21

Anthelmintics

Chapter

21 Anthelmintics

1. INTRODUCTION

The restrictive application of the terminology **anthelmintic** is invariably meant for such drugs exerting their action locally to expel parasites from the GI tract exclusively. Nevertheless, there exists several varieties of *worms* which are able to penetrate other tissues as well ; therefore, the **'drugs'** that predominantly act on these *parasitic infections* are frequently termed as **anthelmintics.**

At this juncture one may come across *two* more terminologies, namely ;

(*a*) **Vermicides.** *i.e.*, the **'drugs'** that solely kill worms are called **vermicides**, and

(*b*) **Vermifuges.** *i.e.*, the **'drugs'** that specifically affect the worm in such a fashion that either the **peristaltic activity** or **catharsis** expels it from the intestinal tract are commonly known as **vermifuges**.

Importantly, such absolute arbitrary categorization actually affords no useful and gainful objective as a host of **anthelamintics** have been recognized that particularly mainfest both actions equally, as per the strength of dosages employed. Therefore, in a broader sense and perspective the **anthelmintics** are defined more appropriately as—**'drugs used to combat any type of helminthiasis'**.

1.1. Types of Worm Parasites

In fact, the worm parasites of man actually belong to *two* phyla, namely ; (*a*) **Nemathelminthes** (*roundworms*) ; and (*b*) **Platyhelminthes** (*flat worms*).

1.1.1. Roundworms

The roundworms essentially comprise of the following *seven* species which shall be discussed briefly *vis-a-vis* the disease they produce in humans in the sections that follows :

(*a*) **Hookworm :** These are of *two* types, namely :

(*i*) **American Variety.** *Necator americanus,* and

(*ii*) **European Variety.** *Ancylostoma duodenale.*

They are found to attach themselves to the mucosa of the duodenum and subsequently obtain their nourishment (for survival) by sucking blood from the surrounding blood vessels.

(*b*) **Roundworm.** The most prevalent human helminths belonging to this category is *Ascaris lumbricoides.* It is observed to inhabit in the upper segment of the small intestine ; and, therefore, it is vomitted up quite often.

(*c*) **Whipworm.** It exactly resembles a tiny whip and the causative species in *Trichuris trichiura.* It is mostly inhabitated in the **cecum,** but is also located in the lower segment of the ileum and the appendix.

(*d*) **Pinworm (Threadworm).** It is only 1.5 to 3mm long, *Enterobius vermicularis,* and resides mostly in the small intestine, cecum and colon.

(*e*) **Strongyloides stercoralis.** It inhabits in the duodenum mostly but may also be located in various other parts, for instance : biliary passages, pancreatic ducts, stomach, various segments of the intestinal passage.

(*f*) **Trichinella spiralis.** The infection usually caused with *T. spiralis* is known as **trichinosis** *i.e.,* a condition which comes into being due to the ingestion of partially cooked pork meat profusely infested with the larvae of the worm. The intake of such meat allows the cysts to dissolve, the parasites get matured which eventually gives rise to a new crop of larvae that not only develops but also penetrates right into the intestinal mucosa and ultimately lodge in the muscles.

(*g*) **Wuchereria bancrofti.** It is one of the most vital filarial worms that is particularly transmitted by the bite of the mosquito. The prevailing symptoms are the blocking of the lymphatic ducts with the adult worms.

1.1.2. Flat worms

The flatworms are normally of *two* kinds, namely : (*a*) **Segmented** (*cestodes*) ; and (*b*) **non segmented** (*trematodes*).

(*i*) **Cestodes.** They include the **'tapeworms',** which are of *four* categories commonly found in humans *viz.,* **beef tapeworm** (*Taenia saginata*) ; **pork tapeworm** (*Taenia solium*) ; **fish tapeworm** (*Diphyllobothrium latum*) ; and **dwarf tapeworm** (*Hymenolepis nana*). In reality, the larval stage of all the four tapeworms is invariably spent in the muscles of the intermediate host, and human infection usually takes place by means of eating partially (improperly) cooked meat and fish.

(*ii*) **Trematodes.** They mostly include the **flukes ;** and in man they occur in *three* varieties that solely inhabit the blood stream thereby causing prominently **schistosomiasis.** These blood flukes are, namely : *S. haematobium ; S. mansoni ; S. mekongi* ; and *S. japonicum.* Importantly, all these human parasites predominantly produce epigastric distress, abdominal pain, anorexia, diarrhea with blood and mucus in the stools, pyrexia, enlarged and tender liver, and ascites. It has been established that the intermediate host is either a **freshwater snail** or a **freshwater mollusk**. The usual mode of transmission in humans is on account of drinking contaminated water.

2. CLASSIFICATION

The **anthelmintics** are classified based upon their chemical structures invariably, whereas a few '**natural products**' also are used to combat the infections in humans. They may be classified as :

(*i*) Piperazines

(*ii*) Benzimidazoles

(*iii*) Heterocycles

(*iv*) Antimalarials

(*v*) Natural Products

These different categories of '**anthelmintics**' shall now be treated with specific examples in the sections that follows :

2.1. Piperazines

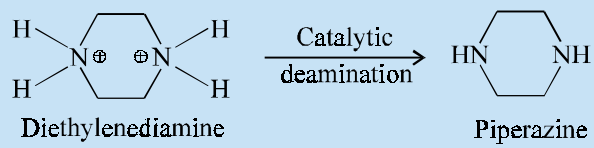
In general, **piperazine** and a good number of its salts *e.g.*, **adipate**, **calcium edetate**, **citrate**, **phosphate** and **tartrate**—have been employed profusely in therapeutic treatment of roundworm and pinworm infections. **Piperazine** itself is a representative example of this class of compounds :

2.1.1. Piperazine BAN, USAN

Hexahydropyrazine ; Diethylenediimine ;

Arthriticine(R) ; Dispermin(R) ;

Synthesis

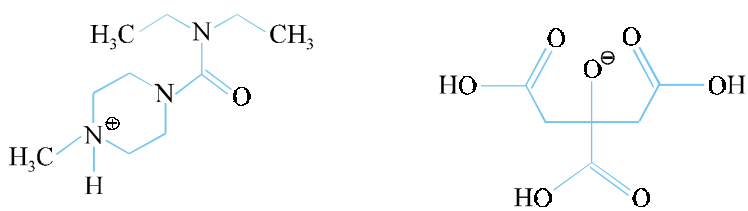


Piperazine may be prepared by the catalytic deamination of diethyldiamine (US Pat 2, 267, 686).

It is used as an **anthelmintic** for the management and treatment of *pinworm* and *roundworm* infestations.

Mechanism of Action. The **'drug'** blocks the response of the ascaris muscle to ACh, thereby affording flaccid paralysis in the worm, which is eventually dislodged from the intestinal inner lumen and ultimately get expelled in the faeces.

2.1.2. Diethylcarbamazine Citrate BAN, USAN



N, N-Diethyl-1, 4-methyl-1-piperazinecarboxamide citrate ; 1-Diethylcarbamyl-4-methyl-piperazine dihydrogen citrate ; USP ;

Hetrazan(R) ;

It is effective against various forms of *filariasis,* including *Bancroft's onchocerciasis,* and *laviasis.* It is also found to be active against *ascariasis.*

Mechanism of Action. The explicite mechanism of action of **diethylcarbamazine (DEC)** is not yet known. It has been observed that **DEC** seems to be the **'active form'** of the drug having a very fast onset of action ; however, interestingly the drug is absolutely **inactive** *in vitro* thereby affirming the glaring fact that activation of a cellular component is a must for the ensuing **filaricidal action.**

In a broader perspective the following *three* most probable mechanisms have been proposed, namely :

(*a*) direct involvement of blood platelets triggered by the action of filarial excretory antigens, thereby accomplishing a rather **'complex reaction'** taking place amongst the '**drug**', **antigen** and **platelets**,*

(*b*) **inhibition** of *microtubule polymerization* and **disruption** of *preformed microtubules,*** and

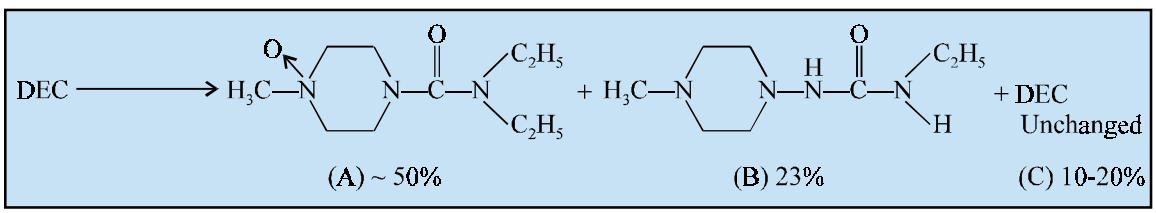
(*c*) interference with *arachadonic acid metabolism.****

Besides, **DEC** also exerts antiinflammatory activity which action is caused solely due to the bockade occurring at *cyclooxygenase* and LTA_4 *synthase* **(leukotriene synthesis),** whereby two predominant activity takes place :

- To change vascular and cellular adhesiveness, and
- To alter cell activation.

Perhaps the latter biological action may propose a plausible suggestion that a possible relationship would prevail between the *first* (*a*) and the *third* (*c*) mechanism stated above.

Metabolism. DEC undergoes metabolic reactions (degradations) to yield *three* products *viz.*, A, B and C as shown below :



Besides, the traces of **piperazine** and **methylpiperazine** are also obtained. In fact, all these metabolites are finally excreted through the urine. Based on the fact that the '**drug**' possesses a very rapid onset of action one may believe that none of these metabolites are virtually involved in exerting the therapeutic action of **DEC**.

2.2. Benzimidazoles

In 1960s, a broad-spectrum group of drugs, known as **benzimidazoles,** were discovered with a big-bang having specific activity against the *gastrointestinal helminths.* In fact, out of several thousand benzimidazoles synthesized and evaluated for their anthelmintic profile only **three** members of this family have gained enormous recognition and wide acceptance, namely : **Albendazole**, **Mebendazole** and **Thiabendazole**. These drugs shall now be discussed individually in the sections that follows :

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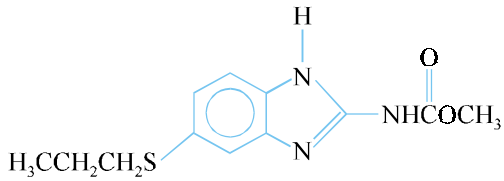
*Cesbron JY *et al. Nature*, **325 :** 533-536, 1987.

*Fujimaki Y *et al. Biochem Phamacol*, **39,** 851-856, 1992.

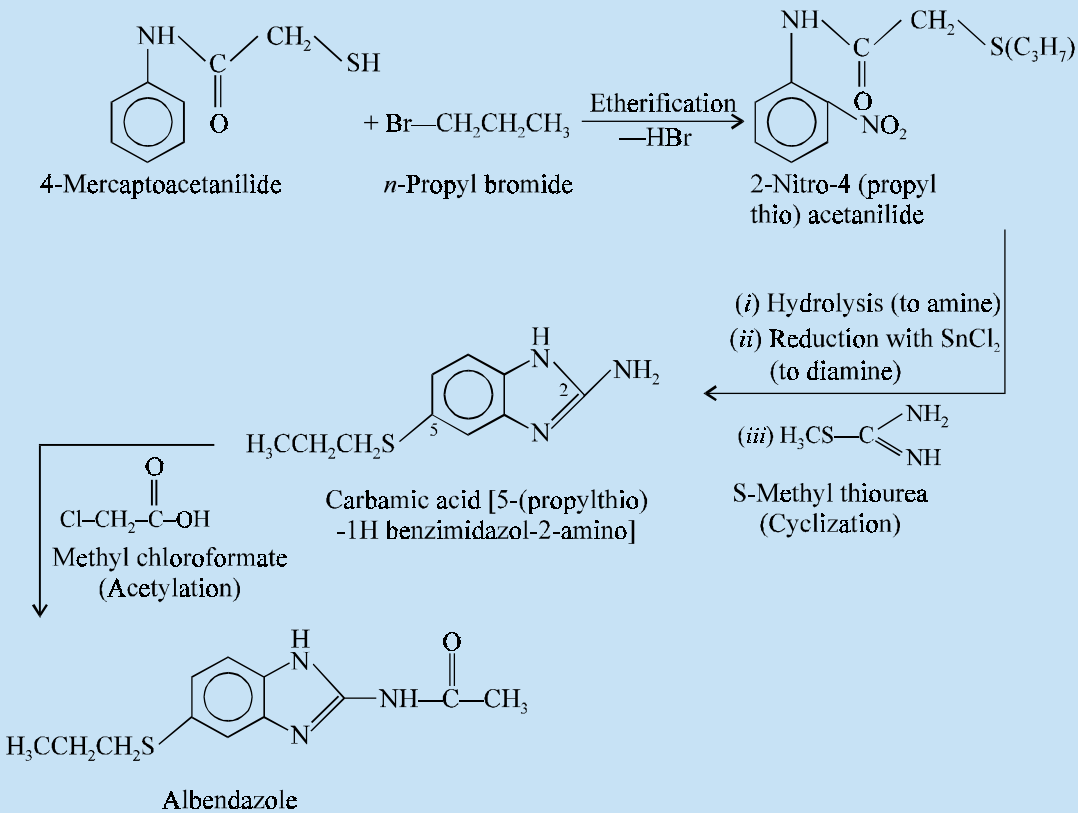
Maizels RM *et al. Parasitol*, **105, S49-S60, 1992.

2.2.1. Albendazole BAN, USAN, INN



Carbamic acid, [5-(propylthio)-1H benzimidazol-2yl-] methyl ester ; USP ; IP ; BP ; Albenza(R) ; Eskazole(R) ; Zentel(R) ;

Synthesis



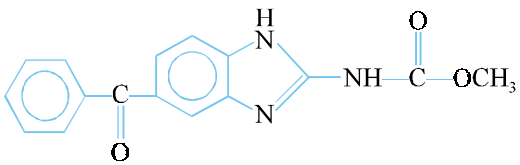
The etherification of 4-mercaptoacetanilide with *n*-propyl bromide gives rise to the formation of 2-nitro-4 (propylthio) **acetanilide** with the elimination of one mole of HBr. The resulting product upon hydrolysis converts it to an amine, reduction with $SnCl_2$ to a diamine, and finally interaction with S-methyl thiourea affords cyclization to yield carbamic acid [5-(propylthio)-1H benzimidazol-2-amino]. This upon acetylation with methyl chloroformate affords the official compound **albendazole**.

It is widely used across the globe for the management and treatment of *intestinal nematode infection.* It is also quite effective as a single-dose-treatment for *ascariasis, New and Old World hookworm infections,* and *trichuriasis.* It has been observed that a recommended multi-dose therapy with **albendazol** may help in the complete eradication of pinworm, threadworm, capillariasis, chlonorchiasis, and hydated disease as well. However, the overall observed effectiveness of albendazole against tapeworms (cestodes) is obviously more variable and less impressive apparently.

Mechanism of Action. The precise mechanism of action of the '**drug**' is not propely understood ; however, it seems to afford its primary anthelmintic effect by binding to the free (**3-tubulin** present in the parasite cells, thereby causing a more or less selective **inhibition of parasite micotubule polymerization,** and inhibition of **micotubule-dependent glucose-up-take** significantly. Besides, the effective inhibition of parasite **β-tubulin** usually takes place at rather lower strengths of the '**drug**' than those that are normally needed to check and suppress human microtubule polymerization.

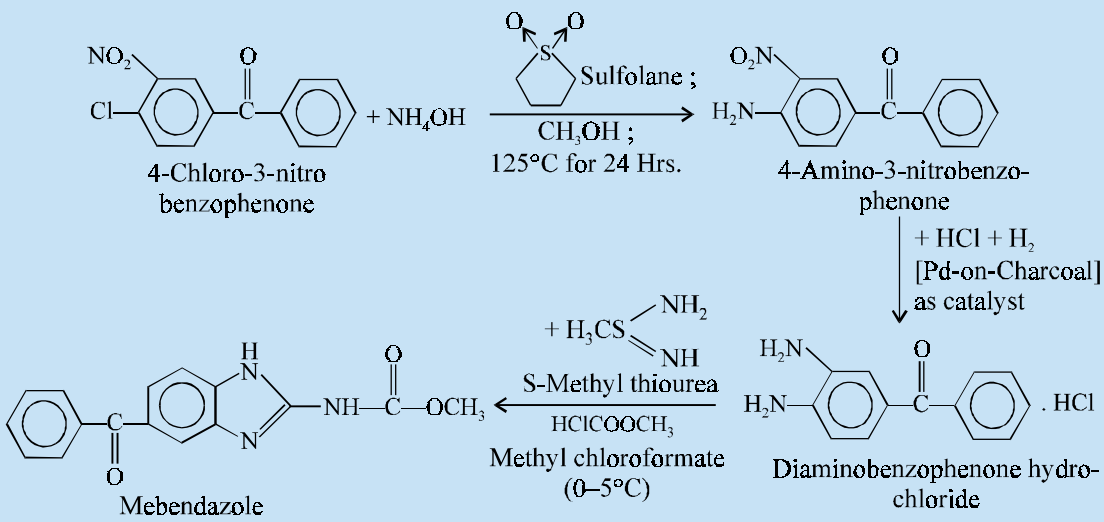
Interestingly, the drug's bioavailability gets enhanced in the presence of fat *e.g.,* the presence of 40g fat helps to enhance the plasma concentrations of **albendazole** to nearly five fold in comparison to that observed in the *'fasting subjects'.*

2.2.2. Mebendazole BAN, USAN, INN



Carbamic acid (5-benzoyl-1H-benzimidazol-2-yl), methyl ester ; IP., BP, USP ;
Vermox(R) ; Antiminth(R) ;

Synthesis

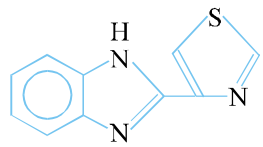


By heating together 4-chloro-3-nitro benzophenone and ammonia at 125°C for 24 hours in the presence of sulfolane yields 4-amino-3-nitrobenzophenone. The resulting product on being treated with hydrochloric acid and hydrogenation with **Pd-on-charcoal** as a catalyst yields **diaminobenzophenone hydrochloride**. This on being treated with S-methyl thiourea in the presence of methyl chloroformate at 0-5°C gives rise to the desired official drug.

It is the anthelmintic drug of first choice in hookworm, pinworm, roundworm, whipworm, guinea worm, in filariasis, and also as an alternative drug for *Visceral Larva Migrans.* It is also employed as an adjunct to steroids for curing *trichinosis.*

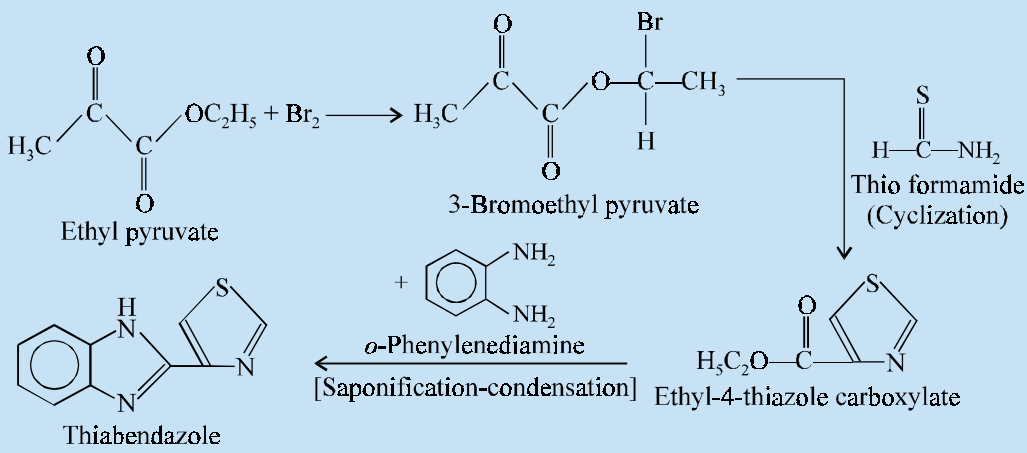
Mechanism of Action. The **'drug'** specifically blocks the glucose uptake by susceptible heliminths, thereby depleting the stored glycogen within the parasite. Obviously, the glycogen depletion invariably causes in an actual decreased generation of **adenosine triphosphate (ATP)**, the latter is essentially needed for the survival and reproduction of the helminth. Besides, it inhibits cell-division in nematodes.*

2.2.3. Thiabendazole USAN, BAN, INN



2-(4-Thiazolyl) benzimidazole ; 1H-Benzimidazole, 2-(4-thiazolyl)- ; USP ; IP ; Mintezol(R) ; Thibenzole(R) ;

Synthesis



*Dessan A *et al. Science*, **267** : 1638, 1995.

Ethyl pyruvate is first brominated, and the resulting 2-bromo ester derivative is treated with thioformamide when cyclization takes place with the formation of ethyl-4-thiazole carboxylate. The ester thus obtained is further saponified and condensed with *o*-phenylenediamine so as to introduce the benzimidazole heterocyclic nucleus and obtain the official drug.

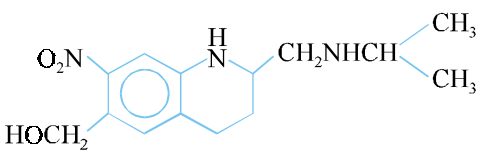
The **'drug'** exhibits broad-spectrum anthelmintic activity. It is mostly employed for the management and treatment of enterobiasis, strongloidiasis (causing threadworm infection) ; ascariasis, uncinariasis (causing hookworm infection) ; and trichuriasis (causing whipworm infection). Besides, it also finds its usefulness to get rid of symptoms associated with cutaneous larva migrans (*i.e.*, the creeping eruption*), and the ensuing invasive phase of trichinosis.

Mechanism of Action. The **'drug'** exerts its anthelmintic action by inhibiting the *helminth-specific enzyme* **fumarate reductase.**** However, it has not yet been fully established whether metal ions are involved in the inhibition mechanism or if the inhibition of the enzyme is exclusively associated with the anthelmintic effect of **thiabendazole**. It has also been established beyond any reasonable doubt that, in general, the **benzimidazole anthelmintic drugs** *viz.*, **thiabendazole**, mebendazole in helping to arrest totally the **nematode-cell division** particularly in the **metaphase state** by directly interfering with the microtubule assembly.*** In fact, the two aforesaid compounds are responsible for exhibiting a high affinity for **tubulin****** *i.e.*, the well-known **precursor protein** essential for the **microtubule synthesis**.

2.3. Heterocyclics

There are some compounds structurally based upon the heterocyclic necleus *viz.*, pyridine (**oxamniquine**), pyrimidine (**pyrantel pamoate**) which are also found to exert anthelmintic activities. These *two* compounds shall now be discussed as under.

