaps the most time-effective way of searching for information relating to reported biological or chemical properties of plants is to use electronic data stored in a range of databases. One key example of this is the NAPRALERT (Natural Products Alert) database (Loub *et al*., 1985). This system was initiated and is maintained at the University of Illinois at Chicago. It contains a wealth of information in the form of a huge number of references relating to reports of biological activity in the scientific literature, ethnobotanical reports and phytochemical data.

For chemical information, databases such as the Chapman and Hall *Dictionary of Natural Products* (2000) can be useful tools. This database contains information on well over 100 000 natural products, often including the species from which the compound originates. Searches can be carried out on the basis of chemical structure, sub-structure, structural similarity, presence of particular functional groups, etc. in order to build a set of organisms reported to produce these compounds. Alternatively, the database can be searched on the basis of species or genus, so as to build a list of compounds reported to derive from particular organism groups. This can be particularly useful in the process of de-replication and compound identification (see later). In addition, searches can be carried out on a range of other fields, such as molecular weight, which are also useful in compound identification following identification of a molecular ion by mass spectrometry analysis, reported uses of the natural product, literature references, log P, etc. The database comprises not just plant metabolites but compounds from all natural sources, and although the data are not complete, it can represent a valuable starting point by which to build chemically focused sets of samples for screening.

In the case of preparing samples that might be expected to contain specific chemical entities, phytochemical procedures reported in the literature can be used to generate semi-purified extracts, or extracts enriched in the compound or chemical class of interest. In the case of plants with an ethnomedical use, it might be possible to prepare extracts using methodology as recommended for use in traditional medicine.

These 'targeted' approaches are likely to involve smaller numbers of plant samples than a high throughput, random screening programme. However, the actual numbers of plants selected and screened can be tailored by the scientist, by making the selection criteria more or less stringent. For example, a selection process may result in a small number of plants reported to produce specific compounds of interest, but if the assay is capable of screening much larger numbers of samples, the set can be extended to include all those plants reported to produce metabolites of the much broader chemical class. Similarly, a set can be extended taxonomically to an optimum size by including plants of related species or genera, on the basis that they might also produce related chemistry. Making the selection criteria slightly more 'fuzzy' in this manner also allows a greater role for the element of luck—always important in the drug discovery process!

SAMPLE PREPARATION

Preparation methods are tailored towards the type of natural material being processed and the strategy for analysis being undertaken. For plant samples, the standard approach is to acquire and store dried plant material. In rare cases, for example if an ethnomedical report dictates use of fresh material, then this may be undertaken, but would not be the norm. Although a very small amount of plant material—less than half a gram—may provide sufficient extract to allow testing in many hundreds of bioassays, it is only sensible to collect a larger amount of material. For natural product samples to remain competitive sources of lead compounds, it is necessary to be able to very rapidly follow-up any active extracts. For this reason, collection of a few hundred grams of dry material is more typical. Plant material is finely ground using techniques ranging from pestle and mortar to industrial grinding apparatus, as appropriate.

Microbial strain collections are maintained either as freeze-dried cultures or are preserved by low-temperature storage. Required strains are generally revived by inoculation onto agar and/or growth media, and then are cultivated on a medium—or, more often, a range of media—that have been developed with the aim of promoting secondary metabolite production. Factors like incubation times and temperature, media composition and agitation rates can all have a significant effect on growth and metabolite synthesis. The next step is to generate an extract for analysis in a range of bioactivity screens. Often, there will be no prior knowledge of which chemical types may be present in the samples, and which will be active in any given bioassay. Techniques will therefore be aimed at solubilizing as wide a range of compounds as possible, typically using an alcoholic solvent, such as methanol or ethanol, or an aqueous alcoholic solvent. Extraction may take the form of a cold infusion or it may involve hot-solvent extraction using a Soxhlet apparatus (Silva *et al*., 1998). If the natural source material is under investigation because it has been reported to contain a desired compound or chemical class, then a bespoke extraction procedure will be adopted, probably using a literature report. If the target molecule is less specific, for example if the aim is to access any alkaloid molecules that might be present, then alkaloid-enriched extracts can be generated by following a method such as acid–base partitioning.

An alternative approach might favour the development of fractionation methods, so that several fractions originating from a single sample are generated prior to biological testing. Although more labour intensive, this can have the benefit of eliminating many of the problems that can be seen when testing crude extracts in some bioassays, e.g. frequent actives caused by commonly occurring interfering compounds such as tannins, saponins, flavonoids, etc. Such actives can take a significant time to de-replicate amd eliminate and, in some circumstances, it may be desirable to reduce this resource by spending time before biological testing to do some semi-purification of extracts. Techniques used can include solvent partitioning or chromatographic fractionation.

DE-REPLICATION AND ISOLATION OF ACTIVE COMPONENTS

Biological analysis is likely to yield a number of active extracts, as defined by showing a certain level of activity in a given screen. A process then needs to begin that will either lead to a full identification of a fully characterized, active compound or to a partial identification of activity to the level of a family of known compounds.

Before commencing full bioassay-guided fractionation of the active samples it is necessary to review the tolerance of a given assay to crude or semi-purified extracts of natural materials. The aim of evaluating such samples in a biological assay is to identify compounds that interact with a particular biological target, e.g. an enzyme or receptor. However, in practice, most assays utilize a measurable system, such as colour, light or radioactivity, enabling a high throughput of samples. This leads to the possibility of detection of non-specific interactions, which are particularly problematic when investigating multicomponent, uncharacterized extracts as opposed to single chemical entities. Examples of natural products that generate such effects are detergent-like compounds, which disrupt cell membranes, polyphenolics, which form complexes with a wide array of proteins, antioxidants and ultraviolet (UV) quenchers. It is vital to be able to detect, and eliminate, such actives as rapidly as possible (VanMiddlesworth and Cannell, 1998). This process can be speeded up by testing a standard set of known interfering compounds and extracts in an assay prior to the full screen. This provides data to indicate the tolerance of the assay to such samples, and can at times lead to a decision not to proceed with testing of crude extracts against that target. The physicochemical properties of a compound can give useful clues as to its identity. The most commonly used properties include high-performance liquid chromatography (HPLC) retention time and UV spectra data that are readily acquired through standard analytical techniques. By comparing these data with those of known compounds, it may be possible to characterize the components of a mixture without the need for full isolation—or at least, it may be possible to narrow the possibilities. If a library of such data from a relatively large number of natural products is used, this can be very effective in identifying those compounds that are present in more than one organism. If full isolation of the active component is warranted, various methods may be adopted. There is no single, best isolation technique, nor is there any single, correct method for any given compound. Most separation methods involve some form of chromatography—typically preparative HPLC. An isolation method would normally involve solvent partitioning, followed by a crude chromatography step such as a silica column or counter-current chromatography with a relatively small number of fractions based on polarity, followed by final purification through a high-resolution separation step such as HPLC. At each stage of the purification, the active compound is tracked by bioassay of the fractions. The only sure way to identify the structure of a bioactive metabolite is to demonstrate activity using the isolated compound and then to determine its structure by nuclear magnetic resonance (NMR) and mass spectrometry (MS). Until a compound is isolated, it is also impossible to determine its concentration and, hence, its potency in a given assay. It is also prudent to check that the concentration of the metabolite in the extract and the activity in the unpurified extract tally, so that a minor but active component does not go unaccounted for. Secondary testing will then be undertaken on the active compound to determine the mechanism and the selectivity of action and eventually, to evaluate *in vivo* activity. The chemical structure of the compound will also be evaluated to give some indication of the classical 'drug-like' qualities of the molecule. These are to some extent subjective but include consideration of whether the compound is of sufficiently low molecular weight to allow ready chemical modification, and not prevent drug uptake; of whether the compound is likely to be stable with respect to oral uptake, whether it has sites that may be suitable for modification, whether it is likely to have a suitable log P, and so on. In fact, some classes of natural product are not suitable as drug candidates by these criteria, as they are too big and complex or possess unsuitable redox properties. If the molecule is deemed to be of interest, related metabolites from the sample, or related species, may be accessed for structure activity determination. Even if the structure of the metabolite is not novel, this does not preclude it becoming a lead molecule, particularly if the mode of action is novel.