2.3.1. Oxamniquine USAN, INN



6-Quinolinemethanol, 1, 2, 3, 4-tetrahydro-2-[[(1-Methylethyl) amino]-methyl]-7-nitro ; USP ; Vansil(R) ;

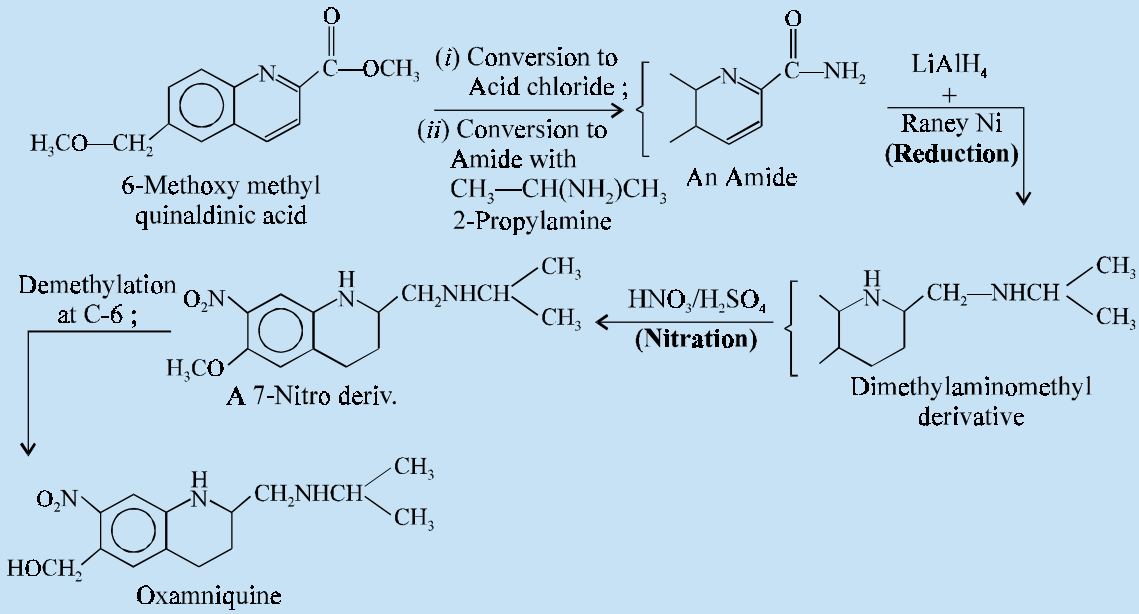
*Creeping eruption caused by *Angiostrongylus costaricensis.*

Prichard RK, *Nature*, **228 : 684, 1970.

***Friedman PA *et al. Biochim. Biophys Acta*, **544** : 605, 1978.

****A colchicine-binding protein.

Synthesis



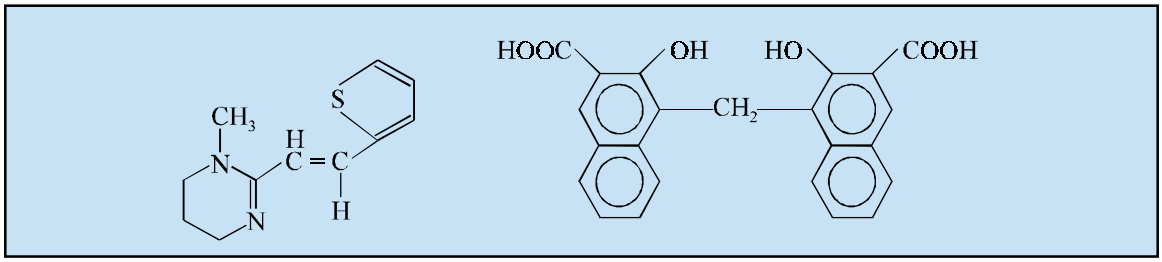
First of all the 6-methoxymethyl quinaldinic acid is converted to an acid chloride, followed by its conversion to an amide with diethylamine to produce an *'amide'* derivative, which upon reduction with $LiAlH_4$ and Raney Nickel to yield the dimethylaminomethyl derivative wherein the pyridine ring also gets saturated. The resulting product is subjected to nitration in the presence of HNO_3/H_2SO_4 to give rise to the formation of the corresponding 7-nitro derivative ; and finally the demethylation at C-6 yields the desired product **oxamniquine**.

Oxamniquine is a potent antischistosomal agent which is specifically indicated for the treatment of *intestinal schistosomiasis* caused by *S. mansoni*, including the severe and the chronic phase with hepatosplenic involvement. It is found to minimize appreciably the *egg-load* of *Schistosoma mansoni*.

Mechanism of Action. The **'drug'** is observed to critically cause inhibition of DNA, RNA and protein synthesis schistosomes. The oral bioavailability of oxamniquine is fairly good, and effective plasma levels are accomplished within a span of 1 to 1.5 hours. Interestingly, the **'drug'** gets metabolized to its corresponding inactive metabolites predominantly, of which the major component is the 6-carboxy derivative.

SAR of Oxamniquine. The most critical and vital entity present in this **'drug'** is the presence of the 6-hydroxymethyl moiety ; and the subsequent metabolic activation of the precursor 6-methyl derivatives is equally critical in nature.

2.3.2. Pyrantel Pamoate USAN, BAN



(E)-1, 4, 5, 6-Tetrahydro-1-methyl-2-[2-(2-thienyl) ethenyl]-, compound with 4, 4′-methylenebis [3-hydroxy-2-naphthalenecarboxylic acid] (1:1) ; USP ;

Antiminth$^{(R)}$;

It enjoys the reputation of being one of the anthelmintics of choice in the treatment of *ascariasis* (roundworm infection), *enterobiasis* (pinworm infection). Recently, it is under intensive and extensive investigation with regard to this potential for the treatment of **hookworm, moniliformis,** and **trichostrongylus** infections.

Mechanism of Action. The **'drug'** exerts its action as a depolarizing blocking agent that particularly affords spastic paralysis in susceptible helminths. More than half of the oral dosage gets excreted in the faeces.

Note. As the action of pyrantel pamoate is just the reverse of piperiazine ; therefore, these two drug substances must not be administered simultaneously to a patient.

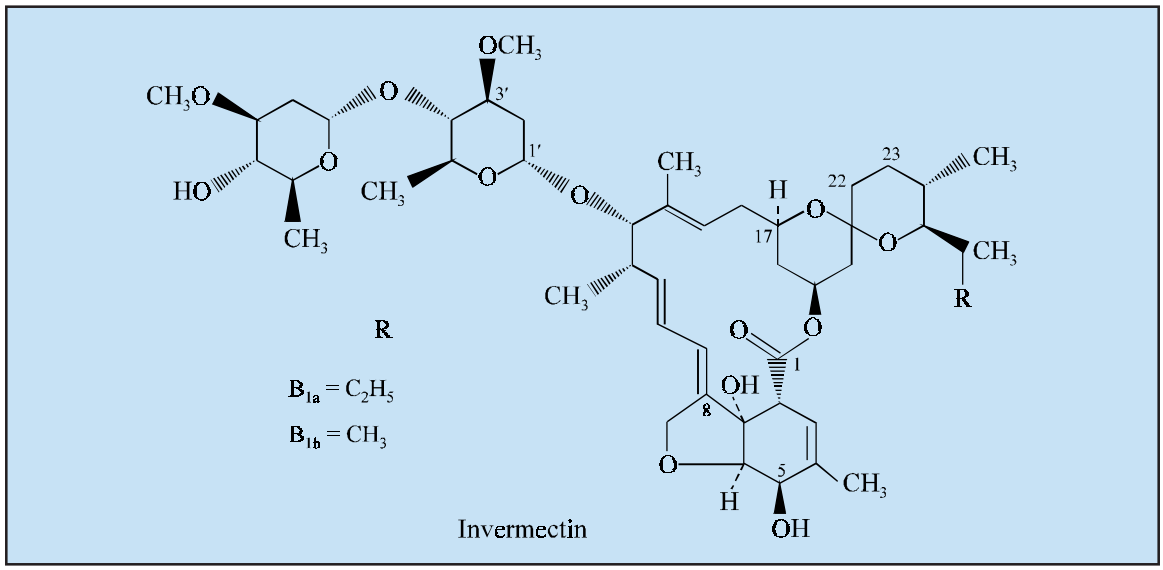
2.4. Antimalarials

Some of the **'antimalarials'**, discussed in Chapter-20, are also used as *'anthelmintics'* at different dosage regimen.

2.5. Natural Products

There are quite a few natural products that are used extensively as **anthelmintics** *i.e.*, as anti-infective agents. A few important as well as typical examples shall be discussed in the sections that follows :

2.5.1. Invermectin USAN, BAN, INN



Invermectin

USP ; Int. P. ; BP ;

Invomec(R) ; Cardomec(R) ; Equalan(R) ; Mectizan(R) ;

Invermectin is usually extracted from the soil of actinomycete *Streptomyces avermitilis*, the **natural avermectins** are 16-membered macrocyclic lactones and is found to be a mixture of 22, 23-dihydro structural analogues of avermectins B_{1a} and B_{1b} prepared by catalytic hydrogenation (reduction). In reality, **avermectins** are members of a family of rather structurally **complex antibiotics** obtained by fermentative process with the pure isolated strain of *S. avermitilis*. An intensive screening of cultures for the anthelmintic drugs exclusively from the '**natural products**' ultimately gave birth to this wonderful drug.

It has been amply demonstrated that the natural avermectins invariably exhibit **minimal biologic profile of activity,** whereas **invermectin** has proven to be extermely useful and hence recognized for the management and treatment of good number of nematode infections. Besides, it is found to be active against arthropods that usually parasitize the animal folks.*

Mechanism of Action. There are *two* different modes of '**mechanism of action**' for **invermectin** have been suggested, namely :

(*a*) **Indirect Action.** In this particular instance the motility of *microfalaria* is minimized appreciably which subsequently permits the cytotoxic cells of the host to enable them adhere to the parasite thereby causing an elimination from the host finally. This specific action may be afforded due to the ability of invermectin to either exhibit its action a **GABA agonist** or as an **inducer of Cl^- ion influx** that may ultimately cause *hyperpolarization* and *muscle paralysis.* However, the latter mechanism *i.e.*, the Cl^- ion influx seems to be the more logical and plausible explanation.** The overall net result of this action is a rapid lowering in the prevailing *microfilarial concentrations.*

*Burg RW *et al. Antimicrob. Agents Chemother* : **15 :** 361, 1979.

Campbell WC, *Science*, **221, 823, 1983.

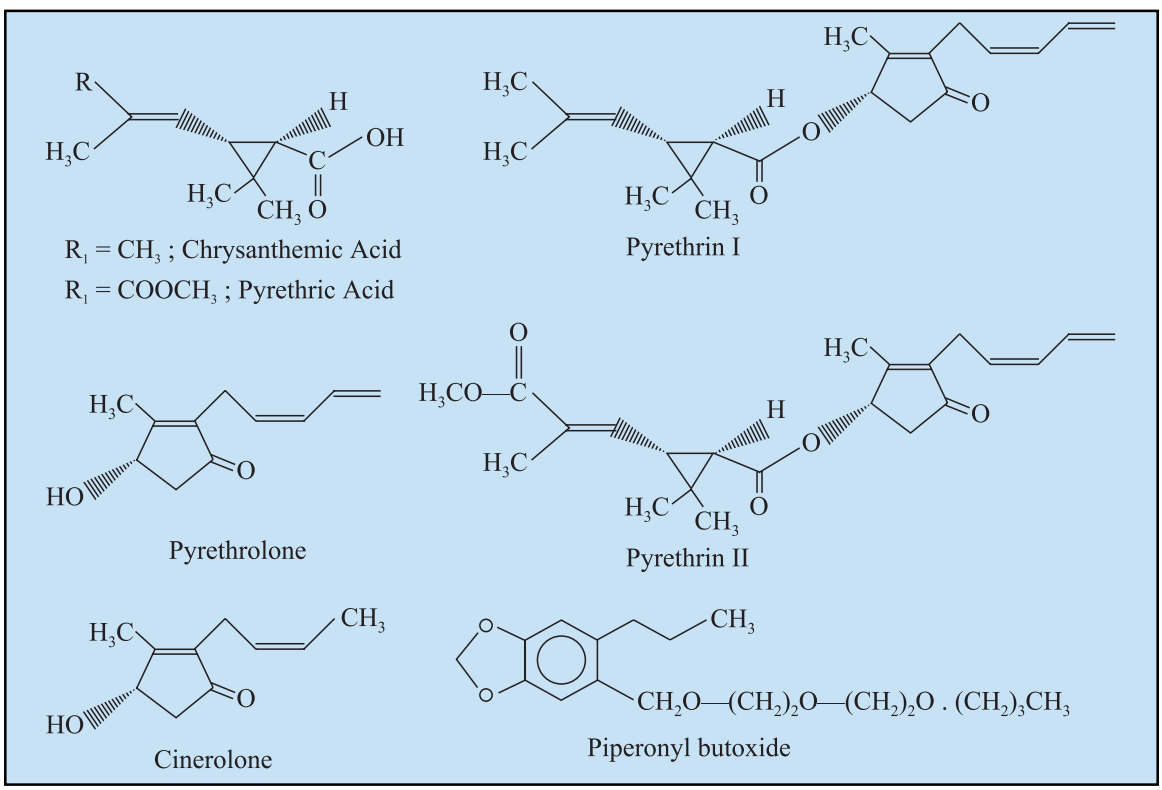
(*b*) **Degeneration of Microfilaria *in uterio*.** This specific action essentially would give rise to relatively fewer microfilaria being released from the female worms and normally extends over a longer duration of time interval. The overall effect caused due to the presence of the *degenerated microfilaria' in uterio* directly prevents **fertilization phenomenon** and the **production of microfilaria.**

Metabolism. The **'drug'** gets absorbed rapidly, bound to an appreciable extent to *plasma protein ;* and excreted ultimately either through the urine or faeces in *two* forms, namely : (*i*) **unchanged invermectin** ; and (*ii*) **3′-O-demethyl-22, 23-dihydro-avermectin B_{1a} or as dihydroavermectin B_{1b} monosaccharide**. Ethanol is found to aggravate the absorption of the **'drug'** even upto 100%.

2.5.2. Pyrethrum and Pyrethroids

In fact, a plethora of naturally occurring pyrethrums have been used quite extensively as viabally potent insecticides since the 1800s. A number of potent chemical entities have been successfully isolated from the extract of the flowering portion of the **Chrysanthemum** plant. Importantly, the plants grown in Kenya (East Africa) contain upto **1.3% pyrethrins.** The **'pyrethrum extracts'** earn a sizable agricultural revenue for the country.

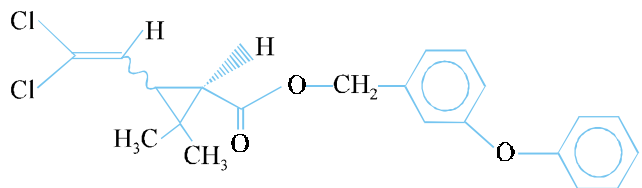
The **Chrysanthemum Extract** comprise of a mixture of *ester e.g.,* **chrysanthemic and pyrethric acids** ; *alcohols e.g.,* **cinerolone** and **pyrethrolone**. As the *'esters'* are usually more prone to get *hydrolyzed* and *oxidized,* hence it must be stored in sealed light-proof containers in a cool place.



Mechanism of Action. The mechanism of action of **pyrethrins** and **pyrethroids (permethrin)** are due to their inherent characteristic feature as *nerve membrane sodium channel toxins* that fail to exert any action upon the potassium channels. In reality, most of these chemical entities get bound to

specific sodium-channel proteins and thereby slow down the rate of inactivation of the sodium current elicited by membrane depolarlization.. The net overall affect being the prolongation of the 'open time' of the sodium channel.

However, at low concentrations the **pyrethroids** (**permethrin**) is observed to display **repetitive action**



Permethrin

potentials and also afford **neuron firing ;** whereas, at relatively higher concentrations the nerve membrane gets depolarized almost completely thereby causing a blockade of excitation.

Stereospecific Aspects. It has been well established that the ensuing receptor interaction of the pyrethrums with the sodium channel complex is absolutely **stereospecific ;** and, therefore, solely dependent on the stereochemistry of the carboxylic acid in question. Interestingly, in the case of **permethrin** the **most active isomers** are the **IR, 3-cis-** and **IR, 3-*trans*-cyclopropane-carboxylates**. However, the IS *cis*- and IR *trans*-isomers are **inactive ;** and are found to serve as antagonists to the therapeutic action of the corresponding IR-isomers.

Metabolism. The wide acceptance and enormous usefulness of the **pyrethrum and pyrethroids** are that they pose to be highly toxic particularly to the **ectoparasites,** whereas they prove to be comparatively much less toxic (*i.e.*, **nontoxic**) to mammals in case absorbed. The magnificent notoxic characteristic feature is associated with the excellent and rapid metabolism of these drug substances either **via** hydrolysis or oxidation. More specifically, the extent of either hydrolysis or oxidation is exclusively dependent upon the structure of the prevailing **pyrethrins or pyrethroids.**

Besides, the rapid breakdown of these drug substances also accounts for their low persistence in the surrounding environment.

Probable Questions for B. Pharm. Examinations

1. Give a brief account on **'Anthelmintics'** and provide suitable examples wherever necessary.
2. How would you classify **'Anthelmintics'** on the basis of chemical structures ? Give the structure, chemical name and uses of **one** example from each category.
3. Discuss the synthesis of any **one** of the following drugs :
   (*a*) Albendazole
   (*b*) Thiabendazole.
4. Describe the synthesis, uses, mechanism of action, and SAR of **oxamniquine.**
5. Write a short note on any **one** of the following potent **'Anthelmintics'** :
   (*i*) Pyrantel Pamoate
   (*ii*) Pyrethrum and Pyrethroids.
6. What is Invermectin ? Discuss its metabolism and mechanism of action.

RECOMMENDED READINGS

1. Block JH and Beale JM Jr., (eds), **'Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'**, Lippincott Williams and Wilkins, New York, 5th edn., 2004.
2. Freeman CD *et al.* : **Metronidazole : A Therapeutic Review and Update,** *Drugs*, **54** : 679-708, 1997.
3. Gennaro AR : **Remington : The Science and Practice of Phramacy**, Vol. I and II, Lippincott Williams and Wilkins, New York, 21st edn., 2006.
4. Mandel GL *et al.* (eds.) **Principles and Practices of Infectious Diseases**, Vol. I, Churchill-Livingstone, New York, 4th edn, 1995.
5. Testa B (ed.), **Advances in Drug Research**, Vol. 21, Academic Press, New York, 1991.
6. Yamaguchi H *et al.* (eds.) **Recent Advances in Antifungal Chemotherapy**, Mercell Dekker, New York, 1992.
7. Williams DA and Lemke TL, **Foye's Principles of Medicinal Chemistry**, Lippincott Williams and Wilkins, New York, 5th edn, 2002.
8. Wilson JD *et al.* (eds.), **Harrison's Principles of Internal Medicine**, McGraw Hill, New York, 12th edn., 772, 1992.

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22

Insulin and Oral Hypoglycemic Agents

Chapter

22 Insulin and Oral Hypoglycemic Agents

1. INTRODUCTION

A major portion of the **pancreas*** essentially comprises of glandular tissue which specially contains acinar cells that predominantly gives rise to the secretion of certain **digestive enzymes.** Besides, there also exist some '**isolated groups of pancreatic cells**' commonly known as the **islets of Langerhans** which usually made up of *four* cell types, each of which generates a **distinct polypeptide hormone**, namley :

(*a*) **Insulin** — in the beta (β) cells,

(*b*) **Glucagon** — in the alpha (α) cells,

(*c*) **Somatostatin** — in the delta (δ) cells, and

(*d*) **Pancreatic polypeptide** — in the PP or F cell.

Interestingly, the **β-cells** made up 60-80% of the **islets of Langerhans** most predominantly and distinctly.

Diabetes — a general term for diseases marked by excessive urination ; and is usually refers to *diabetes mellitus.*

However, the *clinical diabetes mellitus* invariably occurs in *two* forms, associated with different causes and methods of therapy.

Type 1 Diabetes : The **insulin-dependent diabetes mellitus (IDDM)**, normally takes place when the β-*cells* of the prevailing pancreatic *islets of Langerhans* are destroyed, perhaps by an **autoimmune, mechanism,** as a consequence of which the '**insulin production**' *in vivo* is overwhelmingly insufficient. Subjects undergoing such abnormalities in biological functions may show appreciable metabolic irregularity that may ultimately lead to develop **diabetic β-ketoacidosis** together with other manifestations of acute diabetes. Therapeutically Type-I diabetes is largely treated with **insulin.**

Type 2 Diabetes : The **noninsulin-dependent diabetes mellitus (NIDDM)**, *i.e.*, type 2 diabetes, is most abundantly linked with obesity in its adult patients largely. In such a situation, the **insulin** levels could be either elevated or normal ; and therefore, in short, it is nothing but a disease of abnormal **'insulin resistance'.** However, it has been duly observed that the impact of the disease is relatively

*Both an *exocrine* and *endocrine* orgin ; a compound acinotubular gland situated behind the stomach in front of the first and second lumbar vertebrae in a horizontal position, its head attached to the duodenum and its tail reaching to the spleen.

milder, occasionally leaving to **β-ketoacidosis** and may also be accompanied by certain other degenerative phenomena *in vivo.* The etiology of the condition bears a *strong genetic hereditary ;* and, hence, **insulin therapy** may not prove to be quite effective.

2. INSULIN-PRIMARY STRUCTURE

Sanger (in 1950s) put forward the primary structure of **insulin** as illustrated below in Fig. 22.1.

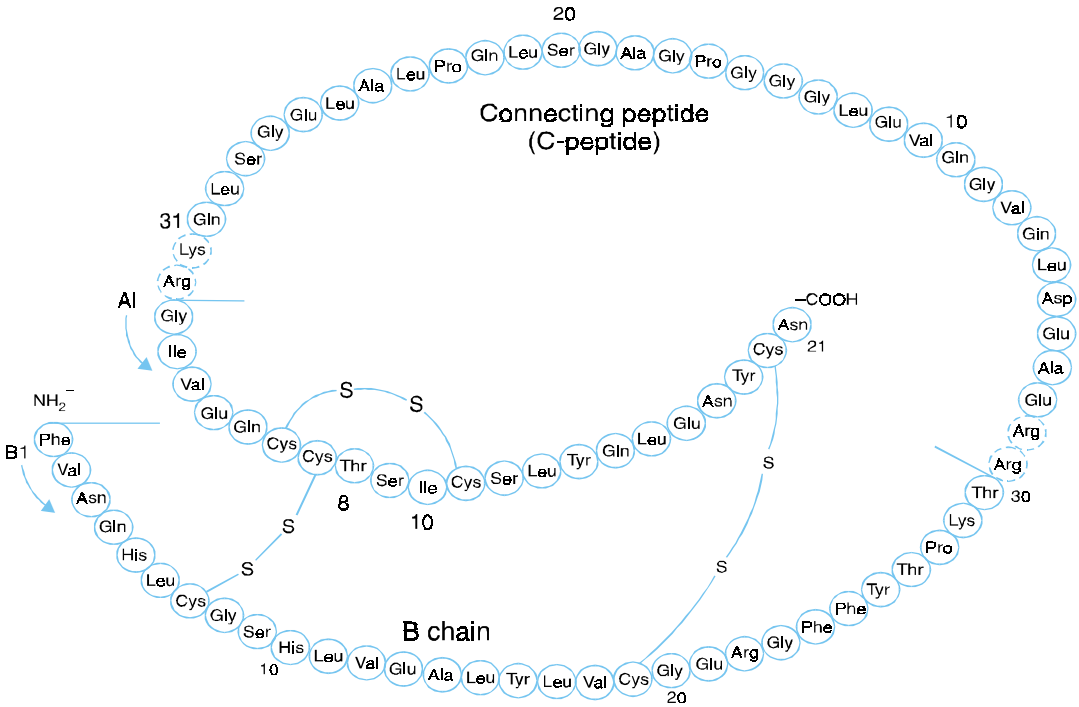


Fig. 22.1. Primary structure of proinsulin, depicting cleavage sites to produce insulin.