AN EXAMPLE OF THE SUCCESS OF HIGH-THROUGHPUT SCREENING OF PLANTS FOR NEW LEAD COMPOUNDS

The discovery of a series of novel and highly potent euphane triterpenes illustrates the potential of plant extracts to generate useful chemical leads in a high-throughput screening programme.

During a 'random' screening programme to search for novel inhibitors of human thrombin, which were capable of blocking the formation of blood clots and hence could be of value in treating and preventing deep vein thrombosis, some 150 000 samples, including synthetic compounds and bacterial, fungal and plant extracts, were evaluated. Methanolic extracts of *Lantana camara* (Verbenaceae) leaves, obtained from a UK garden, were found to display potent activity.

Large-scale extraction and bioassay-guided chromatographic fractionation led to the identification of a series of novel compounds, which were characterized by NMR and MS as 5,5-*trans*-fused cyclic lactone-containing euphane triterpenes (O'Neill *et al*., 1998). The compounds showed IC_{so} values of the order of 50 nm against thrombin.

After the initial activity was detected, literature searches on *Lantana camara* revealed this plant species to be reported to be toxic to grazing animals, which, on ingestion of the leaves, develop hepatotoxicity and photosensitization (Sharma and Sharma, 1989). These toxic effects have been attributed to the lantadenes, a series of pentacyclic triterpenes. A further study of haematological changes in sheep following *Lantana* poisoning demonstrated a significant increase in blood coagulation time and prothrombin time, with an associated decrease in blood sedimentation rate, total plasma protein and fibrinogen (Uppal and Paul, 1982). This observation might be associated with the thrombin inhibitory translactone-containing euphane triterpenes described above.

The biological activity of these compounds has been reported in detail (Weir *et al*., 1998). Their mechanism of action as inhibitors of blood clotting is via acylation of the active site Ser 195 residue of thrombin. This acylating activity has been found to be generic against other serine protease enzymes and this finding forms the basis for exploitation in drug discovery.

Further reading

Amman RL, Ludwig W, Schleifer KH 1995 Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiological Reviews 59: 143–169

Chang H-M, But P-H 1986 Pharmacology and applications of Chinese materia medica. World Scientific Publishing Co, Singapore

Cox P 1994 The ethnobotanical approach to drug discovery: strengths and limitations. In: Ethnobotany and the search for new drugs. Wiley, Chichester (Ciba Foundation Symposium 185), pp 25–41

Dictionary of natural products, 5th edn, 2000 Chapman and Hall/CRC Press

Kinsman OS, Chalk PA, Jackson HC *et al* 1998 Isolation and characterisation of an antifungal antibiotic (GR 135402) with protein synthesis inhibition. Journal of Antibiotics 51 (I): 41–49

Loub WD, Farnsworth NR, Soejarto DD, Quinn ML 1985 NAPRALERT: Computer handling of natural product research data. Journal of Chemical Information and Computing Sciences 25: 99–103

Martindale, the complete drug reference. Internet version: http//www.csi.micromedex.com
O'Neill MJ, Lewis JA, Noble HM *et al* 1998 Isolation of translactone containing triterpenes with thrombin inhibitory activity from the leaves of *Lantana camara.* Journal of Natural Products 61(11): 1328–1331
Pezzuto JM 1997 Plant derived anticancer agents. Biochemical Pharmacology 53(2): 121–133 *and references therein*
Sampson JH, Phillipson JD, Bowery N *et al* 2000 Ethnomedically selected plants as sources of potential analgesic compounds: indications of in vitro biological activity in receptor binding assays. Phytotherapy Research 14: 24–29
Sharma OMP, Sharma PD 1989 Natural products of the Lantana plant—the present and prospects. Journal of Scientific Industrial Research 48: 471–478
Silva GL, Lee I-S, Kinghorn AD 1998 Special problems with the extraction of plants. In: Cannell RJP (ed) Natural products isolation. Humana Press, Totowa, NJ, pp 343–363
Strohl WR 2000 The role of natural products in a modern drug discovery program. Drug Discovery Today 5(2): 39–41
Tait RM 1992 Development of a radiometric spot-wash assay for squalene synthase. Analytical Biochemistry 203(2): 310–316
Tan GT, Lee S, Lee I-S *et al* 1996 Natural product inhibitors of human DNA ligase I. Biochemical Journal 314: 993–1000
Uppal RP, Paul BS 1982 Haematological changes in experimental Lantana poisoning in sheep. Indian Veterinary Journal 18–24
VanMiddlesworth FW, Cannell RJP 1998 Dereplication and partial identification of natural products. In: Cannell RJP (ed) Natural products isolation. Humana Press, Totowa, NJ, pp 343–363
Weir MP, Bethell SS, Cleasby A 1998 Novel natural product 5,5-*trans*-lactone inhibitors of human a-thrombin: mechanism of action and structural studies. Biochemistry 37: 6645–6657

PART 3

Principles related to the commercial production, quality and standardization of natural products

10. COMMERCE IN CRUDE DRUGS 83

11. PRODUCTION OF CRUDE DRUGS 87

12. PLANT GROWTH REGULATORS 93

13. PLANT CELL AND TISSUE CULTURE; BIOCHEMICAL CONVERSIONS; CLONAL PROPAGATION 98

14. PHYTOCHEMICAL VARIATION WITHIN A SPECIES 106

15. DETERIORATION OF STORED DRUGS 117

16. QUALITY CONTROL 121

This page intentionally left blank

10

Commerce in crude drugs

R. Baker

HISTORICAL DEVELOPMENTS 83
CURRENT ASPECTS 84
CHANGING DEMANDS 86
THE FUTURE 86

Like almost all other basic commodities, the trade in crude drugs is of great antiquity. The necessity for goods to be collected, graded, transported and distributed effectively has rarely been considered by the pharmacist as part of his or her remit, and thus it has been left to the trader or merchant to perform this less scientific, but by the same token important, group of tasks. The essentials of the trade are still very much as they were 10, 100 or 1000 years ago, although the speed and efficiency with which they can be performed has improved exponentially with time. It is well within quite young living memory that one had to book a telephone call to one's supplier in Brazil, China or India some hours or perhaps a day in advance: now communications by fax or more frequently now by e-mail take moments to perform, and replies come with similar alacrity. The advent of 'VOIP' (Voice Over Internet Protocol) and the various messaging services, which are essentially free of charge, has improved at least the availability of means of communication.

HISTORICAL DEVELOPMENTS

The absolute origins of the trade in crude drugs are lost in the mists of time. One supposes the first contract for the collection and supply of a drug with a third party, whether in exchange for specie or not, came about when physicians or pharmacists found themselves too busy to do this relatively menial task themselves. The trust that physicians had in the collectors must have been remarkable, as unadulterated drug was essential, if only to avoid poisoning the patient! It is probable that the first commercial dealers in botanical drugs were apprentices or freed slaves, who preferred to take on that role rather than becoming pharmacists themselves: we shall never know for certain. References from antiquity to the drug trade are rare, although some mural inscriptions from Ancient Egypt, dating back to 3000 BC, evidence knowledge of the effect of medicinal plants and, in the British Museum, clay tablets from the library of King Ashurbanipal (668–626 BC) of Assyria suggest that, around 2500 BC, the Sumerians had a form of Herbal. By 660 BC, around 250 drugs were recognized by the Assyrians themselves, some of which were actively cultivated. Hippocrates (467 BC) was well acquainted with a variety of drugs (although it is improbable that any of the works attributed to him are actually 'of his hand'). Theophrastus, like Alexander the Great, was a pupil of Aristotle, and later became chief of the Aristotelian school. He listed some 500 plants known to him, and distinguished cinnamon from cassia (an art that, apparently, is being lost in this day and age, at least by some manufacturers of foodstuffs!). It is instructive to note that the use of the Mercury's or Hermes' winged staff with entwined snakes or caduceus, nowadays a widely understood symbol for medicine, was originally a symbol for commerce.[1] The whole economy of the ancient city state of Kyrene (Cyrene, near Shahhat in present-day Libya) was predicated on the supply of silphium, a now (probably) extinct species of giant fennel.[2] The importance of this plant was so great that it was habitually depicted on the coins of this city, both in the form of the plant itself and also as its heart-shaped seeds. It is said by some that the last specimen of a stalk of this plant was presented to the Emperor Nero, who promptly ate it. Mohammed was said to be a spice trader, and at that time spice traders were invariably concerned with crude drugs, particularly as many products were used for both culinary and medicinal purposes, as they are today. The adventures of all of the major explorers, such as Marco Polo,