[Adapted from : **Foye's Principle of Medicinal Chemistry**, 5th International Student Edition, Lippincott Williams and Wlikin, New York, 2002]

The above Fig. 1 has the following **Salient Features,** namely :

(1) **Proinsulin** is the immediate precursor to **insulin** in the single-chain peptide.

(2) **Proinsulin** folds to adopt the 'correct orientation of the prevailing '**disulphide bonds**' plus other relevant conformational constraints whatsoever on account of its primary structure exclusively.

(3) **Proinsulin** in reality, has a precursor of its own, ***preproinsulin***–a **peptide**, that essentially comprises of hundreds of **'additional residues'.**

(4) At an emerging critical situation the **insulin** gets generated from ***proinsulin*** due to the ensuing cleavage of **proinsulin** at the *two points indicated.* This eventually produces **insulin,** that comprises of a **21-residue A chain** and strategically linked with **two disulphide bonds**

ultimately to a **30-residue B chain.** Interestingly, these bondages between the two aforesaid residual chains 'A' and 'B' are invariably oriented almost perfectly and correctly by virtue of the prempted nature of **proinsulin folding**.

2.1. Variants of Insulin Products

There are a number of variants of **insulin** products that are available as a **'drug'**, namely :

2.1.1. Insulin Injection

[***Synonyms*** **: Regular Insulin ; Crystalline Zinc Insulin**]

It is available as a sterile, acidified or neutral solution of **insulin**. The solution has a potency of 40, 80, 100 or 500 USP **Insulin Units** in each ml.

Mechanism of Action. It is a rapid-action insulin. The time interval from a hypodermic injection of this 'drug' until its action may be observed ranges between 1/2 to 1 hour. It has been observed that the duration of action is comparatively short but evidently a little longer than the plasma half-life that stands at nearly 9 minutes. Importantly, the duration of action is not linearly proportional to the size of the dose, but it is a simple function of the logarithm of the dose *i.e.*, if 1 unit exerts its action for 4 hours then 10 units will last 8 hours. In usual practice the duration is from 8 to 12 hour after the subcutaneous injection, which is particularly timed a few minutes before the ingestion of food so as to avoid any possible untoward fall in the prevailing blood-glucose level.

2.1.2. Isophane Insulin Suspension

[***Synonyms*** **: Isophane Insulin ; Isophane Insulin Injection ; NPH Insulin ; NPH Iletin** ;] :

The **'drug'** is a sterile suspension of **Zinc-insulin crystals** and **protamine sulphate** in buffered water for injection, usually combined in such a fashion that the *'solid phase of the suspension'* essentially comprises of crystals composed of **insulin**, **protamine***, and **zinc**.

Each mL is prepared from enough insulin to provide either 40, 80, or **100 USP Insulin units of insulin activity**.

Mechanism of Action. The **'drug'** exerts its action as an intermediate-acting insulin for being insoluble and obtained as repository form of insulin. In reality, the action commences in 1–1.5 hour, attains a peak-level in 4 to 12 hour, and usually lasts upto 24 hours, with an exception that '**human isophane insulin**' exerts a rather shorter duration of action. It is, however, never to be administered IV.

Note : Incidence of occasional hypersensitivity may occur due to the presence of 'protamine'.

2.1.3. Insulin Zinc Suspension

It is invariably obtained as a sterile suspension of insulin in buffered water for injection, carefully modified by the addition of zinc chloride ($ZnCl_2$) in such a manner that the *'solid-phase of the suspension'* comprises of a mixture of **crystalline** as well as **amorphous** insulin present approximately in a ratio of 7 portions of crystals and 3 portions of amorphous substance. Each mL is obtained from sufficient **insulin** to provide either 40, 80, or 100 USP Insulin Units of the **Insulin Activity.**

Mechanism of Action. It has been duly observed that the **'amorphous zinc-insulin component'** exerts a duration of action ranging between 6–8 hours, whereas the **'crystalline zinc-insulin component'**

---

*The **protamine sulphate** is usually prepared from the sperm or from the mature testes of fish belonging to the genera **Oncorhynchus** Suckley, or **Salmo** Linne (*Family : Salmonidae*).

a duration of action more than 36 hour, certainly due to the sluggishness and slowness with which the larger crystals get dissolved. However, an appropriate dosage of the 3 : 7 mixture employed usually displays an onset of action of 1 to 2.5 hour and an intermediate duration of action which is very near to that of '**isophane insulin suspension**' (24 hour), with which preparation this **'drug'** could be employed interchangeably without any problem whatsoever. However, it must not be administered IV.

Note : The major advantage of 'zinc insulin' is its absolute freedom from '*foreign proteinous matter*', such as : *globin*, or *protamine*, to which certain subjects are sensitive.

2.1.4. Extended Insulin Zinc Suspension

[***Synonyms*** **: Ultra-Lente Iletin ; Ultralente Insulin/Ultratard**]

Mechanism of Action : The actual **'crystalline profile'** in this specific form are of sufficient size to afford a slow rate of dissolution. It is found to exert its *long-acting action* having an onset of action ranging between 4 to 8 hours, an optimal attainable peak varying between 10-30 hours, and its overall duration of action normally in excesss of 36 hours, which being a little longer than that of **Protamine Zinc Insulin.**

Note : Because the '*drug*' is free of both protamine and other foreign proteins, the eventual incidence of allergic reactions gets minimized to a significant extent.

2.1.5. Prompt Insulin Zinc Suspension

[**Synonyms : Semi-Lente Iletin ; Semitard**]

The '**drug**' is usually a sterile preparation of insulin in **'buffered water for injection'**, strategically modified by the addition of zinc chloride ($ZnCl_2$) in such a manner that the **'solid phase of the prevailing suspension'** is rendered amorphous absolutely.

Each mL of this preparation provides sufficient insulin either 40, 80, or 100 USP Insulin Units.

Mechanism of Action. The zinc-insulin in this particular form is a mixture of amorphous and extremely fine crystalline materials. As a result, the **'drug'** serves as a rapid-acting insulin with an onset of 1 to 1.5 hour, an attainable peak of 5-10 hours, and a duration of action ranging between 12-16 hours.

Note : Since this specific form of insulin is essentially free of any foreign proteins, the incidence of allergic reactions is found to be extremely low.

2.1.6. Lispro Insulin

[**Synonyms : Human Insulin Analog ; Humalog**] : It is a **human insulin** analogue of *r* DNA origin meticulously synthesized from a special nonpathogenic strain of *E. coli*, genetically altered by the addition of the gene for insulin lispro ; Lys (B28), Pro (B29). In fact, the prevailing amino acids at position 28 and 29 of **human insulin** have been reversed altogether.

Mechanism of Action. The *'drug'* is a very **rapid-acting insulin** which may be injected conveniently just prior to a meal. It exhibits an onset of action within a short span of 15 minutes besides having a relatively much shorter peak ranging between 0.5 to 1.5 hour, and having duration of action varying between 6 to 8 hours in comparison to the '**regular insulin injection**'.

2.1.7. Protamine Zinc Insulin Suspension

[***Synonyms :*** **Zinc Insulin ; Protamine Zinc Insulin Injection ; Protamine Zinc and Iletin** ;] :

The '**drug**' is a sterile suspension of insulin in buffered water for injection, that has been adequately modified by the addition of **zinc chloride ($ZnCl_2$)** and **protamine sulphate**. The **protamine sulphate**

is usually prepared from the sperm or from the mature testes of fish belonging to the genus *Oncorhynchus* Suckley or *Salmo* Linne (Family : *Salmonidae*). Each mL of the suspension prepared from sufficient insulin to provide wither 40, 80, or 100 USP Insulin Units.

Mechanism of Action. The '**drug**' exerts a long-acting action having an onset of action of 4 to 8 hour, a peak at 14 to 24 hour, and a duration of action nearly 36 hour. As a result this **'drug'** need not be administered with any definite time relation frame to the corresponding food intake. Besides, it should not be depended upon solely when a very prompt action is required, such as : in **diabetic acidosis** and **coma.** Since the *'drug'* possesses an inherent prolonged action, it must not be administered more frequently than once a day. It has been duly observed that **'low levels'** invariably persists for 3 o 4 days ; and, therefore, the dose must be adjusted at intervals of not less than 3 days. It is given by injection, normally into the **loose subcutaneous tissue.**

Note : The *'drug'* should never be administered IV.

3. ORAL HYPOGLYCEMIC AGENTS

The synthetic **oral hypoglycemic agents** have been added to the therapeutic armamentarium over the last five decades in lieu of the various **'insulin variants'** discussed earlier. In this particular section the focus shall be made on the different categories of **synthetic oral hypoglycemic agents** based on their chemical structures, namely :

(*i*) Sulfonylureas,

(*ii*) Non sulfonylureas,

(*iii*) Thiazolindiones,

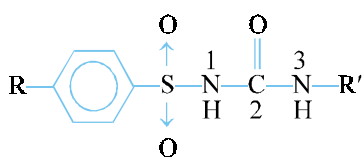
(*iv*) Bisguanides, and

(*v*) α-Glucosidase Inhibitors

The important **'drugs'** belonging to each of the above categories shall now be discussed individually in the sections that follows :

3.1. Sulfonylureas

The **sulfonylurea hypoglycemic agents** are basically sulphonamide structural analogues but they do not essentially possess any *'antibacterial activity'* whatsoever. In fact, out of 12,000 **sulfonylureas** have been synthesized and clinically screened, and approximately 10 compounds are being used currently across the globe for lowering blood-sugar levels significantly and safely. The **sulfonylureas** may be represented by the following general chemical structure :



Salient Features : The salient features of the **'sulfonylureas'** are as given below :

(1) These are urea derivatives having an arylsulfonyl moiety in the 1 position and an aliphatic function at the 3-position.

(2) The aliphatic moiety, R′, essentially confers lipophilic characteristic properties to the newer drug molecule.

(3) Optimal therapeutic activity often results when R′ comprises of 3 to 6 carbon atoms, as in **acetohexamide**, **chlorpropamide** and **tolbutamide**.

(4) Aryl functional moieties at R′ invariably give rise to toxic compounds.

(5) The R moiety strategically positioned on the **'aromatic ring'** is primarily responsible for the duration of action of the compound.

However, these agents are now divided into *two* sub-groups, namely :

(*a*) First-generation sulfonylureas, and

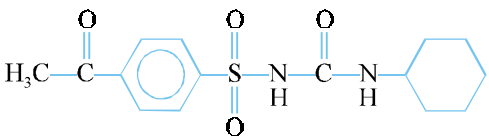
(*b*) Second-generation sulfonylureas.

These two aforesaid classes of **sulfonylureas** will be dealt with separately as under :

3.1.1. First-Generation Sulfonylureas

The various important drugs that belong to this category are, namely : **Acetohexamide** ; **Chlorpropamide** ; **Tolazamide** ; and **Tolbutamide**. These drugs shall be treated individually as under :

3.1.1.1. Acetohexamide BAN, USAN, INN



1-[(*p*-Acetylphenyl) Sulfonyl]-3-cyclohexyl urea ; USP ;

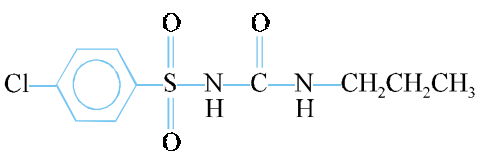
Dymelor(R) ;

It lowers the blood-sugar level particularly by causing stimulation for the release of endogenous insulin.

Mechanism of Action. The '**drug**' gets metabolized in the liver solely to a reduced entity, the corresponding **α-hydroxymethyl structural analogue**, which is present predominantly in humans, shares the prime responsibility for the ensuing hypoglycemic activity.

SAR of Acetohexamide. It is found to be an intermediate between **'tolbutamide'** and **'chlorpropamide'** *i.e.*, in the former the cyclohexyl ring is replaced by butyl moiety and *p*-acetyl group with methyl group ; while in the latter the cyclohexyl group is replaced by propyl moiety and the *p*-acetyl function with chloro moiety.

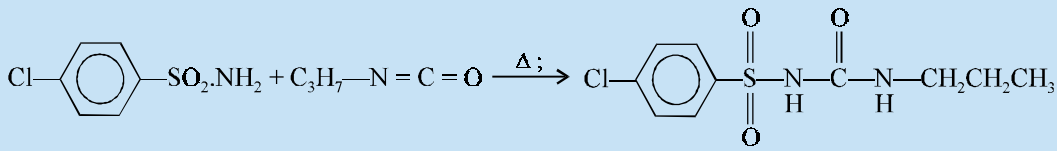
3.1.1.2. Chlorpropamide USAN, BAN, INN,



1-[(*p*-Chlorophenyl)-Sulphonyl]-3-propyl urea ;

Diabinese(R) ;

Synthesis



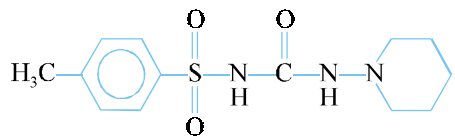
p-Chlorobenzene Sulphonamide — Propylisocyanate — Chlorpropamide

The interaction between *p*-chlorobenzenesulphonamide and phenyl isocyanate in equimolar concentrations under the influence of heat undergoes **addition reaction** to yield the desired official compound.

The therapeutic application of this **'drug'** is limited to such subjects having a history of stable, mild to mderately severe diabetes melitus who still retain residual pancreatic β-cell function to a certain extent.

Mechanism of Action. The **'drug'** is found to be more resistant to conversion to its corresponding **inactive metabolites** than is **'tolbutamide' ;** and, therefore, it exhibits a much longer duration of action. It has also been reported that almost 50% of the **'drug'** gets usually excreted as metabolites, with the principal one being hydroxylated at the C-2 position of the **propyl-side chain**.*

3.1.1.3. Tolazamide USAN, BAN, INN



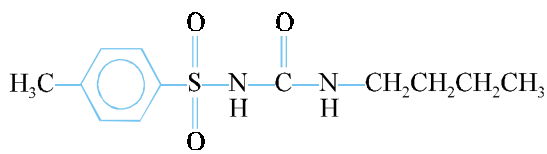
1-(Hexahydro-1H-azepin-1-yl)-3-(*p*-tolylsulphonyl) urea ;

Tolinase(R) ;

It is found to be more potent in comparison to **'tolbutamide'**, and is almost equal in potency to **chlorpropamide**.

Mechanism of Action. Based on the radiactive studies it has been observed that nearly 85% of an oral dose usually appears in the urine as its corresponding metabolites which were certainly more water-soluble than the parent **tolazamide** itself.

3.1.1.4. Tolbutamide

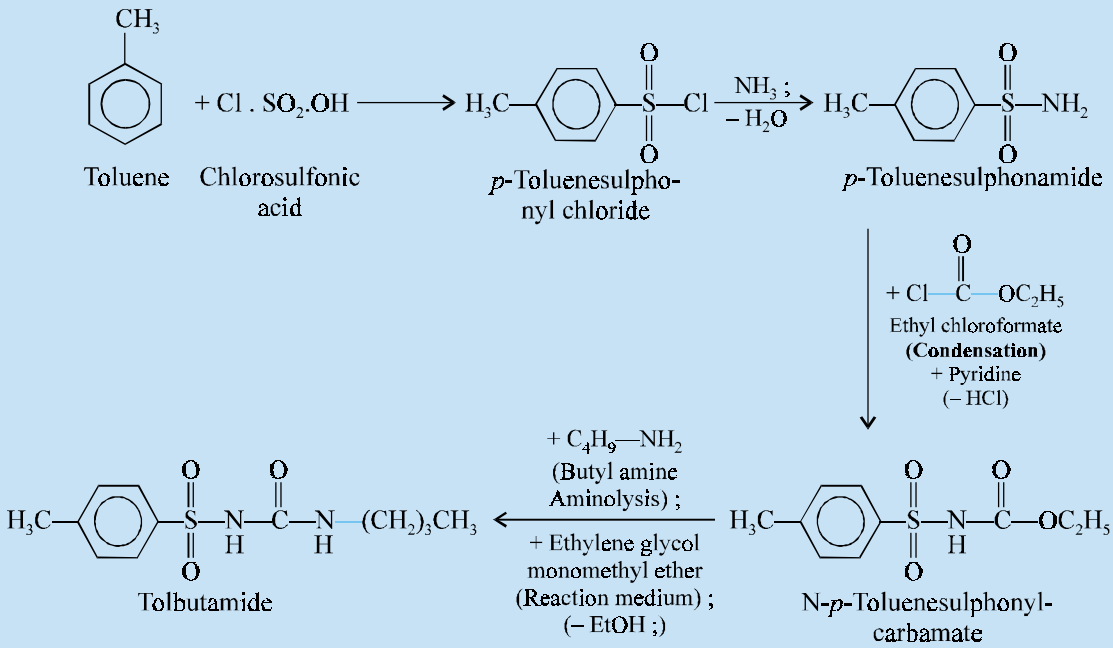


Benzenesulphonamide, N-[(butylamino) carbonyl]-4-methyl- ;

Orinase(R) ;

*Thomas RC *et al. J Med Chem*, **15,** 964, 1972**.**

Synthesis



First of all toluene is treated with chlorosulfonic acid to yield *p*-toluenesulphonyl chloride, which on treatment with ammonia gives rise to the formation of *p*-toluenesulphonamide. The resulting product on condensation with ethyl chloroformate in the presence of pyridine produces N-*p*-toluenesulphonyl carbamate with the loss of a mole of HCl. Further aminolysis of this product with butyl amine using ethylene glycol monomethyl ether as a reaction medium loses a mole of ethanol and yields **tolbutamide**.

It is mostly beneficial in the treatment of selected cases of **non-insulin-dependent diabetes melitus (NIDDM)**. Interestingly, only such patients having **some residual functional islet β-cells** which may be stimulated by this drug shall afford a positive response. Therefore, it is quite obvious that such subjects who essentially need more than 40 Units of insulin per day normally will not respond to this drug.

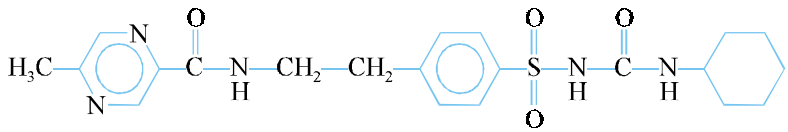
Mechanism of Action. The **'drug'** usually follows the major route of breakdown ultimately leading to the formation of butylamine and *p*-toluene sulphonamide respectively.

Importantly, the observed hypoglycemia induced by rather higher doses of the **'drug'** is mostly not as severe and acute as can be induced by **insulin ;** and, therefore, the chances of severe hypoglycemic reactions is quite lower with tolbutamide ; however, one may observe acute refractory hypoglycemia occasionally does take place. In other words, refractoriness to it often develops.

3.1.2. Second-Generation Sulfonylureas

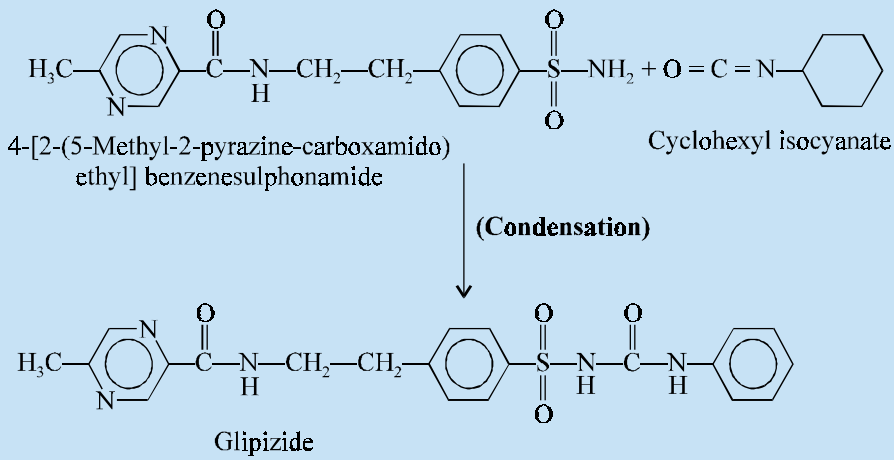
The vital and important members of this class of compounds are, namely : **Glipizide** ; **Glyburide** ; and **Glumepiride**. These drug substances will be dealt with separately in the sections that follows :

3.1.2.1. Glipizide USAN, INN



Pyrazinecarboxamide, N-[2-[4-[[[(cyclohexylamino) carbonyl] amino] sulfonyl] ethyl]-5-methyl-; Glucotrol$^{(R)'}$;

Synthesis



Glipizide may be prepared by the condensation of 4-[2-(5-methyl-2-pyrazine-carboxamido)-ethyl] benzenesulphonamide with cyclohexylisocyanate in equimolar proportions.

It is employed for the treatment of **Type 2 diabetes mellitus** which is found to be 100 folds more potent than **tolbutamide** in evoking the pancreatic secretion of insulin. It essentially differs from other oral hypoglycemic drugs wherein the ensuing tolerance to this specific action evidently does not take place.

Mechanism of Action. The primary hypoglycemic action of this **'drug'** is caused due to the fact that it upregulates the insulin receptors in the periphery. It is also believed that it does not exert a direct effect on **glucagon secretion.**

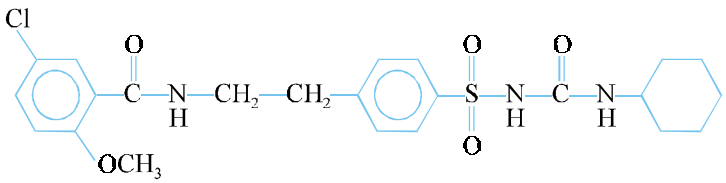
The '**drug**' gets metabolized *via* oxidation of the cyclohexane ring to the corresponding *p*-hydroxy and *m*-hydroxy metabolites. Besides, a *'minor metabolite'* which occurs invariably essentially involves the N-acetyl structural analogue that eventually results, from the acetylation of the primary amine caused due to the hydrolysis of the amide system exclusively by **amidase enzymes.**

Note : The '*drug*' enjoys *two* special status, namely :

(*a*) Treatment of non-insulin dependent diabetes mellitus (NIDDM) since it is effective in most patients who particularly show resistance to all other hypoglycemic drugs ; and

(*b*) Differs from other oral hypoglycemic drug because it is found to be more effective during eating than during fasting.