[1] The true symbol for medicine in this context is the rod of Asclepius, one snake and no wings

[2] It is believed by some to be *Ferula indiana*

Columbus, Henry the Navigator and the like were undertaken partly with a view to the sourcing of botanical crude drugs. The establishment of the great National Trading Companies, for instance The Honourable East India Company, The Netherlands United East India Company and the Danish Asiatic Company, were undertaken with a similar view.

Prior to modern times, there was no real distinction between the drug and spice trades, and thus during London's development as an entrepôt, the Guild of Pepperers of London (later the Grocers' Company) was charged with the overseeing of both trades. The importance of the trade is evidenced on the Grocers' Company coat of arms, which features nine cloves (Flores Eugenia Caryophilia). The foundation of the East India Company in 1600 placed a near monopoly in the import of 'East India produce' into England, and it was from this that the modern general produce trade emerged. Initially in *ad hoc* form, and later by way of the famous 'coffee houses', trade in drugs, even until the Second World War, was conducted in the main by auction. The problem was that the shippers at origin had no direct representation in the consuming countries, and thus had to engage the services of a broker. The usual format was for the goods, whether sent 'on consignment' (i.e. sent speculatively to London in the reasonable hope of a sale) or 'ex stock' (being the property of an importing merchant), to be put up for show in the warehouse prior to auction, or for samples to be drawn and placed on view in the brokers' offices. The various broking firms then attempted to sell the merchandise entrusted to them by public auction (the order in which the various brokers did so being decided by lot). Towards the end of the popularity of auctions in London, it was common for no, or almost no, goods actually to be sold at the auction; instead the auctioneer would invite a customer to see him after the auction and would subsequently negotiate a mutually acceptable price between the shipper and the potential customer.

During the Second World War, when lines of communication with the various origins were disrupted, a certain amount of regulation was imposed by the government. This saw an end both to the auctions and, due to licensing controls, to the supply of goods to London on consignment. The trade then took a new form: samples were displayed in brokers' offices for all to see. The traders, brokers and buyers met in the Corn Exchange and deals were done by word of mouth (and sometimes in covert whispers) in the best traditions of the trade. By the 1960s, with improving communications and increased volumes, this method became impractical and the trade finally took to conducting most of its business by telecommunications.

Obviously, early on, when the trade between the dealers and brokers was small and almost self-regulating, little regulatory interference was required. However, the increasing numbers of firms involved in the trade required a system of settling disputes in an inexpensive and swift manner. Thus, in 1876 the General Produce Brokers' Association (GPBA) of London was formed. This body performed a number of functions: it presented a united voice to those outside the trade, it regulated the trade by means of a system of arbitration and appeal and it provided a forum in which to voice concerns of interest to members in general. The GPBA thrived initially, but suffered as time went on and various trade groups formed their own Associations, leaving the GPBA with only the smaller parts of London Commodity Trading as its remit. More recently, broking as part of the London trade became less relevant and the name was accordingly changed in 1981 to the 'General Produce Association of London'. Finally, in 1985, the name was again changed, this time to the 'International General Produce Association', to reflect the current true nature of the trade. Forms of contract are issued by the Association for the use of members and others (it is probably true to say that the IGPA contracts, terms and conditions are those most generally used world-wide), and there is a thriving, and relatively swift and inexpensive, system of arbitration and appeal.

CURRENT ASPECTS

London's pre-eminence as the drug-trading centre has diminished substantially over the years. Although still undeniably the largest market for the trade in essential oils and aromatic chemicals (a trade misnomer frequently used to denote flavour and perfumery chemicals and isolates such as menthol, camphor, piperonal, vanillin and the like, be they natural or synthetic), its role concerning crude herbs and botanicals (such as rhubarb, ipecacuanha and boldo, for instance) has decreased to Hamburg's benefit, and the trading of spices has moved substantially to Rotterdam. Nonetheless, when disputes need to be settled, most eyes still invariably turn to London to obtain a 'fair deal'.