3.1.2.2. Glyburide USAN, INN



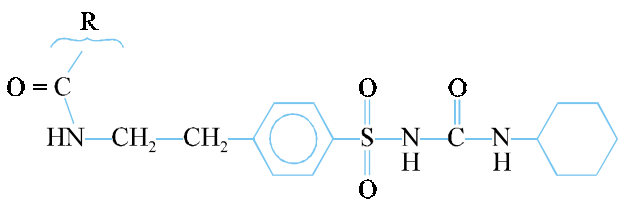
Benzamide, 5-chloro-N-[2-[4-[[[(Cyclohexylamino) carbonyl] amino] sulphonyl] phenyl] ethyl]-2-methoxy- ;

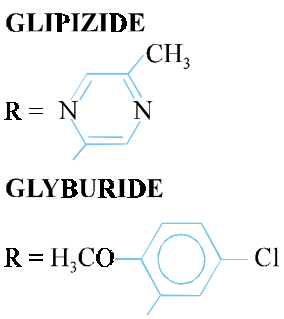
Dia Beta(R) ; Glynase Press Tab(R) ; Micronase(R) ;

It is mostly used for Type 2 diabetes melitus. It is found to be almost 200 times as potent as *tolbutamide* in evoking the release of **insulin** from the pancreatic islets. However, it exerts a rather more effective agent in causing suppression of *fasting* than *postprandial* hyperglycemia.

Mechanism of Action. The **'drug'** gets absorbed upto 90% when administered orally from an empty stomach. About 97% gets bound to plasma albumin in the form of a weak-acid anion ; and, therefore, is found to be more susceptible to displacement by a host of weakly acidic drug substances. Elimination is mostly afforded by *'hepatic metabolism'*. The half-life ranges between 1.5 to 5 hours, and the duration of action lasts upto 24 hours.

SAR of Glyburide. The SAR of **Glyburide** and **Glypizzide** are discussed below :

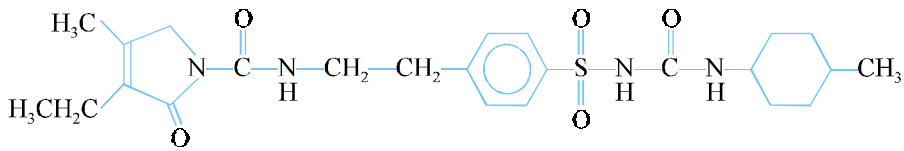




DRUG	pKa	Potency Compared to Tolbutamide
Glipizide	5.9	100 times more potent
Glyburide	5.3	200 times more potent

Obviously the presence of 'R' in **glyburide** potentiates the hypoglycemic activity 200 times, whereas the heterocylic nucleus in **glipizide** potentiates 100 times in comparison to tolbutamide.

3.1.2.3. Glimepiride USAN, INN



1-[[*p*-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulphonyl]-3 (*trans*-4-methylcyclohexyl) urea ;

Amaryl$^{(R)}$;

Its hypoglycemic activity is very much akin to **glipizide**.

Mechanism of Action. The '**drug**' is found to be metabolized primarily through oxidation of the alkyl side chain attached to the pyrrolidine nucleus *via* a minor metabolic path that essentially involves acetylation of the amine function.

SAR of Glimepiride. The only major distinct difference between this '*drug*' and **glipizide** is that the former contains a five-membered **'pyrrolidine ring'** whereas the latter contains a six-membered **'pyrazine ring'.**

3.2. Non-Sulfonylureas-Metaglinides

Metaglinides are nothing but **non sulphonylurea oral hypoglucemic agents** normally employed in the control and management of **type 2 diabetes** (*i.e.*, **non-insulin-dependent diabetes mellitus, NIDDM**). Interestingly, these agents have a tendency to show up a quick and rapid onset and a short duration of action. Just like the **'sulphonylureas'**, they also exert their action by inducing insulin-release from the prevailing functional pancreatic β-cells.

Importantly, the mechanism of action of the **'metaglinides'** is observed to differ from that of the '**sulphonylureas**'. In fact, the mechanism of action could be explained as under :

(*a*) through binding to the particular receptors in the β-cells membrane that ultimately lead to the closure of ATP-dependent K^+ channels, and

(*b*) K^+ channel blockade affords depolarizes the β-cell membrane, which iN turn gives rise to *Ca^{2+} influx, enhanced intracellular Ca^{2+}*, and finally stimulation of **insulin** secretion.

Based on the altogether different mechanism of action from the two aforesaid '**sulphonylureas**' there exist *two* distinct, major and spectacular existing differences between these two apparently similar categories of *'drug substances'*, namely :

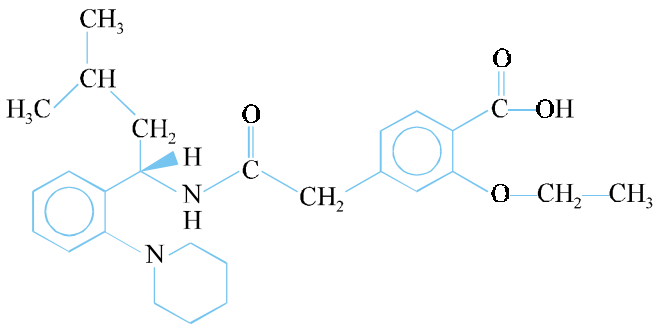
(*i*) **Metaglinides** usually produe substantially faster insulin production in comparison to the '**sulphonyl ureas**', and, therefore, these could be administered in-between meals by virtue of the fact that under these conditions pancreas would produce **insulin** in a relatively much shorter duration, and

(*ii*) **Metaglinides** do not exert a prolonged duration of action as those exhibited by the '**sulphonylureas**'. Its effect lasts for less than 1 hour whereas **sulphonylureas** continue to cause **insulin** generation for several hours.

Note : The glaring advantage of short duration of action by the metaglinides being that they possess comparatively much lesser risk of hypoglycemia in patients.

A few typical examples from this category are : **repaglinide**, **nateglinide** which would be treated as under :

3.2.1. Repaglinide USAN, INN



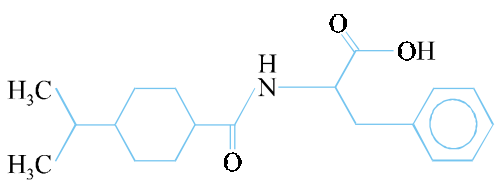
p-Toluic acid, (+)-2-ethoxy-α-[[(S)-α-isobutyl-*o*-piperidino-benzyl] carbamoyl]- ;

Prandin$^{(R)}$;

It is used in the control and management of **Type-2 diabetes mellitus**. It must be taken along with meals.

Mechanism of Action. The **'drug'** is found to exert its action by stimulating **insulin** secretion by binding to and inhibiting the ATP-dependent K^+ channels in the β-cell membrane, resulting ultimately in an opening of Ca^{2+} channels. It gets absorbed more or less rapidly and completely from the GI tract ; and also is exhaustively metabolized in the liver by *two* biochemical phenomena, such as : (*a*) glucuronidation ; and (*b*) oxidative biotransformation. Besides, it has been established that the **hepatic cytochrome P-450 system 3A4** is predominantly involved in the ultimate metabolism of **repaglinide**. However, this specific metabolism may be reasonably inhibited by certain drug substances', for instance : **miconazole**, **ketoconazole**, *and* **erythromycin**.

3.2.2. Nateglinide



N-(4-Isopropylcyclohexanecarbonyl)-D-phenylalanine ;

Starlix$^{(R)}$;

It is a phenylalanine structural analogue and belongs to the class of **'metaglinides'**. It is mostly employed in the control and management of **type 2 diabetes.**

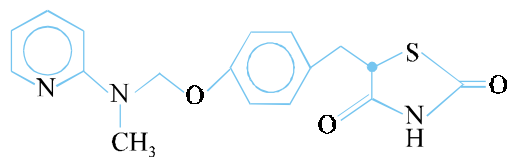
3.3. Thiazolindiones

The **thiazolindiones** exclusively designate a distinct and novel nonsulphonylurea group of potent hypoglycemic agents that are used invariably for the treatment of **NIDDM**. However, these **'drugs'** essentially needs a **'functioning pancreas'** which may give rise to the reasonably adequate secretion of insulin from β-cells, very much akin to the sulphonylureas. It has been observed duly that insulin may be released in *'normal levels'* from the β-cells ; however, the peripheral sensitivity to this particular hormone may be lowered appreciably. It has been amply established that **'thiazolindiones'** are highly selective agonists for the **peroxisome proliferator-activated receptor-r (PPARr)**, that is primarily responsible for improving **'glycemic control'** exclusively *via* the marked and pronounced efficacy of **insulin sensitivity** in the *adipose tissue* and *muscles.* Besides, they also prevent and inhibit the prevailing **hepatic gluconeogenesis.** In short, one may add that **thiazolindiones** invariably help to normalize blood-sugar level in two ways : (*a*) through glucose metabolism ; and (*b*) through reduction of the amount of **insulin** required to accomplish glycemic control.

Note : These agents are effective exclusively in the presence of 'insulin'

A few typical examples belonging to this class of compounds shall be discussed in the sections that follows :

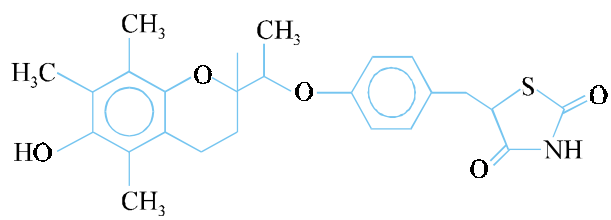
3.3.1. Rosiglitazone USAN



(±)-5-[[4-[2-(Methyl-2-pyridinylamino) ethoxy] phenyl] methyl]-2, 4-thiazolidinedione ; Avandia(R) ;

The **'drug'** has a single chiral centre (marked ●) ; and, therefore, exists as a racemate. Importantly, the enantiomers are found to be **'absolutely indistinguishable'** by virtue of their rapid *interconversion.*

3.3.2. Troglitazone



2, 4-Thiazolidinedione, (±)-5-[[4-[3, 4-dihydro-6-hydroxy-2, 5, 7, 8-tetramethyl-2H-1-benzopyran-2-yl) methoxy] phenyl] methyl]- ;

Rezulin(R) ;

The **'drug'** improves the responsiveness to insulin in such patients that experience **Type 2 diabetes mellitus** problems of **insulin** *resistance* initiated and sustained by a **'unique mechanism of action'** which is fairly comparable with those of other similar drugs. Importantly, it is at present **only approved for use with insulin.**

Mechanism of Action. The **'drug'** exerts its action by decreasing blood glucose in diabetic patients having *hyperglycemia* by improving target organ response to insulin. Besides, in the presence of both exogenous and endogenous insulin the **'drug'** minimizes the hepatic glucose output, enhances insulin-dependent glucose uptake, and finally lowers fatty acid output in adipose tissue.

It also gets bound to the nuclear receptors usually termed as **peroxisome proliferator-activated receptors** (**PPARs**) which predominantly regulate solely the transcription of a host of **insulin-responsive genes** that are found to be critical to **'glucose' and 'lipid' metabolism**.

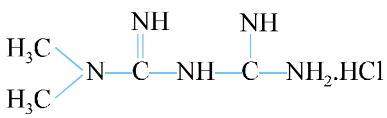
Note : The *'drug'* is not an insulin secretagogue.

Troglitazone is highly bound (> 99%) to serum albumin. It gets metabolized solely in the liver to several **inactive compounds,** including a *sulphate-conjugate*—a major metabolite, and mostly excreted in the faeces.

3.4. Bisguanides

The medicinal compounds included in this classification essentially comprise of two **'guanidine residues'** (*i.e.*, $H_2N—C(=NH)—NH_2$) joined together. A few typical examples belonging to this category, namely ; **metoformin**, **phenoformin**, are described as under :

3.4.1. Metoformin Hydrochloride USAN



Imidodicarbenimidic diamide, N, N-dimethyl-, monohydrochloride ;

Glucophage$^{(R)}$; Metiguanide$^{(R)}$;

It is used as an oral antihyperglycemic drug for the management of **Type 2 diabetes mellitus**. It is invariably recommended either as monotherapy or as an adjunct to diet or with a **sulphonylurea** (combination) to reduce blood-glucose levels.

Mechanism of Action. The **'drug'** is found to lower both basal and postprandial glucose. Interestingly, its mechanism of action is distinct from that of **sulphonylureas** and does not cause hypoglycemia. However, it distinctly lowers hepatic glucose production, reduces intestinal absorption of glucose, and ultimately improves **insulin sensitivity** by enhancing appreciably peripheral glucose uptake and its subsequent utilization. The **'drug'** is mostly eliminated unchanged in the urine, and fails to undergo hepatic metabolism.

3.4.2. Phenoformin

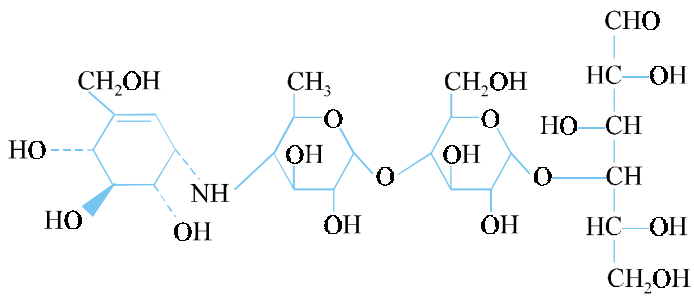
The **'drug'** is obsolete nowadays.

3.5. α-Glucosidase Inhibitors

It is quite well-known that the specific **enzyme α-glucosidase** is strategically located in the *brush-border* of the small intestine ; and, is exclusively responsible for affording cleavage of the dietary carbohydrates and thereby augmenting their rapid absorption into the body. Therefore, any means by which the inhibition of this enzyme is affected would certainly permit less-dietary carbohydrate to be available for absorption ; and, hence, less available in the blood-stream soon after ingestion of an usual meal. It has been observed that the prevailing inhibitory characteristic features of such agents are maximum for **glycoamylase,** followed by **sucrose**, **maltase** and **dextranase** respectively.

A few classical examples are discussed below :

3.5.1. Acarbose USAN, INN



Glucose, *o*-4, 6-dideoxy-4-[[[15-(1α, 4α, 5(3, 6α)]-4, 5, 6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl] amino]-α-*o*-glucopyranosyl-(1-4)-*o*-α-D-glucopyranosyl-(1-4)- ;

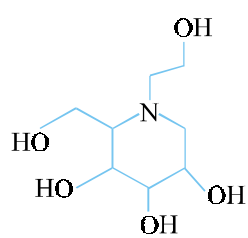
Precose(R) ;

It is used in the control and management of **Type 2 diabetes mellitus.**

Mechanism of Action. The '**drug**', which is obtained from the microorganism *Actinoplanes utahensis*, is found to a **complex oligosaccharide** that specifically delays digestion of indigested carbohydrates, thereby causing in a smaller rise in blood glucose levels soonafter meals. It fails to increase insulin secretion ; and its antihyperglycemic action is usually mediated by a sort of competitive, reversible **inhibition of pancreatic α-amylase membrane-bound intestinal α-glucosidase hydrolase enzymes.**

The **'drug'** is metabolized solely within the GI tract, chiefly by intestinal bacteria but also by diagestive enzymes.

3.5.2. Miglitol USAN, INN



1-(2-Hydroxyethyl)-2-(hydroxy-methyl)-[2R-(2α, 3β, 4α, 3β)]-piperidine ;

Glyset$^{(R)}$;

It also lowers blood-glucose level.

Mechanism of Action. It resembles closely to a sugar, having the heterocyclic nitrogen serving as an isosteric replacement of the *'sugar oxygen'*. The critical alteration in its structure enables its recognition by the **α-glycosidase as a substrate**. The ultimate outcome is the overall competitive inhibition of the enzyme which eventually delays complex carbohydrate absorption from the ensuing GI tract.

Probable Questions for B. Pharm. Examinations

1. (*a*) What are type-I and type-II 'Diabetes' ? Explain with some typical examples.

(*b*) Enumerate the various **'Salient Features'** of the *Insulin-Primary Structure.*

2. What are the various **'Insulin Products'** you have come across ? Discuss briefly any FIVE such products that are used abundantly.

3. How would you classify the 'Oral Hypoglycemic Agents' ? Give the structure, chemical name and uses of at least ONE potent compound that you have studied.

4. Give a brief account of the following with a few typical and important examples :

(*a*) First-Generation Sulfonylureas

(*b*) Second-Generation Sulfonylureas

5. How would synthesize the following 'Drugs' ? Explain the course of reaction(s) involved in the synthesis.

(*i*) Chlorpropamide

(*ii*) Tolbutamide

(*iii*) Glipizide

6. Explain the following :

(*i*) **Glyburide** is 200 times more potent than Tolbutamide.

(*ii*) **Glipizide** is 100 times more potent than Tolbutamide.

(*iii*) SAR of Glymepiride

(*iv*) Mechanism of action of Tolbutamide.

7. (*a*) Discuss the **'Metaglinides'** with regard to their specific 'mechanism of actions'.

(*b*) Give the structure and uses of any ONE of the following drugs :

(*i*) Repaglinide

(*ii*) Nateglinide.

8. Give a comprehensive account on **'Thiazolindiones'** with specific reference to the following potent **drugs** :

(*a*) Rosiglitazone

(*b*) Troglitazone.

9. Write a short note on the following **'oral hypoglycemic agents'** :

(*a*) Bisguanides ; and

(*b*) α-Glucosidase Inhibitors.

RECOMMENDED READINGS

1. Cook NS : ***Potassium Channels : Structure, Classification, Function and Therapeutic Potential***, John Wiley and Sons, New York, 1990.
2. Gennaro AR : ***Remington : The Science and Practice of Pharmacy,*** **Lippincott Williams and Wlikins**, Vol. II, 21st edn., 2006.
3. Meisheri KD : ***Direct Acting Vasodilators : In Singh BJ et al. (eds.) Cardiovascular Pharmacology***, Churchill Livingstone, New York, 1994.

23

Steroids

Chapter

23 Steroids

1. INTRODUCTION

The **steroids** constitute a group of structurally related compounds that are widely distributed both in the plant and the animal kingdom. The basic nucleus of these physiologically potent and biochemically dynamic medicinal compounds do possess a more or less similar stereochemical relationship. The **steroids**, in genreal, have been found to contain either the partly or completely **hydrogenated 17H-cyclopenta-phenanthrene** nucleus.

The **steroids** include a broad-spectrum of important compounds which exhibit remarkable pharmacodynamic properties, namely : **adrenal cortical hormones**, **sex hormones**, **cardiac glycosides**, **antirachitic vitamins (Vitamin D)**, **toad poisons**, **saponins**, **bile acids** and **some alkaloids**.

Broadly speaking both **steroid** hormones and related structural analogues constitute and designate one of the most abundantly employed categories of pharmacologically active and potent agents. These **'medicinal compounds'** are invariably used as first in importance in the control and management of birth control, inflammatory conditions, **hormone-replacement therapy (HRT)**, and above all in the treatment of neoplastic diseases (cancer). Interestingly, the plethora of these agents are exclusively based on a specific common structural nucleus usually termed as the'**steroid backbone'.** However, the different **steroidal variants** essentially attribute to the specific and unique molecular targets.

2. STEROID NOMENCLATURE, NUMBERING, DOUBLE BONDS AND STEREOCHEMISTRY

The general formula for the basic structure of the above cited compounds may be represented as follows :

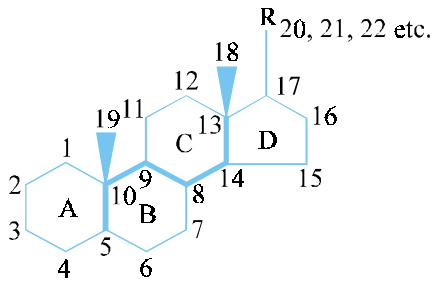


Fig. 2.3. General Steroid Formula.

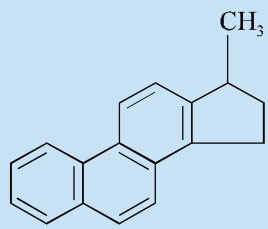
The rings are conventionally lettered and numbered as indicated above. However, in actual conformation the basic structure of **steroid** is not planar. It has also been observed that in the naturally occurring **steroidal compounds** the substitutions in the rings usually occur at C-3, C-7 and C-11 positions.

According to the standard convention the direction of projection from the plane of the ring system of substituting groups located at centres of asymmetry is usally designated by the Greek letters α and β.

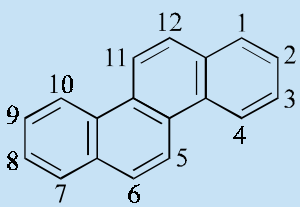
The α-substituting group is viewed as projecting beneath the ring plane and is conventionally represented by a broken line (dotted line).

The **β-substituting groups** is viewed as projecting above the ring plane and is normally represented by a solid line.

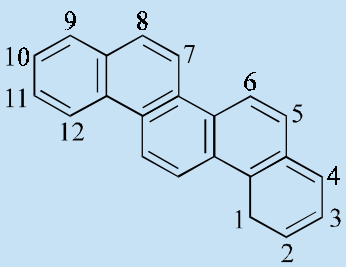
It has been observed that all the steroids on dehydrogenation with selenium at 360°C usually yield **Diel's hydrocarbon**, *i.e.*, **3′-methyl-1:2-cyclopentanophenanthrene**, whereas at 420°C, the **steroids** give mainly **chrysene** and a small amount of **picene**.



Diel's hydrocarbon



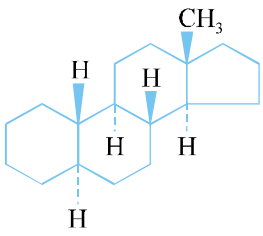
Chrysene
Or
1 : 2-Benzphenanthrene



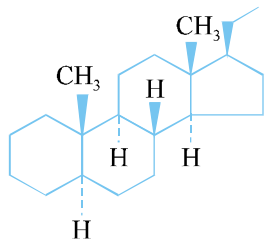
Picene
Or
1 : 2 : 7 : 8-Dibenzphen-anthrene

A few typical examples of **'steroidal drugs'** together with their **nomenclature** and **numbering** are illustrated below :

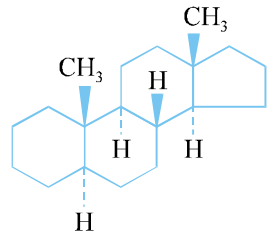
(*a*) **Common and Systematic Nomenclature :**



5α-Estrane

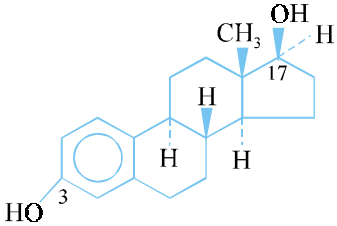


5α-Pregnane

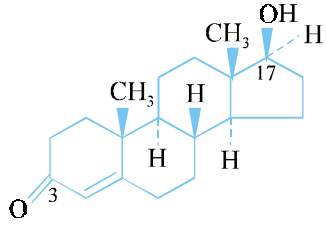


5α-Androstane

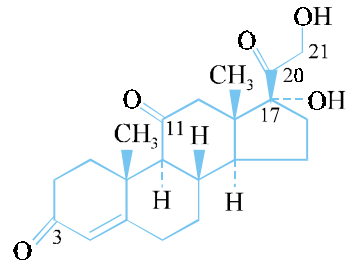
(*b*) **Nomenclature and Numbering :**



17β-Estradiol
[Estra-1, 3, 5(10)-triene-
3, 17β-diol]

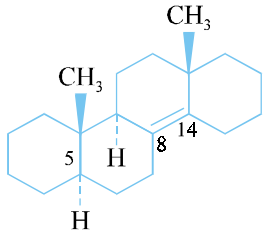


Testosterone
[17β-Hydroxyandrost-
4-en-3-one]

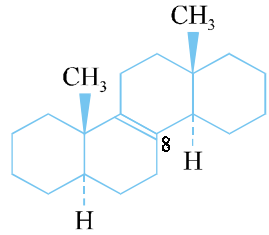


Cortisone
[17, 21-Dihydroxypregn-4-ene
– 3, 11, 20-trione]

(*c*) **Nomenclature and Double Bonds :**

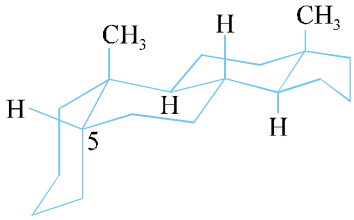


5α-Androst-8(14)-ene
or
5α-$\Delta^{8(14)}$-Androstene

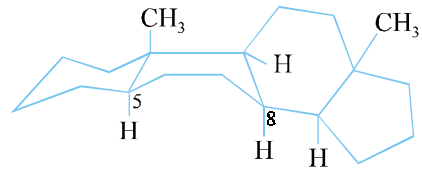


5α-Androst-8-ene
or
5α-Δ^{8}-Androstene

(*d*) **Nomenclature and Stereochemistry :**



5β-Androstane



5α, 8α-Androstane

Salient Features. The salient features with respect to the ***nomenclature*** **(IUPAC)** and **stereochemistry** are as enumerated below :

(1) Stereochemistry of the H-atom at C-5 is invariably incorporated in the 'name' itself,

(2) Stereochemistry of other H-atoms is **not** usually indicated unless and until it essentially happens to differ from **5 α-cholestane,** and

(3) Altering the stereochemistry at any of the **'ring-juncture'** with a heavy-dark line (see 'general steroid formula's Fig. 23.1) changes immensely the prevailing '**shape of the steroid'**, as may be observed in the above cited examples of **5β-androstane** and **5α, 8α-androstane**.