The North American trade is, as it always has been, substantially different. Unlike most of Western Europe, Canada and the United States of America, as well as being large consumers of crude drugs, are also large producers. Many products regularly traded in the European drug markets emanate almost exclusively from North America. A number of American firms hold stocks of drugs (many of native origin, but not exclusively so) speculatively, being confident that there will be demand for the product held on their books in the near term. Granted, there is a trend towards the European market usages of trade, but nonetheless, consumers in North America should be thankful that, because of this pattern of business, it is still usually possible to obtain materials for immediate delivery from a trader's stock, rather than waiting for shipment from origin. There has been a certain amount of consolidation in the trade in the USA and, consequently, the North American market is edging, however reluctantly, towards the European model.

Consuming patterns in Europe have also changed. There has been substantial consolidation of companies concerned with crude and processed botanical drugs, particularly but not exclusively within Germany, to the extent that nowadays one large conglomerate, while not controlling the business, has great influence. A number of recent closures and rationalization have caused concern, particularly on the part of consuming companies. What effect all of this will eventually have on the world markets for crude drugs has yet to be seen, but the current mood of the market could well be described as 'melancholic'.

Trade nowadays has changed substantially. In relatively recent times, many drugs were imported speculatively with a view to selling them either 'afloat' (i.e. once confirmed the goods were aboard ship) or from 'the spot' (i.e. with the goods in store in a European warehouse, available for immediate delivery). With the changing requirements of buyers, be it either for the quality of the goods required or with changing trends in the particular drugs used, this has become less common, and nowadays it is more likely that a customer will request of a trader an offer for a specific quantity of crude drug for shipment from origin and delivery after safe arrival of shipment, be that in one delivery or in parts, either against a sample or (more rarely) a mutually agreed level of quality. Whereas the customer is, of course, at liberty to approach the origin direct for supplies, the advantages of purchasing from a trader are those of transference of risk. If the quality or condition of goods is such that on arrival the goods are either of lower quality than that contracted or damaged in some way, or if delivery is delayed beyond the agreed period, recourse is to the local trader, from whom one is far more likely to obtain settlement than the origin, where satisfaction of a claim might be far more difficult or, in some cases, impossible to perform, due for instance to local currency regulations. Also, if the trader concerned supplies the same material to various buyers it can frequently be possible to rearrange the allocations on his book to provide the buyer with an 'emergency' delivery should production demands require this. In return for this service, the trader asks a small premium over the price paid to origin and requires absolute adherence

to the contract terms, such as delivery dates and terms of payment, for it is on this basis that the price has been calculated and thus, by inference, the margin. Margins in the crude drug trade are currently probably as small as they have ever been. For the customer to delay or cancel an order placed, or to delay payment once goods are delivered, is unacceptable, as would be the trader defaulting, delivering goods late or of poor quality. A fallacy still holds in the consumer industries that 'rapacious' traders make vast sums from their livelihood. This might once have been the case, centuries ago, but almost invariably nowadays margins are confined to low, single-figure percentage levels, representing a small fraction of those that the customer usually makes. This point cannot be overemphasized: contracts (which, after all are based on the concept of 'equity' or fairness) work both ways and, in London at least, the old adage *Verba mea pacta* still holds with the traders; it must also do so with the customer. Is it reasonable that a trader who has purchased a specific product for a customer, for which that trader has no other potential outlet, should without recourse be required not to deliver goods because of an error on the part of the customer, or a whim of the customer's customer?

The question of quality from the trader's point of view is improving. Strong competition at origin has seen a general increase in quality across the board. Many goods are still traded on the old descriptions such as the 'Common Round' or 'Flat' grades of Chinese rhubarb or 'Mossel Bay' or 'Port Elizabeth' aloes from South Africa. One can always be sure that 'Mossel Bay' aloes will pass the requirements of the *British Pharmacopoeia*, whereas 'Port Elizabeth' will rarely, if ever, do so. Nonetheless, more and more trading is being undertaken on the basis of pre-shipment samples, and shippers at origin feel happier to do so now that the world-wide networks of courier services are in place, as a sample of the specific lot in question can be on the desk of the trader two or three days after it was dispatched from origin, rather than the two or three weeks it used to take, or the two or three months before the advent of airmail. Some products are still traded on the basis of out-turn analysis, there being defined a nominal content of isolate on which the price of the contract is calculated, a minimum level below which the parcel is rejectable, and an agreed pro-rata formula for adjusting the invoice value once the quality has been independently established.