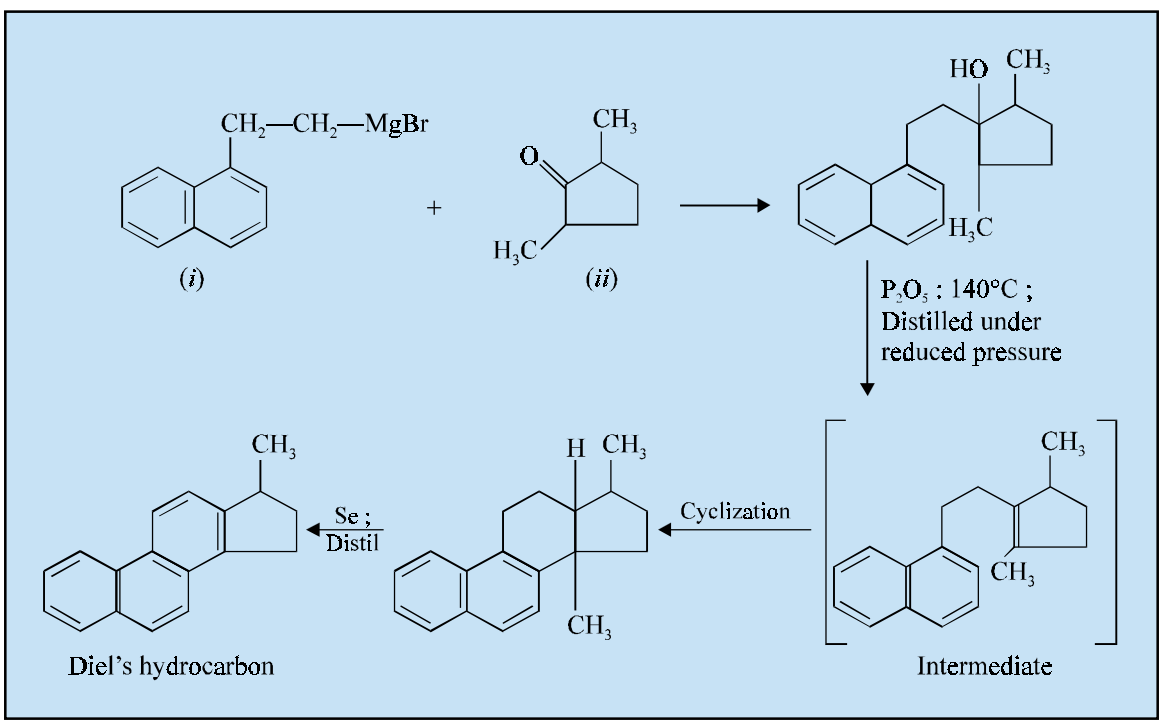
2.1. Diel's Hydrocarbon

It is a solid substance having a melting point 126-127°C and a molecular formula $C_{18}H_{16}$. Based on the results of oxidation reactions, X-rays crystal analysis coupled with absorption spectrum measurements it was revealed that the hydrocarbon in question could be **3′-methyl-1:2-cyclopentanophenanthrene**. The next essential step was to establish the structure of this compound by synthesis, *e.g.*, that of Harper *et al* (1934) who used the **Bogert-Cook method** commencing from :

(*i*) 2-(1-naphthyl)-ethyl-magnesium bromide

(*ii*) 2:5-dimethylcyclopentanone

2-(1-Naphthyl)-ethyl-magnesium bromide and 2:5-dimethyl-cyclopentanone react to give a condensed product which on oxidation with phosphorus pentoxide at 140°C and subsequent distillation under reduced pressure yields an intermediate. This undergoes cyclization first and later on when distilled with selenium gives the **Diel's hydrocarbon**.



3. CLASSIFICATION

Various authors have used slightly different means of classifying the **steroids**, but the one selected here divides them into *five* categories depending solely on the type of substituent group at C-17, *i.e.*, group R.

(*i*) **Sterols**—where R is an aliphatic side chain. They contain usually one or more hydroxyl groups attached in alicyclic linkage.

(*ii*) **Sex Hormones**—where R bears a ketonic or hydroxyl group and mostly possesses a two-carbon side chain.

(*iii*) **Cardiac Glycosides**—where R is a lactone ring. The **glycosides** also contain sugars linked through oxygen in other parts of the molecule. Normally on hydrolysis it yields this sugar together with the cardiac aglycone.

(*iv*) **Bile Acids**—where R is essentially a five-carbon side chain ending with a carboxylic acid moiety.

(*v*) **Sapogenins**—where R contains an oxacyclic (ethereal) ring system.

3.1. Sterols

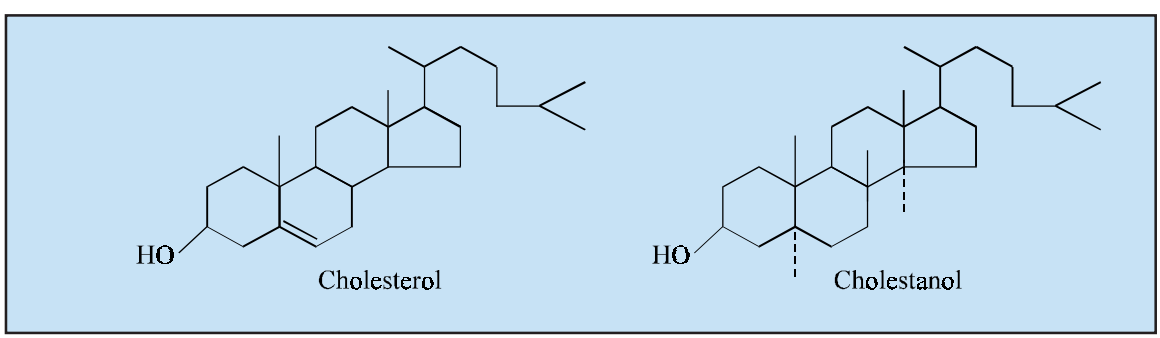
The term **sterols** has been coined from the words **'steroidal alcohols'**. They have been found to occur both in animal and plant oils and fats. These are usually crystalline compounds and mostly bear an alcoholic group. They may occur either as free or as esters of the higher fatty acids, and are isolated from the unsaponifiable fraction of oils and fats.

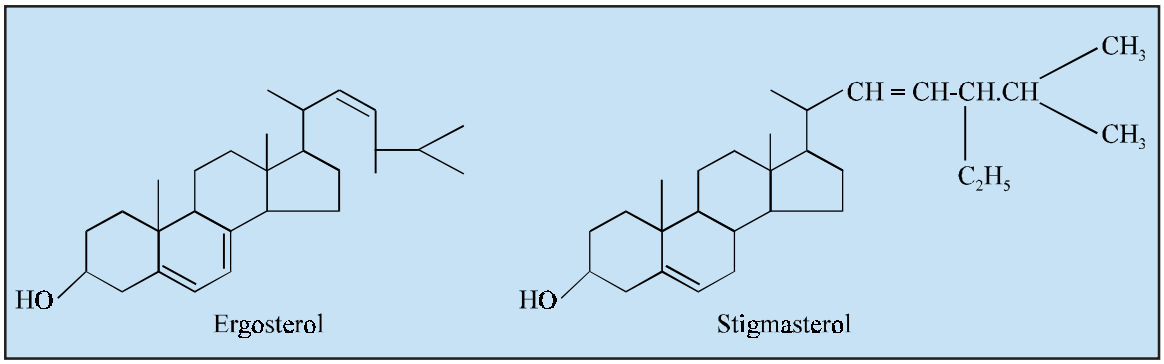
The **sterols** may be further sub-divided into the following ***three*** categories, namely :

(*a*) **Zoosterols**—such sterols those are obtained from the animal kingdom only, *e.g.*, **cholesterol**, **cholestanol**, **coprostanol (coprosterol)**, etc.

(*b*) **Phytosterols**—such sterols those are derived exclusively from the plant sources, *e.g.*, **ergosterol**, **stigmasterol**, **sitosterols**, etc.

(*c*) **Mycosterols**—such **sterols** those are obtained from either yeast or fungi. It is pertinent to mention here that this particular classification is not quite rigid because of the fact that some sterols are obtained from more than one of these groups.





Ergosterol Stigmasterol

3.2. Sex Hormones

Generally, **hormones** are substances that are secreted by the ductless glands, and only minute amounts are necessary to produce the various physiological reaction in the body.

However, the **sex-hormones** belong to the steroid class of compounds and are produced in the gonads, *i.e.*, testes in the male and ovaries in the female. In fact, their activity seems to be controlled and monitored by the hormones that are produced in the anterior lobe of the pituitary glands. Perhaps because of this inherent characteristies the **sex hormones** are invariably termed as the secondary **sex hormones** and the **hormones** of the anterior lobe of the pituitary are called the primary **sex-hormones**.

A general survey of the literature stretching over the past three decades would reveal that a vast number of structural modifications of the steroid hormones have taken place. These newer compounds have been prepared with a view to enhance their biological activities, oral activity and duration of action, besides attributing better solubility properties, minimising the requirement for some essential perimeter functional group of the parent hormone and lastly to effect a marked separation of their biological activities.

These modifications have been duly accomplished through a number of means, for instance, protecting some vital moieties against the metabolic attack or attack by intestinal bacteria, prevention of the conversion *in vivo* of one **steroid hormone** into another steriod and lastly through alteration of physical properties by preparing their respective 19-nor analogues, ester derivatives, enol ethers, acetals and ketals, bringing about conformational changes and electron attracting effect.

3.2.1. Classification

Sex-hormones are usually classified under the following *three* heads, namely :

(*i*) **Androgens** (Male Hormones) *e.g.*, **androsterone**, **testosterone**.

(*ii*) **Oestrogens** (Female or Follicular Hormones), *e.g.*, **oestrone**, **oestriol**, **oestradiol**, **stilbesterol**, **hexesterol**.

(*iii*) **Gestogens** (The Corpus Luteum Hormones) *e.g.*, **progesterone**.

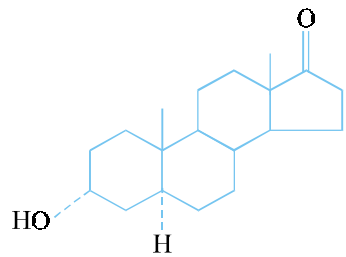
3.2.2. Androgens

Experiments with testicular extract has more or less enjoyed a chequered career. Veronoff successfully transplanted tested from monkeys into elderly men and claimed to rejuvenate them. Likewise, ligature of *vas deferens* which is known to cause atrophy of spermatogenic tissue and indirectly hypertrophy ofintestinal tissue which secrete **testosterone**. Another researcher Steinach carried out similar studies by ligaturing the *vas deferens* and obtained identical results.

Androgens besides showing a specific action on gonads, also stimulate production of elements that are absolutely essential for all tissue growth. This characteristic which the **androgens** share with other **steroidal hormones**, such as : **corticosterones** and **oestrogens** has paved the way towards synthesis of newer steroids that possess mainly metabolic activity without androgenic effect.

The *two* most important **androgens** are **androsterone** and **testosterone**.

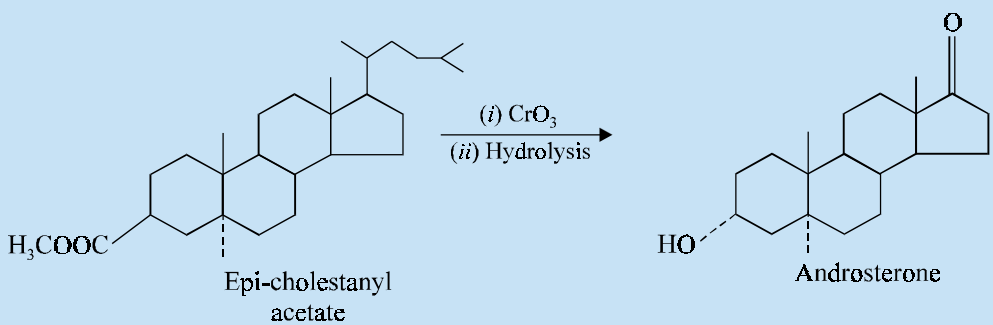
A. Androsterone



3α-Hydroxy-5α-androstan-17-one.

Androsterone (m.p. 185°C) is a **naturally occurring androgen** that may be isolated from male urine. It can also be synthesized from **epi-cholestanyl acetate** as given below :

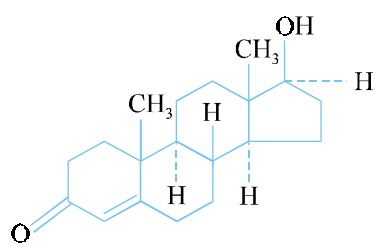
Synthesis



It may be synthesized from epi-cholestanyl acetate at two stages : *first*, by its oxidation with chromium-6-oxide and *secondly*, by subjecting the resulting product to hydrolysis to yield the desired product.

Butanandt and co-workers (1931) first isolated **androsterone** (15 mg) from 15,000 litres of urine.

B. Testosterone INN, BAN, USAN,



Testost. 17β-Hydroxyandrost-4-en-3-one ; Androst-4- en-3-one, 17-hydroxy-, (17β)- ; BP, USP ; Synadrol F(R) (Pfizer) ; Mertestate(R) (Sterling).

Synthesis

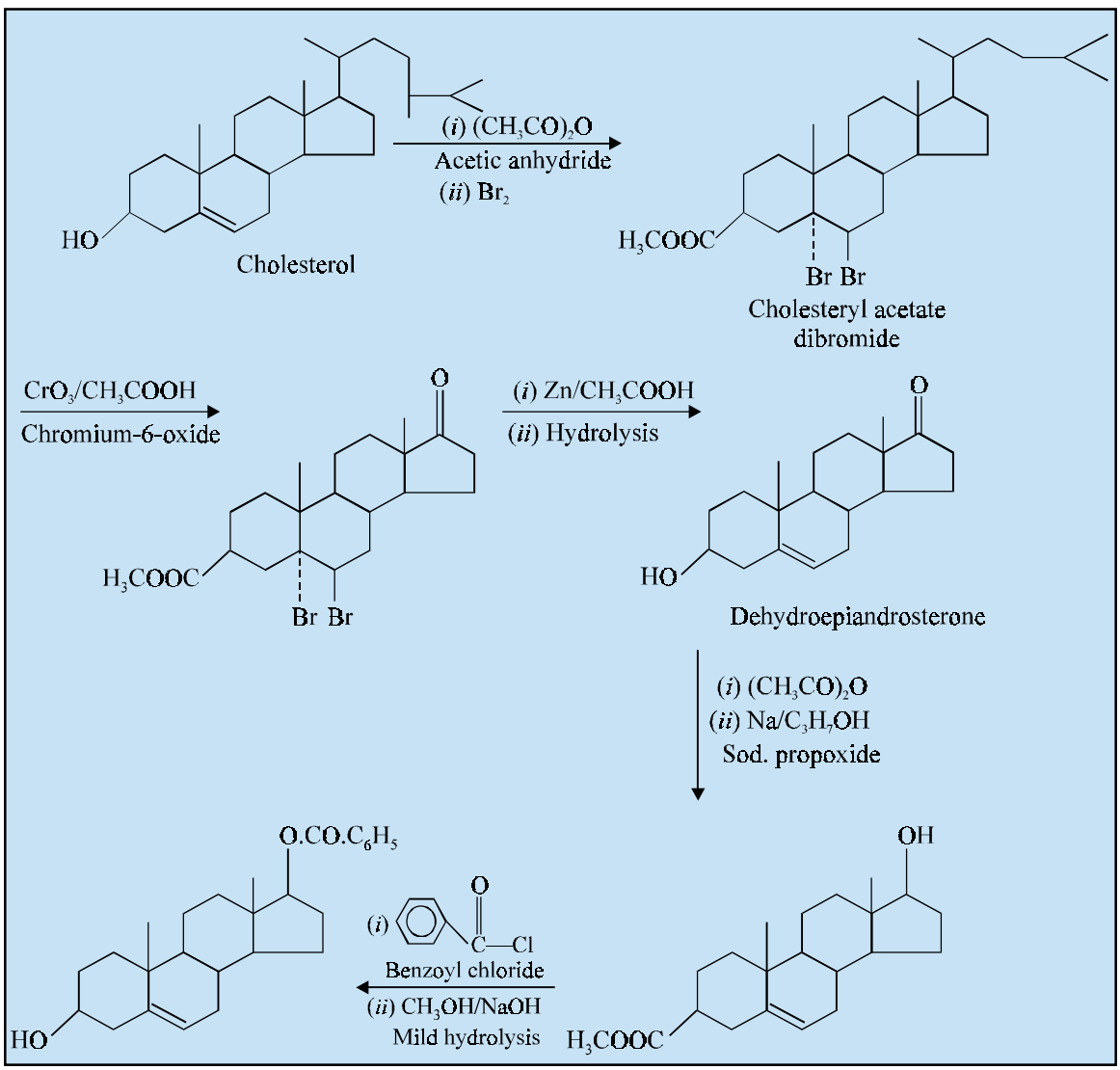
Testosterone may be synthesized from the following *two* starting materials, namely ;

(*i*) From Cholesterol, Butenandt (1935) ; Ruzica (1935) ; Oppenauer (1937) ;

(*ii*) From Dehydroepiandrosterone, Mamoli (1938).

(a) From Cholesterol

Cholesteryl acetate dibromide is first prepared by the acetylation of chloesterol and its subsequent bromination. This on oxidation with chromium-6-oxide reduces the 8-carbon side chain at C-17 to a mere CO moiety, which on reduction followed by hydrolysis yields dehydroepiandro-sterone. The resulting product on acetylation protects the acetyl moiety at C-3 and treatment with sodium propoxide introduces a hydroxy group at C-17. Benzoylation followed by mild hydrolysis causes the reappearances of free OH moiety at C-3 and a benzoxy function at C-17. **Oppeanauer oxidation** cuased by refluxing the resulting secondary alcohol with aluminium tertiary butoxide in excess of acetone affords a ketonic function at C-3, which upon hydrolysis in an alkaline medium yields the official compound.



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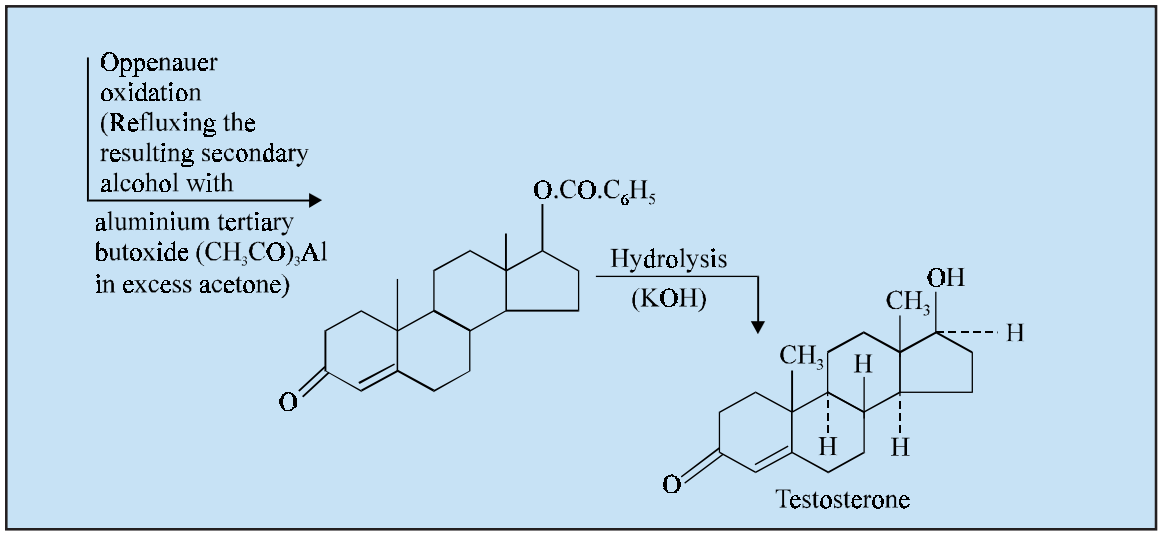
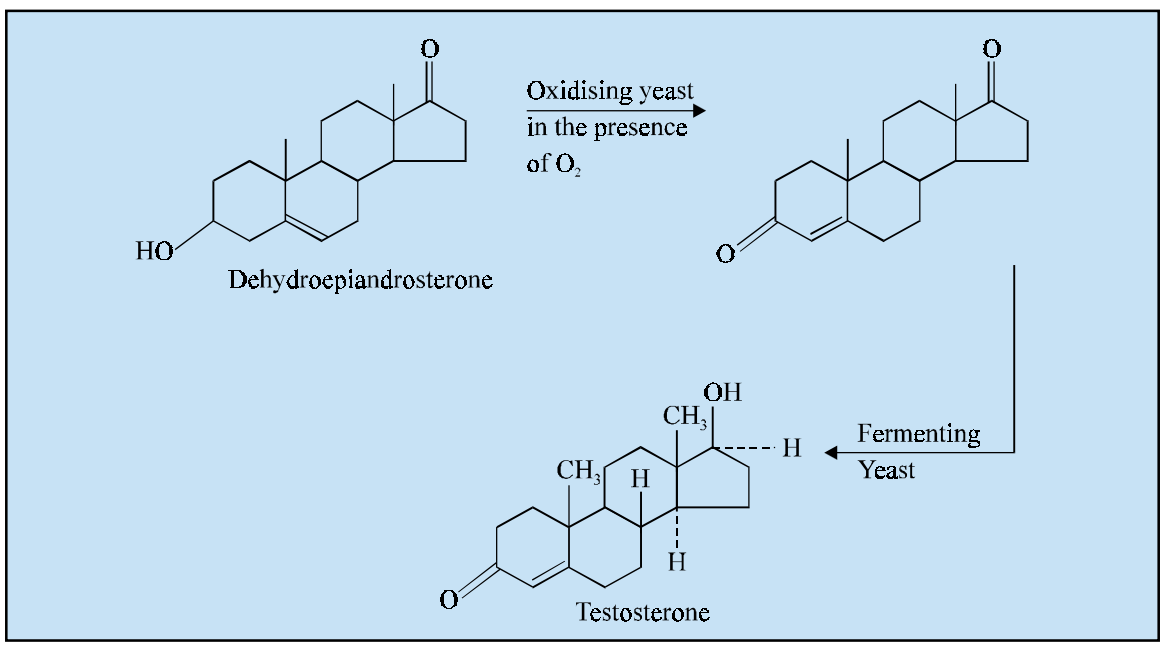


(*b*) *From Dehydroepiandrosterone*



Dehydroepiandrosterone first on being treated with oxidising yeast in the presence of oxygen and secondly by the fermenting yeast yields the desired official compound.