From a commercial point of view, the world has changed dramatically over the past few years. The change in Eastern Europe from state capitalism (less accurately, communism) to free-market economies has also changed supply patterns from these countries. Fifteen years ago, traders had one or two source companies to contact in a country, whereas now there is a plethora of suppliers. Reliability has changed as well: formerly, contracts were sacrosanct but nowadays attitudes are a little more relaxed. Further, to increase income, commercially valuable crude drugs are being produced in countries that previously had not done so; India is an excellent case in point. This country had for many years been a large nett importer of ipecacuanha root (*Cephaelis acuminata*), for both internal consumption and re-export of finished alkaloids. Granted, there has been a small local cultivation of this material, but the quality, until recently, had been relatively poor. Over the past few years, however, India has been able to offer ipecacuanha of high quality and at very competitive levels. These developments all tend to reduce the price of the drugs in question. But at what point does this become deleterious to the market? The answer must be directly related to economics. Subsequent to the changes in Eastern Europe, the prices of some drugs have fallen consistently. Thus, in a place where aspirations for personal income are rising fast (not unreasonably, considering past history and current circumstances), the income from their produce is consistently falling. Perhaps due to increased competition, or conversely from factors at the point of consumption, the price falls. Frequently, the initial effect of lower prices is, in the short term, to raise the quality of the product delivered, with a view to securing the next order. However, if low prices are sustained then eventually the quality of the crop slowly falls as the producer is unable to fertilize or tend the crop to the optimum levels. Adulteration, either with admixed drug or, more subtly, with water (i.e. increasing the moisture content) sometimes takes place. Finally, the farmer or collector is presented with the decision of either continuing the cultivation of the product in the absence of profit or changing crop in favour of one that gives a return. In the worst case, supplies of a drug could well cease for lack of profit to the producer, which, when taken in the context of the price at which the finished product is retailed to the public, is almost insignificant.

It might be that the reader is taken aback by what appears to be a hard-nosed trader making a plea for a fair deal to the producer; this, however, is not the case. The writer would far rather have a steady, sustained business over an extended period than a phenomenal short trading 'boom' and thereafter be out of business.

Be it desirable or otherwise, there has been a move over recent years for the goods to be extracted at origin and the isolate, rather than the bulk drug, being shipped. At first glance this has many advantages for all concerned; the less technically demanding work is performed at origin, where labour and facilities are less expensive and where local regulations as regards, say, effluent are less stringent. This adds value to the goods and increased foreign exchange earnings for the country of origin. It is of advantage to the trader, as there are fewer freight, warehousing and handling costs involved and, finally, it gives the customer an (at least semi-) prepared product with which to work, thus eliminating the expensive and laborious initial extraction processes. However, this concept is a double-edged sword. One may be tempted to be a little more cursory as regards quality control of the inward product, particularly after a long run of good experience. If a parcel of material is found to be of inferior quality for whatever reason, the resulting loss tends to be the greater as concentration of the product frequently leads to the acquisition of fewer, larger parcels and (most important) deprives the consuming country, over time, of the knowledge and equipment with which to perform the complete process. In relatively peaceful times this last consideration might be irrelevant; but if lines of communication with the traditional suppliers are disrupted by war, terrorism or natural disaster, then even though it would be possible to obtain the raw material from elsewhere, this would be of little use if the know-how and/or equipment required for the complete extraction process is lost. Commercially, this is of little moment, but for the greater good it is vital not to lose the expertise that has been built up over many centuries in the consuming countries for a small cost saving. A middle road to this position has evolved in recent years. Goods are procured by the user of the finished product and shipped to an extractor in a third country that usually has low wages and lenient pollution and employment regulations, to be extracted on a 'toll' basis. The drawbacks are two-fold: the 'carbon footprint' of the goods is increased substantially and, again, the expertise of extraction is still maintained out of the country. There is of course a cost saving, but in the long term is this adequate compensation?

Another problem has arisen recently, which also gives cause for concern. It was brought to the author's attention by a friend who is a practitioner in speech and language therapy, that atropine delivered by way of a patch (used for control of the secretion of saliva) was proving difficult to source. A few phone calls led to the conclusion that the reason why this medicine was unavailable was that it was perceived that there was insufficient profit in the manufacture of the said patches, and thus production had ceased. Half a century or more ago this would not have been regarded as serious, for the pharmacist would simply

have obtained a supply of the raw material in question, performed his own extraction and concocted it into the required carrier. Now, with the majority of pharmacists at best unwilling (and at worst unable) to perform this act, the patient will have to go without.

CHANGING DEMANDS

The pattern of demand is changing, as it has always done. Recent inroads into the market by 'nutraceuticals' (bio-active products presented as 'dietary supplements' or similar) have changed the demand for various botanicals. Products of which one would have heard rarely some 10 or 20 years ago are now objects of daily discussion. The lack of licensing of these products is a point of major concern. The majority of the botanicals involved are well documented and every company that the writer has come across to date has been responsible and reputable. However, the relatively high margins that these products attract when offered to the consumer with this cachet, and the comparative lack of technical expertise needed to manufacture the product for presentation to the public, could probably in time attract (or perhaps has already attracted) undesirable elements into the trade, with potentially disastrous consequences. Origins are offering prepared extracts that need little more than mixing with an excipient and tableting or filling into freely available capsules. If nutraceuticals, originally and naturally the domain of the chemist's shop, the supermarket or the health-food store, appear at more unlikely venues (such as open-air markets and car boot sales—as this trader has seen, on occasion) then the potential problems multiply. It is almost inconceivable that well-known branded products within their stated shelf life would emerge at these sites, but given a substantial price advantage to the public, is it not reasonable to speculate, be it by accident or design, that any problem starting with a retail outlet of this type attached to goods of questionable origin would reflect badly on the industry as a whole? Licensing, both of the manufacturer and the retailer, would minimize this risk, even if that licensing were far less stringent than that required for dispensing. EU regulations are currently being shaped to tackle this problem, but their implementation has and will attract substantial opposition. Furthermore, there has been a number of recent examples of products included in various traditions of oriental medicine having found their way into Europe, where the said ingredients are simply prohibited as noxious if not positively poisonous. One practitioner was seen on the news defending the use of the product and pronouncing the prohibition to be 'prejudiced' against that person's ethnic traditions. The author will leave readers to make up their own mind on that score.

Fair Trade is set to become important in this trade. Although one can, of course, see the advantages of the concept, there are a couple of problems. A recent policy change has caused the Fair Trade authorities to begin to charge suppliers at origin for registration and audit. This ensures that only the better-financed suppliers will be able to afford registration, which to my mind rather negates the point of the Fair Trade in the first place. Thus, on the one hand, we in the West are pouring vast sums of money into the less well-developed parts of the world to enable people to reach beyond poverty, but with the other hand we are stopping those who most need our help by placing financial hurdles in the way.

THE FUTURE

In my few years in the trade I have seen the total demise of the telegram as a trading tool; telex is going the same way. If the rate of change continues as it is, by the time this appears in print the fax will be considered a quaint, slow form of communication; e-mail may well be superseded within a short time. The tools of the trade change, but the methods and principles do not. Trade is based on mutual trust. If the supply of a raw material needs to be guaranteed then there is only one possible method of obtaining that guaranteed supply, and that is from one's preferred trader. If the trader from whom you obtain your raw materials says that the goods will be delivered at a certain time then, within the terms of the contract, that trader will move heaven and earth to do so, just as the trader's predecessors did 10, 50 or 100 years ago. Many is the time that I have listened to the complaint that, 'Origin has let me down, I need 5 tons of XYZ root next week'. If support is not forthcoming in ordinary times, then where is the impetus on the trader's part to provide support to those who require it in difficult times? Perhaps accounting and purchasing departments in manufacturing organizations should carefully consider this question when looking at the 2% or so saving they make (or more often appear to make) when purchasing direct from origin. As an insurance policy, the trader is very inexpensive.