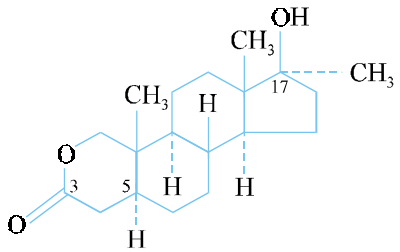
Testosterone controls the development as well as maintenance of the male sex organs and is solely responsible for the male secondary sex characteristics.

It also increases the size of the serotum, phallus, seminal vesicles, prostrate and enhances the sexual activity in adolescent males.

Testosterone *along with other androgens are invariably employed in the male for replacement therapy in hypogonadism, eunuchoidism, and the male climacteric.*

Dose : *For prolonged treatment, subcutaneously, 600 mg; For breast cancer up to 1.5g ; Alternatively 10 to 30 mg per day through the buccal administration.*

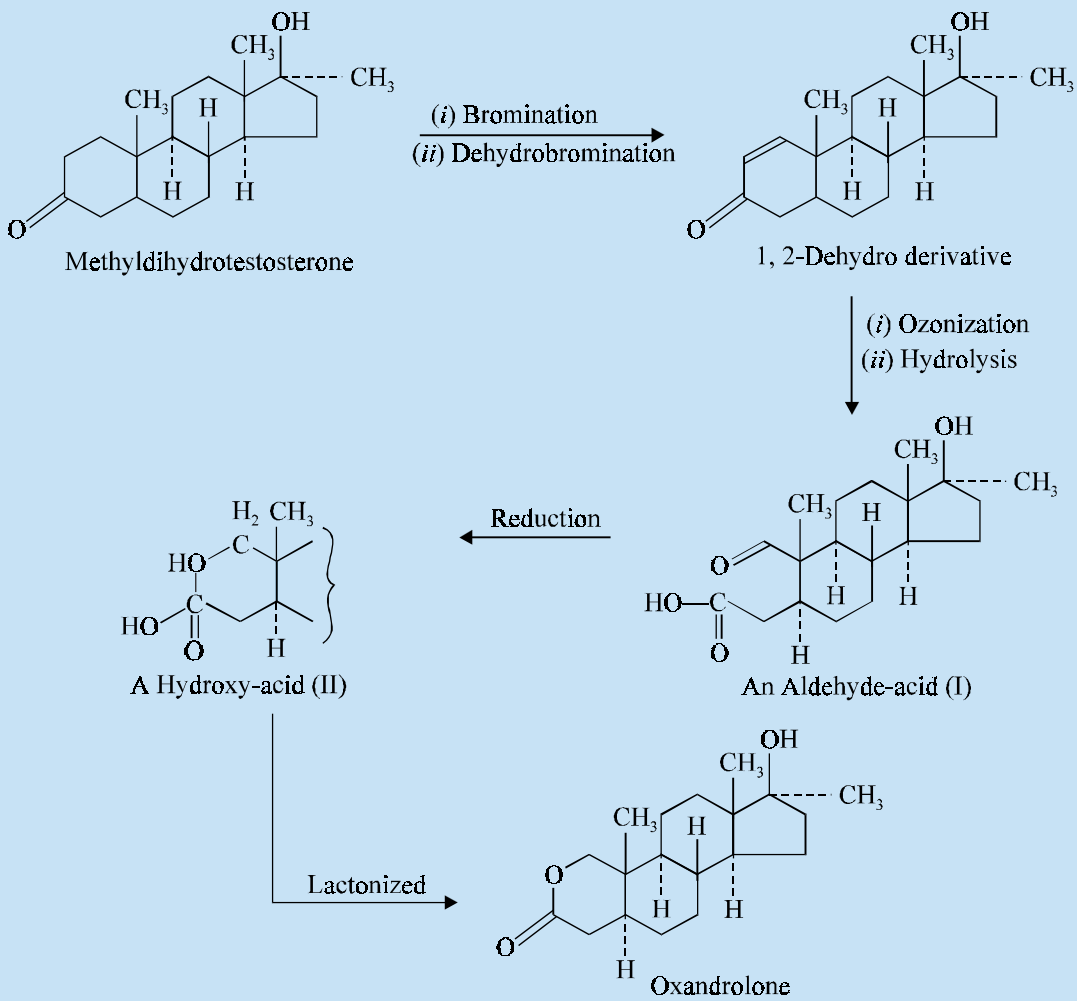
(*c*) *Oxandrolone* USAN, BAN, INN



(5α, 17β)-2-Oxandrostan-3-one, 17-hydroxy-17-methyl-; USP;

Oxandrin(R);

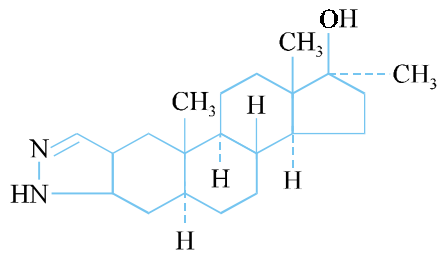
Synthesis



Methyldihydrotestosterone on being subjected to bromination followed by dehydrobromination gives rise to the formation of 1, 2-dehydro derivative, which upon ozonization and hydrolysis yields an aldehyde-acid (I). The resulting acid (I) on reduction produces the corresponding hydroxy-acid (II) which when lactonized produces the desired compound, **oxandrolone**.

It is an androgenic steroid having comparatively higher **anabolic activity** in relation to the **androgenic activity**. Hence, it is used mostly to promote nitrogen anabolism (protein synthesis) and weight-gain in cachexia* and other debilitating diseases and after serious infections, burns, trauma or surgical procedures. It may also be employed to relieve pain in some types of **osteoporosis** thereby augmenting Ca^{2+} retention and hence improving the condition of bone. It also finds its application for its predominant erythropoetic effects in the treatment of both **hypoplastic** and **aplastic anemias**.**

D. *Stanozolol* USAN, BAN, INN



(5α, 17β)-2′H-Androst-2-enol [3, 2-C] pyrazol-17-ol, 17-methyl ; USP ;

Winstrol$^{(R)}$;

It is an androgen having comparatively *strong anabolic* and weak **androgenic activity**. Its uses are almost identical to that of oxandrolone. Besides, it is also employed in the prophylaxis of hereditary angiodema, which is presently the only approved use.

3.2.2.1. Mechanism of Action

The mechanism of action of the various compounds described under Section 23.3.2.2. shall now be treated individually as under :

3.2.2.1.1. Androsterone

Being one of the naturally occurring androgens it exerts widespread anabolic effects. The '**drug**' also affects hypopituitarism and with **Addison's disease**, relief of impotence not associated with evidence of testicular underactivity, pituitary dwarfism to accelerate growth, and in functional dysmenorrhea giving relief through an antiestrogenic action.

3.2.2.1.2. Testosterone

The '**drug**' undergoes metabolism that may lead to either pharmacologically **active steroids** *e.g.*, **estradiol**, **5α-dihydrotestosterone** (or **5α-DHT**), and **androsterone** ; or to **inactive steroids** *e.g.*, **6α-hydroxytestosterone**, **epitestosterone**, and **etiocholanolone**.***

---

*A state of ill-health, malnutrition, and wasting (*e.g.*, chronic diseases, certain malignancies and advanced pulmonary tuberculosis.

***Hypoplastic i.e.*, aplastic anemia ; *Aplastic :* Anemia caused by deficient red cell production due to bone-marrow disorders.

***RW Brueggemier, **Burger's Medicinal Chemistry**, 5th edn, Vol : 3, ME Wolff ed., John Wiley & Sons, NewYork, pp 445-510, 1996.

However, the enzyme **5α-reductase** brings about the following changes, namely :

(*a*) In prostate gland (an androgen target tissue) testosterone gets converted to 5α-DHT, which enjoys the reputation of being the *most potent endogenous androgen metabolite of* **testosterone**, *and*

(*b*) It helps to catalyze an irreversible reaction for which it essentially needs NADPH as a cofactor that strategically provides the H-atom at C-5.*

It is found to be not effective when administered orally as it almost gets destroyed in the liver on absorption. Its plasma half-life ranges between 10-20 minutes.

3.2.2.1.3. Oxandrolone

The '**drug**' exerts its action by virtue of its inherited protein catabolism associated with long-term usage of **corticosteroid**. Besides, it is also indicated in HIV wasting syndrome and alcoholic hepatitis.

Note. Strictly speaking it is not a 'steroid', and its configuration is that of a 17-methyl androgenic steroid.

3.2.2.1.4. Stanzolol

The '**drug**' acts by significantly lowering the frequency and severity of attacks in angioedema ; and it is now the only approved application.

3.2.2.2. Derivatives of Testosterone

There are, in fact, quite a few improtant derivatives of testosterone that have been used extensively in therapy ; and these are summarized in Table 23.1.

Table 23.1. Derivatives of Testosterone

Approved Names	Official Status	Proprietary Names	Dose
Testosterone Acetate	—	Cetovister(R) (Substancia, Spain)	—
Testosterone Cypionate	USP ;	dep Andro 100(R) (Forest)	50 to 200 mg/ml in oil solution
Testosterone Decanoate	BP ;	—	—
Testosterone Enanthate	BP ; USP ;	Delatestryl(R) (Squibb)	100 to 400 mg every 2 to 4 weeks
Testosterone Isocaproate	BP ;	—	—
Testosterone Ketilaurate	—	—	—
Testosterone Phenylacetate	—	—	—
Testosterone	BP ;	Tess PP(R)	—

(*Contd...*)

*P Ofner, *Vit Horm*., **26,** 237, 1968.

Phenylpropionate		(Organon)	
Testosterone Propionate	BP ; USP ; Eur. P. ; Int. P. ; IP. ;	Synadrol(R) (Pfizer)	5 to 20 mg daily as buccal tablets
Testosterone Undecanoate	—	Restandol(R) (Organon, UK)	40 to 160 mg daily

3.2.3. Oestrogens

The **oestrogens** are mainly concerned with growth and function of the sex organs.

In general, they are classified under *two* sub-heads, namely :

(*a*) Steroidal Oestrogens

(*b*) Non-steroidal Oestrogens

(*a*) Steroidal Oestrogens

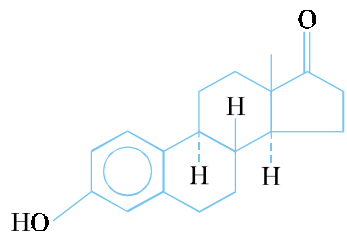
All of them essentially possess a steroidal nucleus and attribute oestrogenic activity.

Examples : Oestrone, oestriol, oestradiol.

***A.* Estrone INN, USAN Oestrone, BAN,**

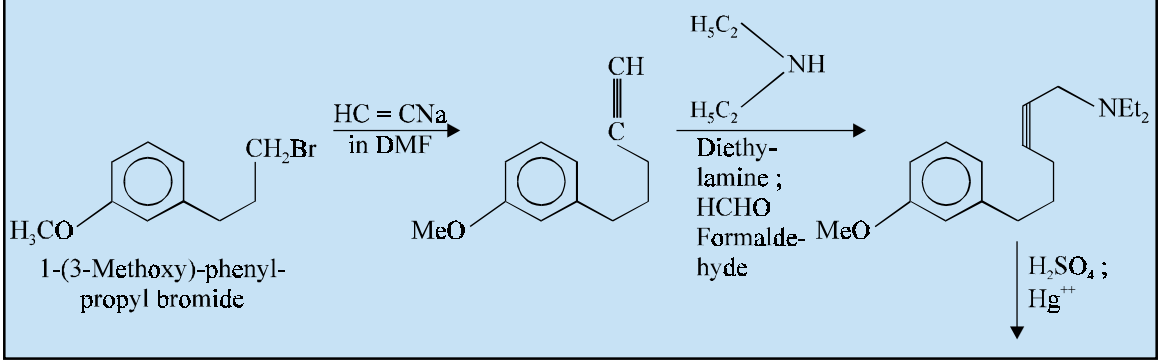
3-Hydroxyestra-1, 3, 5 (10)-trien-17-one ; Estra-1-3, 5(10)-trien-17-one, 3 hydroxy-; Oestrone Eur. P. ; Estrone USP ;

Theelin(R) (Parke-Davis).



Synthesis

Johnson and co-workers (1958, 1962) have carried out a total synthesis of **oestrone** ; each step in their synthesis was **stereoselective**, but Hughs and co-workers (1960) have put forward a total synthesis of oestrone which appear to be comparatively simpler than any other previous method.



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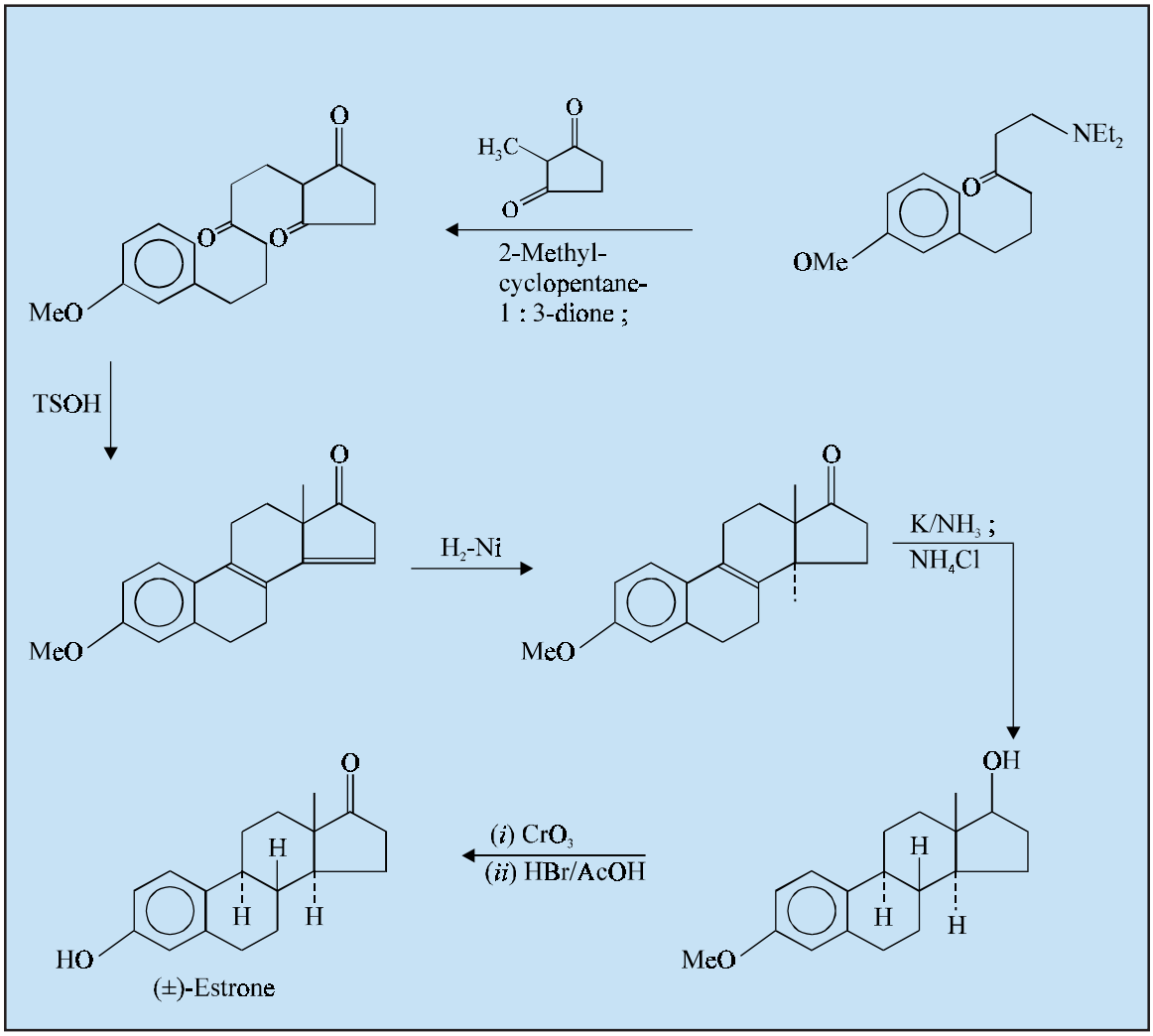
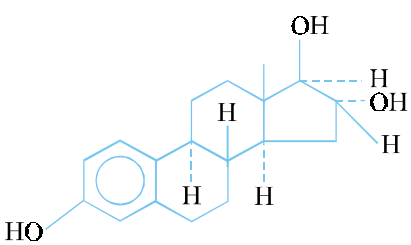


It is mainly used for the replacement therapy in deficiency states, e.g., primary amenorrhoea, delayed onset of puberty, control and management of menopausal syndrome, malignant neoplasms of the prostate.

Dose : *0.1 to 5mg per day.*

B. Estriol INN, USAN, Oestriol, BAN,



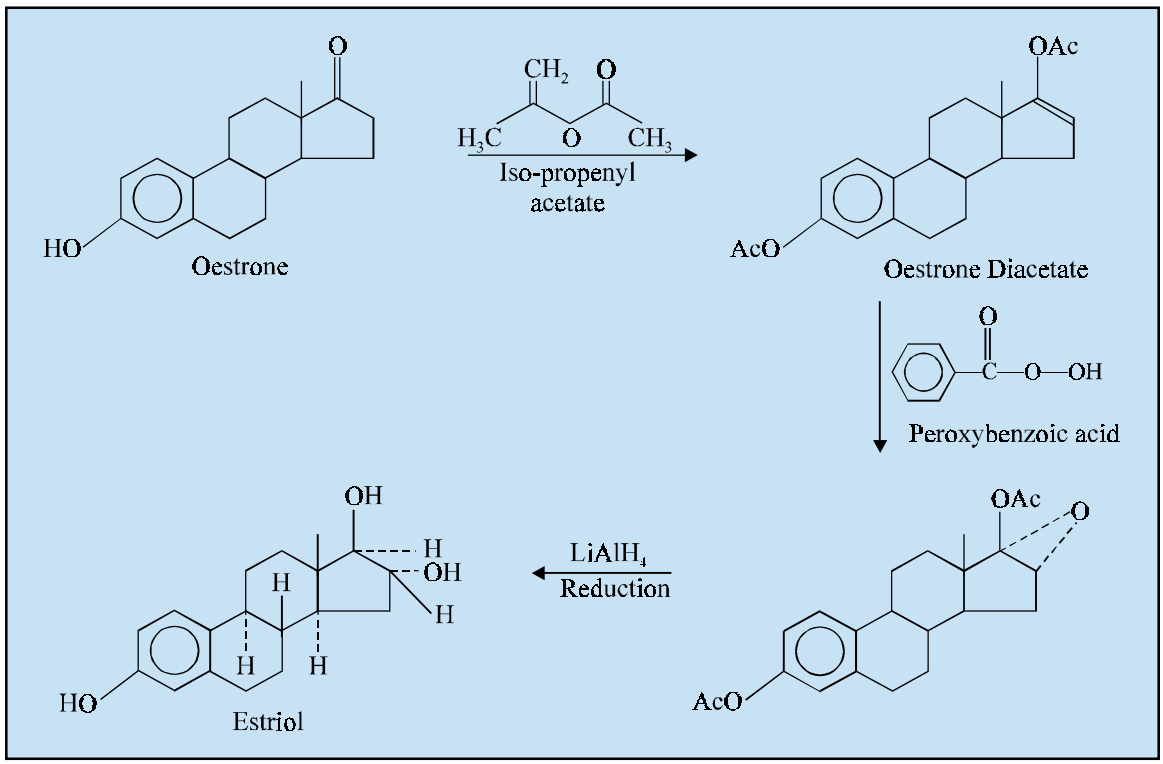
Estra-1,3,5 (10-triene-3,16α,17β- triol ; Estriol USP ;

Ovestin(R) (Organon, UK).

Soon after the discovery of oestrone *two* other hormones were isolated, namely : **oestriol** and **oestradiol**. **Oestriol** was first isolated from human pregnancy urine.

Synthesis

Leeds et al. (1954) have converted **oestrone** into **oestriol** by a simple method as discussed below.

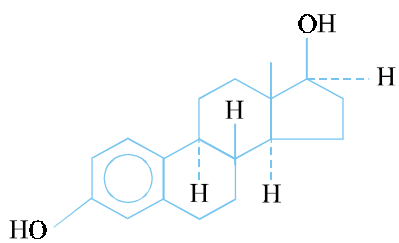


Oestrone on treatment with iso-propenyl acetate yields the corresponding diacetate which on reaction with peroxybenzoic acid removes the double bond between C-16 and C-17 and introduces an oxygen bridge having alpha configuration between the said two carbon atoms. This on reduction with lithium aluminium hydride yields the official compound.

Oestriol *is more potent than either* **oestrone** *or* **oestradiol** *in its oestrogenic activity when administered orally. It is reported to possess a selective action on the vagina and cervix.*

Dose. *For menopausal symptoms, 250 to 500 mcg per day.*

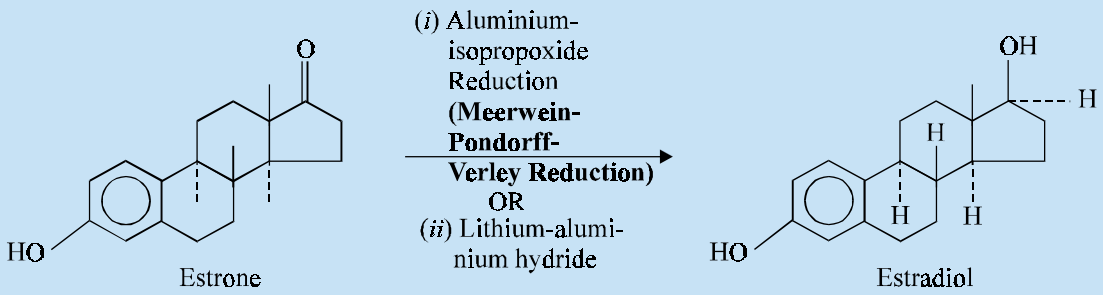
C. Estradiol INN, USAN, Oestradiol BAN,



Estra-1, 3, 5 (10)-triene-3, 17β-diol ; Beta-oestradiol; Estradiol USP ; Oestradiol (BPC1968) ; Diogyn(R) (Pfizer) ; Oestradiol Implants(R) (Organon, U.K.).

Estradiol was first obtained by the reduction of **oestrone**, but later it was isolated from the ovaries of cows.

Synthesis



Estradiol may be prepared conveniently by the reduction of **estrone** either with aluminium isopropoxide or with lithium-aluminium-hydride.

Estradiol *is found to be the most active of the naturally occurring oestrogenic hormones produced in the ovarian follicles under the influence of the pituitary. It helps to regulate and subsequent maintenance of the female sex organs, certain functions of the human uterus and above all the secondary sex features, and the mammary glands.*

Dose. *Oral, 2 mg per day ; intramuscular, 1.5 mg 2 or 3 times weekly ; implantation, 20 to 100 mg.*

A number of derivatives of **estradiol** have been employed in the control and management of oestrogenic activity and these are summarized in Table 23.2.

Table 23.2. Derivatives of Estradiol

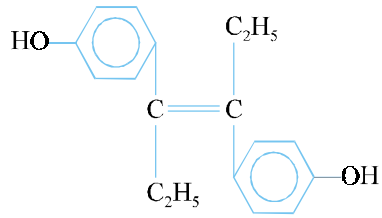
Approved Names	Official Status	Proprietary Names	Dose
Oestradiol Benzoate	BP; Eur. P. ;	**Benzotrone**(R) (Paines & Byrne, U.K.)	1 to 5 mg daily
Estradiol Cypionate	USP ;	**Depo-Estradiol Cypionare**(R) (Upjohn, USA)	1 to 5mg intramuscular every 3 to 4 weeks
Oestradiol Dipropionate	BPC (1954) ; Ind. P. ;	**Ovocyclin**(R) (Ciba-Geigy, Switz),	1 to 5mg i.m. every 1 to 2 weeks
Estradiol Enanthate	—	—	10 mg
Oestradiol Undecanoate	—	**Primogyn Depot**(R) (Schering)	100 to 200 mg every 2 to 3 weeks
Estradiol Valerate	USP ;	**Delestrogen**(R) (Squibb) ;	5 to 40 mg every 1 to 3 weeks

(*b*) Non-Steroidal Oestrogens

A large number of medicinal compounds possessing remarkable oestrogenic activity,but not of steroidal structure (nucleus), have been prepared *synthetically*.

Examples. Diethylstibesterol : **Hexestrol** ; **Dienestrol**.

A. Diethylstilbesterol INN, USAN, Stilbesterol BAN,



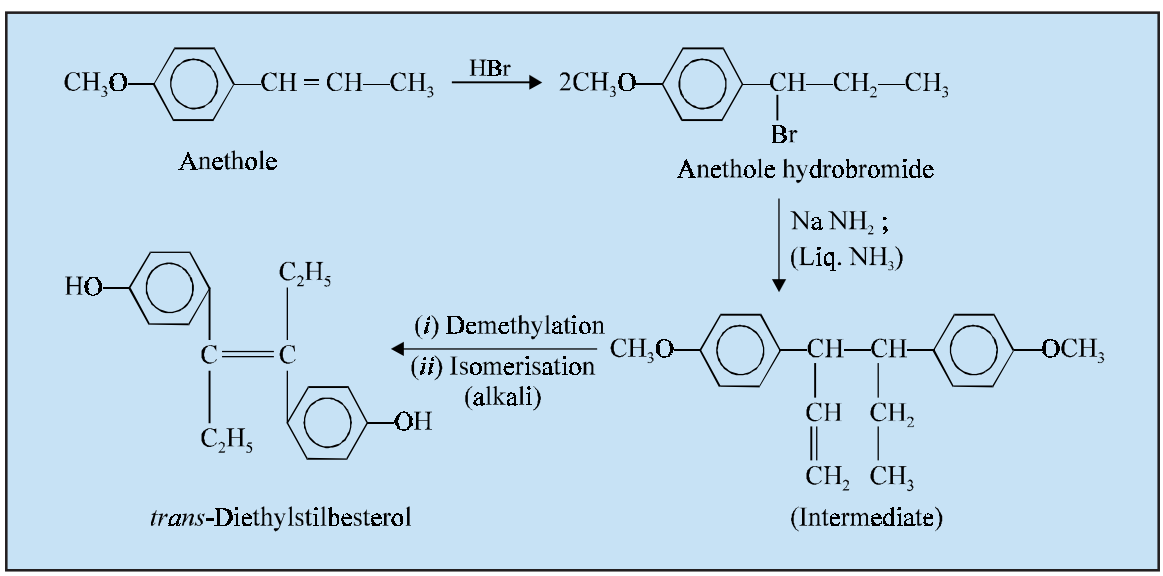
(E)-αβ-Diethylstilbene-4-4′ diol ; Phenol 4,4′-(1,2-diethyl-1,2-ethenediyl) *bis*-(E)- ; Diethylstilbesterol (USP) ; Stilboesterol (BP ; Eur. P. ; Int. P. ; Ind. P.) ;

Stilbetin$^{(R)}$ (Squibb).

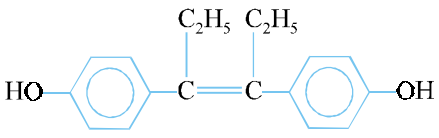
Synthesis

Diethylstilbesterol may be synthesized by *two* different methods. *First*, from anisaldehyde (Dodds and Lawson, 1939) ; and *secondly*, from anethole (Kharasch *et al.* 1943). The latter shall be discussed here.

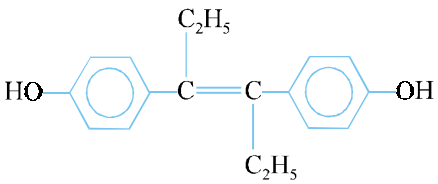
Anethole on treatment with hydrogen bromide undergoes **Markownikoff's addition** to yield anethole hydrobromide. The resulting product in the presence of sodamide and liquid ammonia gives an intermediate product which on subsequent demethylation followed by isomerization in alkali yields the official compound.



There exists **two geometrical isomeric** forms of **diethyl-stilbesterol** ; *cis*- and *trans*-out of which only the latter exhibits potent oestrogenic activity.



cis-Stilbesterol

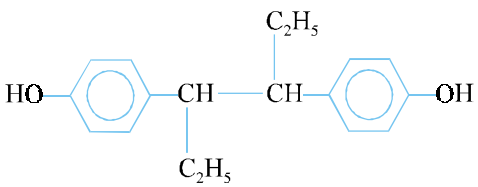


trans-Stilbesterol

It is a synthetic **non-steroidal oestrogen** having similar actions and uses to those of oestradiol. It is used in the *treatment of menopausal symptoms and in secondary amenorhoea due to ovarian insufficiency. It has also been recommended for the inhibition of lactation, in the palliative treatment of malignant neoplasms of the breast, in carcinoma of the prostate and for postcoital contraception.*

Dose. *For menopausal symptoms, oral, 0.1 to 2 mg; for secondary amenorrhoea, 0.2 to 0.5 mg ; for carcinoma of prostate, 3 mg per day.*

B. Hexestrol INN, Hexoestrol BAN,

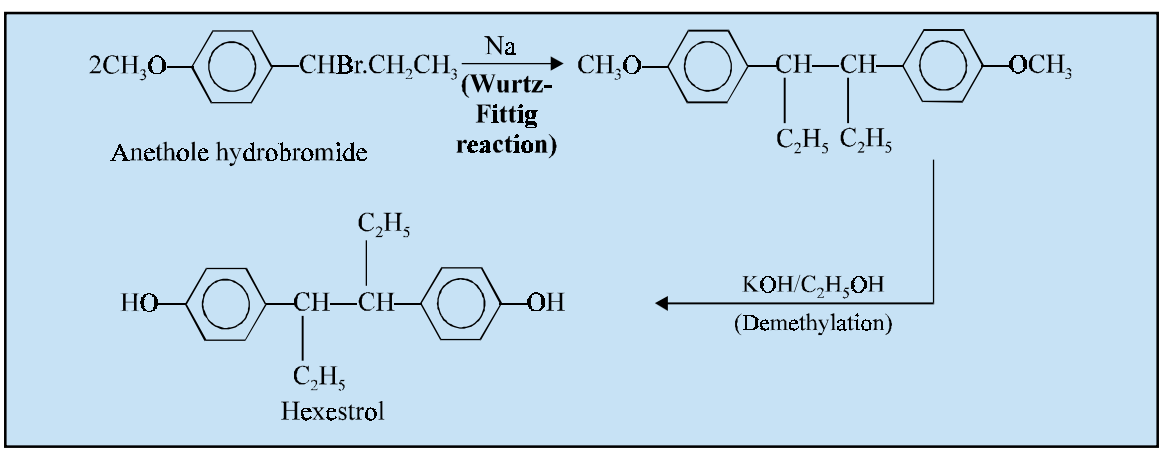


meso-4, 4′-(1,2-Dimethylethylene) diphenol ; Dihydrostilboestrol ; Hexanoestrol ; BPC (1968) ; Ind. P. ;

Hormoestrol(R) (Siegfried, Switz).

Synthesis

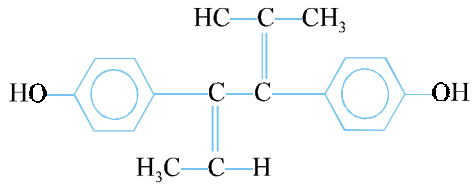
Hexestrol is prepared by subjecting anethole hydrobromide to the **Wurtz-Fittig Reaction** in the presence of sodium to get the corresponding diethyl derivative. This on further demethylation in the presence of alcoholic potassium hydroxide yields the official compound.



It is used for menopausal symptoms and also for the treatment of neoplasms of the breast and prostate.

Dose. *Oral, usual, 1 to 5 mg.*

C. Dienestrol INN, USAN, Dienoestrol BAN,



(E.E)-4, 4′-Di (ethylidene) ethylene diphenol ; Phenol, 4, 4′-(1, 2-diethyl-idene-1, 2-ethanediyl) *bis*-, (E,E)- ; Dienoestrol (BP ; Eur.P., Int. P ; Ind. P ; Dienoestrol (USP) ;

Estraguard(R) (Reid-Provident).

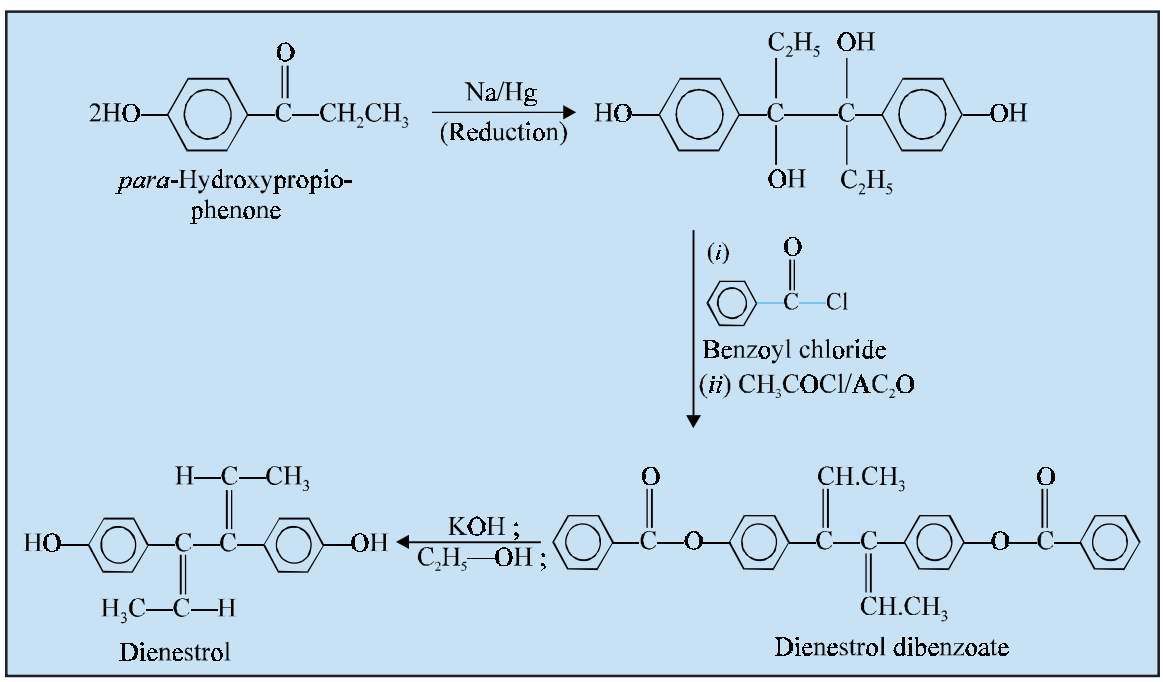
Synthesis

Dienoestrol may be synthesized by various methods. The synthesis put forward by Dodds *et al.* is described here.

Reduction of *para*-hydroxypropiophenone yields a diphenol derivative which upon benzoylation with benzoyl chloride followed by acetylation with a mixture of acetylchloride and acetic anhydride gives the dienestrol dibenzoate. This on treatment with alcoholic KOH yields the official product.

Its actions and uses are similar to those of oestradiol. Besides, it is also employed by local application in creams.

Dose. *For menopausal symptoms, 0.5 to 5 mg per day ; For mammary or prostatic carcinoma, 15 to 30 mg per day.*



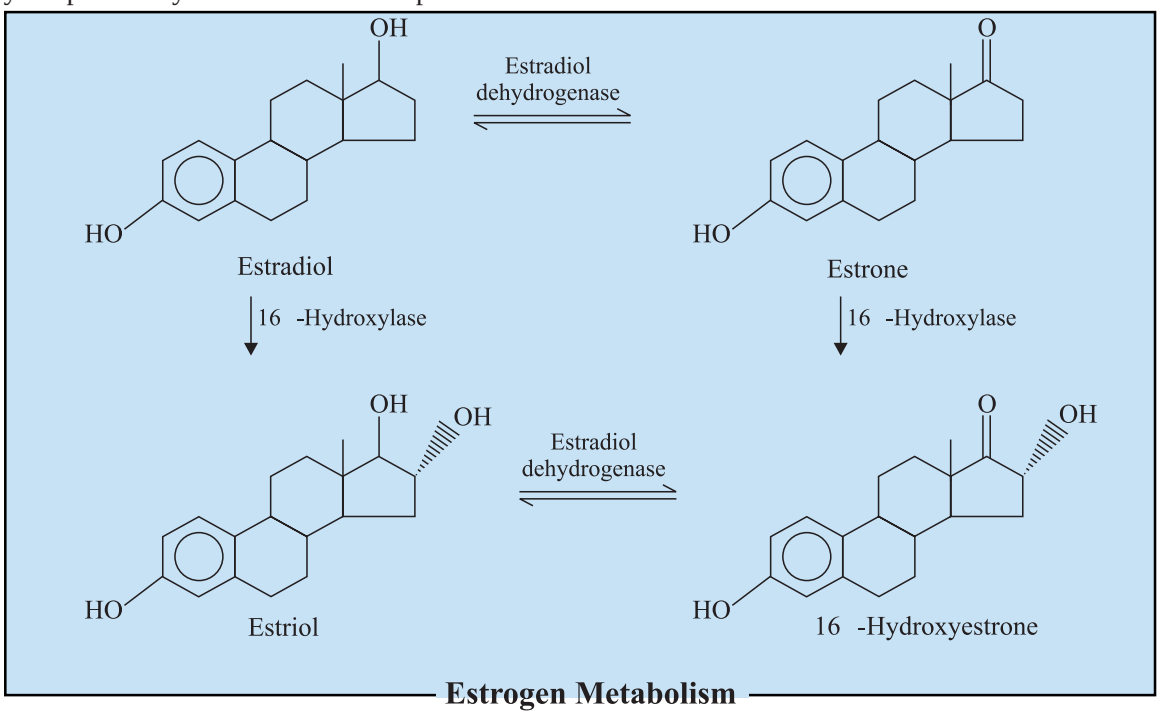
3.2.3.1. Mechanism of Action

The mechanism of action of '**oestrogens'** shall be dealt with separately under the following *two* heads, namely :

A. ***Steroidal Oestrogens***

3.2.3.1. A-1. Estrone

The **endogenous oestrogens**, for instance : **estrone** and **17β-estradiol** are observed to be interconvertible biochemically in the presence of the specific enzyme **estradiol dehydrogenase** and yield practically the same metabolic products as illustrated below :



Estrogen Metabolism

Importantly, these hormones including estradiol dehydrogenase are chiefly metabolized in the liver and mostly get excreted as water-soluble **glucuronide** and **sulphate conjugates. Estrone** is regarded as a less active (1/12) metabolite of **estradiol**.

3.2.3.1.A-2. Estriol

This specific steroid is most abundantly synthesized in the human placenta. It has been observed that in both pregnant and nonpregnant women **estriol** (along-with estrone and **estradiol**) are duly metabolized to small quantum of other structural analogues *viz.*, **2-hydroxyestrone** ; **2-methoxyestrone** ; **4-hydroxyestrone** ; and **16β-hydroxy-17β-estradiol**.

The **'drug'** affords a proliferation of the breast ductile system. It also stimulates the development of lipid and other tissues which essentially contributes to breast shape and function. Fluid retention in the breasts particularly in the later-stages of the menstrual cycle is found to be a common feature of **estriol**.

3.2.3.1.A-3. Estradiol

The '**drug**' distinctly possesses a **high presystemic elimination rate**; and, therefore, gives rise to a low bioavailability by the oral route. The drug gets appreciably converted to estrone *in vivo*. The plasma half-life stands at 1 hour.

Note. Both transdermal and micronized preparations are employed effectively for the replacement therapy.

B. Non-Steroidal Oestrogens

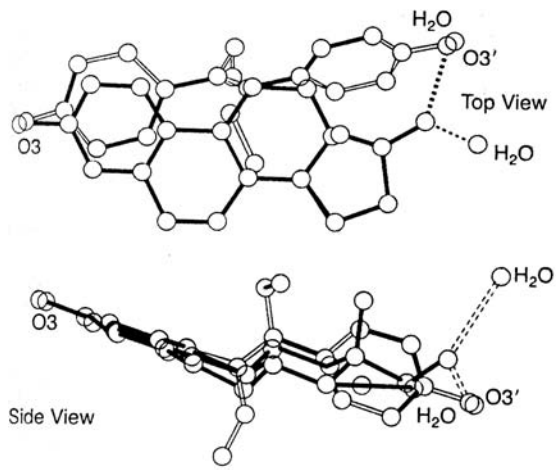
3.2.3.1.B-1. Diethylstilbesterol (DES)

The '**drug**' has an advantage over the other estrogens (*e.g.*, **estrone**, **estriol** and **estradiol**) by virtue of the fact that it gets absorbed quite effectively through the oral administration. Besides, its *rate of inactivation* is very slow and sluggish. **DES** was found to be appreciably cheaper in comparison to the naturally occurring estrogens and still can produce all the same pharmacological estrogenic activities. Interestingly, **DES** has exhibited 10 fold the estrogenic potency of its corresponding *cis*-isomer due to the fact that the *trans*-isomer bears a close relationship to **estradiol.***

Note. Due to the high incidence of 'uterine cancers' as replacement therapy in menopausal women its usage in women has been banned. However, its use in men for the treatment of 'prostatic cancer' still continues.

SAR of DES. One may consider **DES** as another form of estradiol wherein the two 6-membered rings 'B' and 'C' open up and a 6-membered aromatic ring 'D' introduced in place of the cyclopentane ring. It was further suggested that the actual distance prevailing between the two **DES** phenol OH moieties was virtually the same as the C-3 OH to C-17 OH distance existing in estradiol ; and, hence, these two entities may prove to be a **'perfect fit'** to the same receptor site. **Recently, with the advent of latest computer softwares the medicinal chemist has established the distance between the two OH moieties in DES to be 12.1Å and in estradiol 10.9Å**.

The following Figure 23.1 is the '**computer generated graphics**' illustrating explicitely the *top-view* and the *side-view* of the actual superimposition of **estradiol** $(H_2O)_2$ shown by **dark-lines** with **DES** represented by **light-lines.** It is, however, pertinent to state here that in an aqueous medium *estradiol* essentially has two water moles which are hydrogen-bonded to the 17-OH moiety. In case, one of the two water moles is considered in the distance measurement of the hydroxyl groups, there exists a '**perfect fit**' associated with the two OH moieties of **DES** as may be observed in Fig. 1. Hence, it may be implied juistifiably that water may play a vital pivotal role for *estradiol* in its **receptor site**.



ESTRADIOL $(H_2O)_2$: DARK LINES
DIETHYLSTILBESTEROL (DES) : LIGHT LINES

Fig. 23.1. Computer Generated Graphics Showing Superimposition of Estradiol and DES.

*UV Solmssen, *Chem Rev.*, **37**, 481, 1945.

[Adapted from Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, Lippincott Williams and Wiltzns, New York, 5th edn. 2004.]

3.2.3.1. B-2. Hexestrol

The '**drug**' represents the *meso* form of 3, 4-*bis* (*p*-hydroxyphenyl)-*n*-hexane that distinctly possesses the greatest estrogenic potency of the three stereoisomers belonging to the corresponding dihydro analogue of **DES**. Interestingly, it is found to be less potent than **DES**.

3.2.3.1. B-3. Dienestrol

The '**drug**' is a potent estrogen which finds its abundant use only topically, for the treatment of *atrophic vaginitis* and *kraurosis vulvae.*

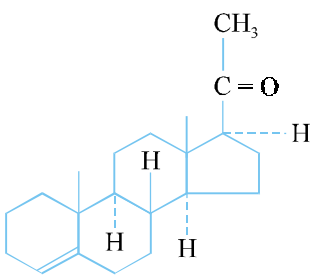
CAUTION. Not recommended in patients with known or suspected cancer of the breast ; known or suspected estrogen-dependent neoplasia; undiagnosed abnormal genital bleeding ; thrombophlebitis or thromboembolic disorders or a previous case-history of such typical conditions ; and hypersensitivity to the ingredients of the cream or suppositories of dienestrol or during pregnancy.

3.2.4. Gestogens

Gestogens or **corpus luteum hormones** are mostly secreted by the corpus luteum portion of the ovary and the metabolized to various inactive products, *e.g.*, **pregnanediol**. The metabolities are esentially excreted through urine.

Example : Progesterone.

A. *Progesterone* INN, BAN, USAN,



Pregn-4-ene-3, 20-dione ; BP ; USP ; Eur. P. ; Int. P. ; Ind. P. ;

Syngesterone(R) (Pfizer) ; Gesterol 50(R) (Forest).

Synthesis

Progesterone has been synthesized by various researchers from different starting materials as indicated below :

(*i*) From Pregnanediol (Butenandt *et al.* 1930)

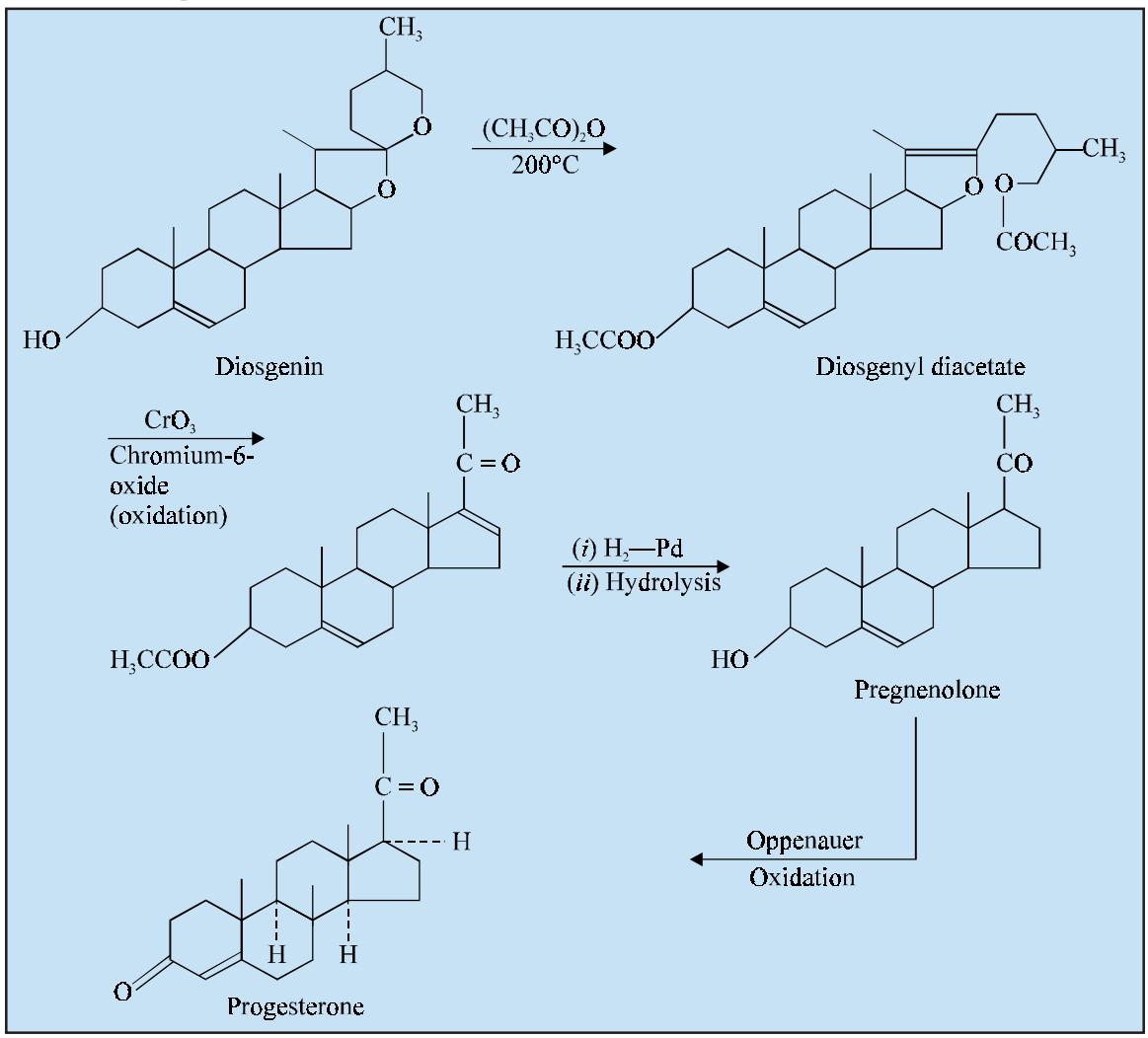
(*ii*) From Stigmasterol (Butenandt *et al.* 1934)

(*iii*) From Cholesterol (Butenandt *et al.* 1939)

(*iv*) From Ergosterol (Shephard *et al.* 1955)

It has also been synthesized from **diosgenine** by Marker *et al.* (1940-1941) which will be discussed here.

Acetylation of diosgenin at 200°C gives the corresponding diosgenyl diacetate which upon oxidation with chromium-6-oxide removes the side-chain at C-17 and the resulting product on reduction followed by hydrolysis yields pregnenolone. This on being subjected to **Oppenauer oxidation** affords the official compound.



It is employed in the treatment of functional uterine bleeding. It is also used in conjuction with an oestrogen in the treatment of menstrual disorders, neoplasms of the breast and endometrium. Sometimes it also finds its use in habitual and threatened abortion.

Dose. *For uterine bleeding, 5 to 10 mg injected per day up to 5 to 10 days ; For habitual abortion, 5 to 20 mg twice or thrice per week by intramuscular injection.*

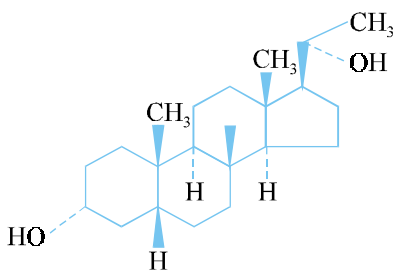
3.2.4.1. Mechanism of Action

The **mechanism of action** of **progesterone** shall now be discussed as under :

3.2.4.1.1. Progesterone

One school of thought considered it to be the '*drug of choice*' specifically in the **luteal-phase dysfunction,** a disorder that gives rise to either *infertility* or *repetitive early* abortion.

The **'drug'** gets metabolized rapidly when adminstered orally showing a plasma half-life of only 5 minutes. It usually undergoes transformation leading to a plethora of steroidal metabolic products. However, the principal excretory product of the **progesterone metabolism** is nothing but **5β-pregnane-3α-20α-diol(I)** and its **corresponding conjugates**.



(I)

A few salient-features of the aforesaid metabolism are :

(*a*) reduction of the double bond between C-4 and C-5,

(*b*) reduction of the ketone (—C—, with O above the bond) function at C-3 giving rise to 3α-ol, and

(*c*) reduction of the ketone moiety at C-20 to provide the 20α-ol.

It has been duly observed that the prevailing reduction invariably taking place at C-5 must precede the reduction of the C-3 ketone. Besides, the characteristic structural features which may specifically cause blockade of the reduction either at C-5 or C-20 have enormously enhanced the half-lives of the corresponding **progesterone derivatives**.

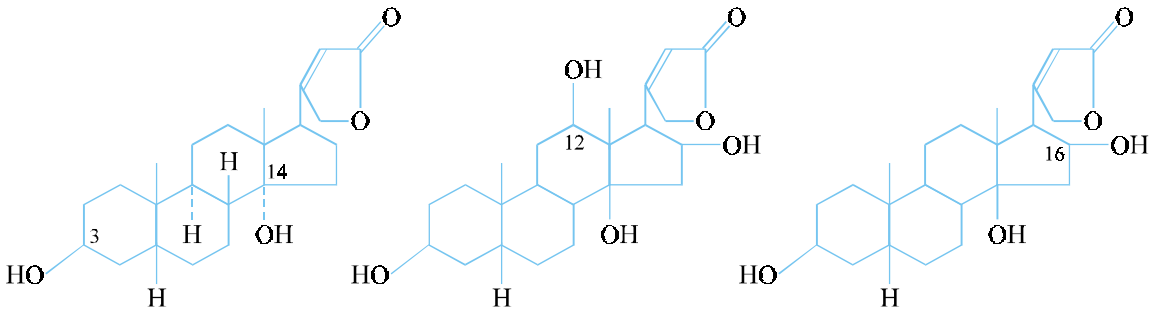
[Note. Progestasert, Alza(R) an intrauterine contraceptive device consists of 38mg of progesterone in silicone oil. In this instance, the 'drug' is believed to increase the contraceptive effectiveness of the said device by a local effect on the endometrium followed by effects upon the motility of sperm, capacitation and metabolism.]

3.3. Cardiac Glycosides

Plant extracts containing **cardiac glycosides** were invariably employed as poisons in the medieval trial by both African and South American natives for the preparation of their lethal arrow and spear poisons for use in fighting as well as hunting.

'Digitalis' a preparation made by extraction of dried seeds and leaves of the **purple foxglove** *Digitalis purpurea*, found certain application in the control and management of dropsy. Later on, in 1785, a noted Scottish physician William Withering first introduced the use of '**digitalis**' in heart therapy and this became a spectacular success and tremendous achievement for curing heart patients.

The active components of **digitalis** are glycosides of **digitoxigenin**, **digoxigenin** and **gitoxigenin** and afford these glycones on acid hydrolysis. The free **aglycones** or **genins** are only weekly active.



The **salient features** of the abvoe *three* **genins** are enumerated below :

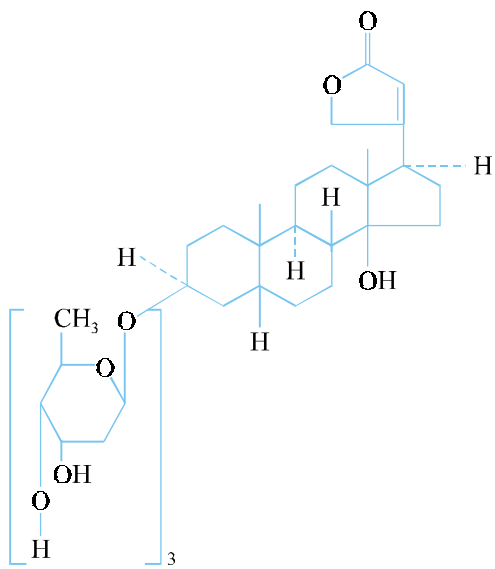
(*i*) All the above three genins have an α, β-unsaturated five-membered lactone ring.

(*ii*) All of them have 3β and 14β hydroxy groups ; **digoxigenin** has an additional 12β-hydroxy group and gitoxigenin a 16β-hydroxy group.

(*iii*) The unsaturated lactone ring and the 14β-hydroxy group are both essential to cardiac activity.

The corresponding glycosides **digitoxin** and **digoxin** are all **triosides** of comparable high cardiotonic activity. They are described briefly here :

A. *Digitoxin* INN, BAN, USAN,



Card-20 (22)-enolide, 3-[(*o*-2, 6-dideoxy-β-D-*ribo*-hexopyranosyl-(1 → 4)-*o*-2, 6-dideoxy-β-D-*ribo*-hexopyranosyl-(1 → 4)-2, 6-dideoxy-β-D-hexopyranosyl) oxy]- 14-hydroxy, (3β, 5β)- ; BP ; USP ; Eur. P ; Ind. P ;

Crystodigin(R) (Lilly).

Digitoxin is the most potent of the **digitalis glycosides** besides being the most cumulative in action.

It enhances the force of myocardial contraction and in the case of heart failure this dominating inotropic effect results in a much modified cardiac output with regard to more complete emptying of the ventricle at systole, an apparent decrease in the elevated end-diastolic ventricular pressure, and above all a positive reduction in the size of the dilated heart. It is used in the treatment of congestive heart failure.

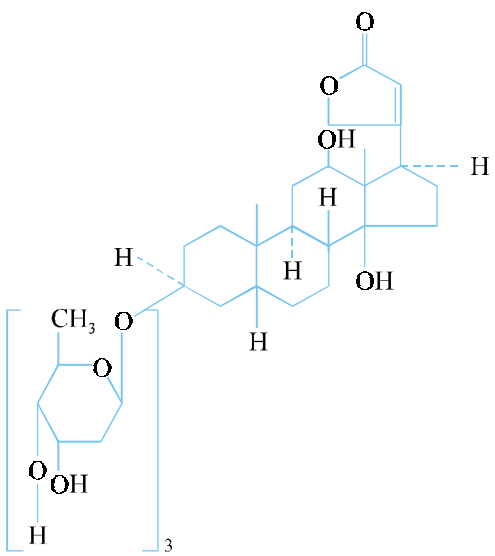
Dose. *Adult, initial, 600 mcg, followed by doses of 200 to 400 mcg every 6 hours as necessary; For slow digitalisation, 300 mcg has been given twice daily for 4 days ; Maintenance dose ranges from 50 to 200 mcg per day.*

B. Digoxin INN, BAN, USAN,

3β-[*o*-2, 6-Dideoxy-β-D-*ribo*-hexopyranosyl-(1 → 4)-*o*, 2, 6-dideoxy-β-D-*ribo*-hexopyranosyl-(1 → 4)-2, 6-dideoxy-β-*ribo* hexopyranosyl) oxy]-12β,-14-dihydroxy-5β-card-20 (22)-enolide ; BP ; USP ; Eur. P. ; Int. P ; Ind. P ;

Lanoxicaps(R) (Burroughs Wellcome) ;

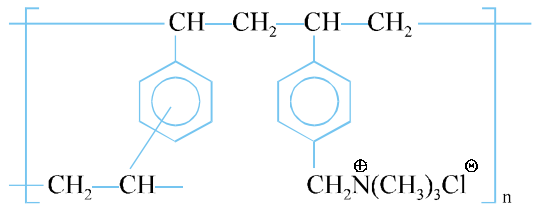
Its uses and actions are very similar to those of **digitoxin**.



Dose. *For rapid digitialization, 0.75 to 1.5 mg orally followed by 250 mcg, every 6 hours until the desired therapeutic effect is achieved.*

3.3.1. Mechansim of Action

The **mechansim of action** of **digitoxin** and **digoxin** are treated in the sections that follows :



(I)

3.3.1.1. Digitoxin

The '**drug**' usually gets absorbed almost completely after oral administration ; of course, with an exception when **cholestyramine(I)** is used concomitantly. It is found to exhibit its optimal activity within a span of 4 to 12 hour. However, after **full digitalization,** the duration of action extends upto 14 days. It gets protein bound in plasma upto almost 97%. Its volume of distribution (v_d^{ss}) is approximately 0.6 mL g^{-1}. It has been duly established that a plasma concentration of 15-25mg mL^{-1} are regarded to be therapeutic range ; whereas, 35-40 ng mL^{-1}, or even more to be toxic. However, significant variation in the plasma concentration may be afforded by plasma K^+ and Ca^{2+} levels along with other such factors. It has been osberved that the ensuing '**hepatic metabolism**' usually accounts for 52-70% of the entire elimination of this '**drug**'. The β-half-life varies between 2.4 to 9.6 (average 7.6) days.

CAUTION. Phenytoin. (anticonvulsant) and phenobarbital (long-acting barbiturate) can induce hepatic microsomal enzymes and thereby retard the half-life significantly ; and, therefore, ultimately interfering with the prevalent efficacy of the 'drug'.

3.2.1.2. Digoxin

The '**drug**' is invariably used IV for accomplishing rapid digitalization because of its high degree of purity ; and its action becomes manifest within a span of 15-30 minutes, eventually attaining its peak in 2-5 hours. However, after full digitalization its duration of action extends upto 6 days (unlike 14 days for digitoxin). The '**drug**' is bound to protein in plasma between 20-30%. Its volume of distribution (v_d^{ss}) stands at 5.1 L. kg^{-1} in normal adults ; whereas, in patients with a history of renal failure v_d^{ss} is nearly 3.3 L. kg^{-1}. It has been observed that an observed **extensive intracellular binding** is usually responsible for the large volume of distribution.

The renal excretion in adults normally accounts for 60-90% of its total elimination ; a small quantum gets converted in the liver to **dihydrodigoxin**. The elimination half-life in adults (normal) ranges between 29 to 135 hours (normally 36-41 hour). It has been observed that an enhanced GI motility lowers and decreased motility augments absorption.

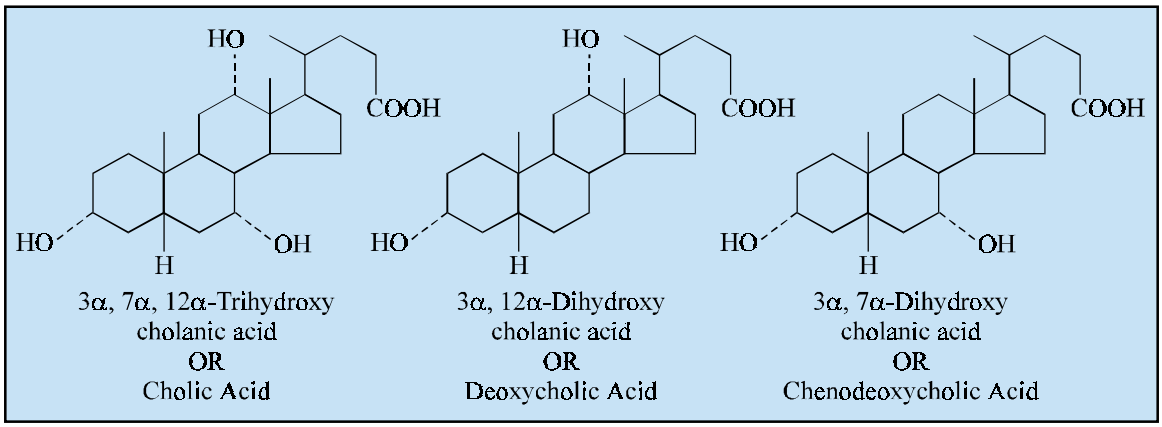
CAUTION. Bioavailability of 'digoxin' gets altered due to the presence of such drugs as : antacids, antineoplastic agents, cholestyramine resins, dietary fibre, erythromycin, neomycin, tetracyclines, metoclopramide, sulphasalazine and propantheline.

3.4. Bile Acids

The liver secretes a clear, golden yellow viscous liquid known as '**bile**'. It is stored in gall bladder and is solely useful for the digestive system. It mainly consists of the inorganic ions like HCO_3^-, Cl^- Na^+, K^+, etc., in addition to organic compounds such as bile acids, bile pigments, liquid fatty acids and cholesterol. **Cholic Acid ; Deoxycholic Acid ; Chenodeoxycholic Acid.**

The bile acids are usually present as the salt of amide with either glycine or taurine, for instance ; **sodium glycocholate** (**glycine + cholic acid**), and **sodium taurocholate** (**taurine + cholic acid**).

In all twelve natural bile acids have been identified and characterised duly. Of these the most abundant bile acids in human bile are : **cholic acid** (26-60% of total bile acids) ; **deoxycholic acid** (5-25%), and **chenodeoxycholic acids** (30-35%), whose structures and chemical names are stated below :



The **bile acids** may be isolated from the bile by cleaving the peptide linkage present in them by hydrolysis with alkali. From the resulting solution the bile acids are conveniently isolated either by crystallization from organic solvent or by treating the ethereal solution of the acids with various concentration of hydrochloric acid, for instance ; the trihydroxy, dihydroxy and the monohydroxy acids may be isolated by treating the ethereal solution with 15%, 25% and concentrated hydrochloric acid respectively.

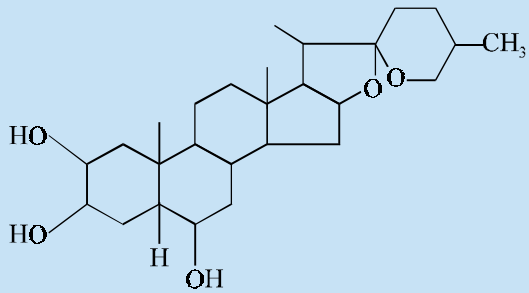
3.5. Sapogenins

Saponins are the plant glycosides that have the characteristic of forming colloidal aqueous solutions which normally foam upon shaking. Like other glycosides, **saponins** usually vary in their chemical structures. **Saponins**, in general possess an unique property to effect hydrolysis of red-blood cells (RBC) even in high dilutions. In this respect, they are very toxic to cold-blooded animals. In general, **saponins** have a bitter taste and are very irritating to the eyes and the nose. The more commonly and abundantly occurring saponins are those found in soap bark, soap root, snake root, similax and cacti.

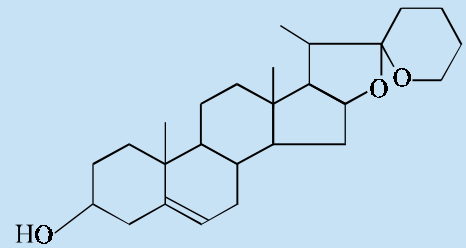
Saponins on hydrolysis yield sugars such as glucose, galactose, rhamnose and xylose together with an aglycone (**sapogenin**) *i.e.*, the non-sugar moiety. They have been used extensively in medicine, as foaming agents in fire extinguishers and as fish poisons.

Following are a few examples of **steroidal saponins** with their respective sources :

Source	Saponin	Sapogenin	Sugars
Digitalis purpurea or ***Digitalis lanata***	Digitonin	Digitogenin ($C_{27}H_{44}O_5$)	Glucose, Galactose
Trillium erectum	Trillin	Diosgenin ($C_{27}H_{42}O_3$)	Glucose



Digitogenin



Diosgenin

Probable Questions for B. Pharm. Examinations

1. Write short notes on the following :

(*a*) Nomenclature of Steroids

(*b*) Diel's hydrocarbon

(*c*) Sterols.

2. Give a brief account of the ANDROGENS. How would you synthesize Testosterone from :

(*a*) Cholesterol

(*b*) Dehydroepiandrosterone.

3. What are Follicular Hormones ? Classify them and describe the synthesis of one potent drug from each class.

4. Name a prominent **Corpus Luteum Hormone** and discuss its synthesis from a glycoside obtained from *Digitalis lanata.*

5. Discuss **'Cardiac Glycosides'** by giving its plant source, **three** important known genins, structure of the corresponding glycosides and their uses.

6. Give a comprehensive account of the **'Bile Acids'**. How are they isolated from the natural bile ? Support your answer with the structure of known bile acids.

7. Naturally occurring plant sources yield **'Sapogenins'.** Discuss their importance and usage in medicine and steroidal chemistry.

8. Hugh's total synthesis of **OESTRONE** from 1-(3-methoxy)-phenyl propyl bromide offers a comparatively simpler method than others. Explain.

9. Give the names and official status of at least **five** derivatives of :

(*a*) Testosterone

(*b*) Estradiol

which are used in medicine.

RECOMMENDED READINGS

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24

Adrenocortical Steroids

Chapter

24 Adrenocortical Steroids

1. INTRODUCTION

It has been emphatically and logically determined to classify the steroidal hormone products belonging specifically to the **adrenal cortex** (*i.e.,* **adrenocortical steroids**) into *two* major groups : the **corticosteroids** (*viz.,* **glucocorticoids** and **mineralocorticoids,** which essentially possess 21 C-atoms, and the **androgens** having 19 C-atoms.

In general, the **adrenocortical steroids** (or **adrenal corticosteroids**) distinctly differ in their respective **glucocorticoid activities** (*i.e.,* **carbohydrate-regulating**), and **mineralocorticoid activities** (*i.e.,* **electrolyte-regulating**). It has been observed that in human beings the following two compounds occur commonly :

Glucocorticoid : Hydrocortisone ; and **Mineralocorticoid : Aldosterone.**

Structure-Activity Relationship (SAR) : Intensive and extensive clinical investigations have duly revealed that the anti-inflammatory activity emanated by the **adrenal cortical steroids** in humans invariably correlates intimately with their respective **glucocorticoid activity.** The advent of glaring and outstanding researches have evolved several **synthetic steroids** that exclusively possess distinctly **higher glucocorticoid,** and relatively **lower mineralocorticosteroid activity** in comparison to **cortisone** or **cortisol** that have been duly prepared, biologically screened, and marketed.

Table : 24.1 records the Cardinal **Adrenal Corticosteroids** (or **Adrenocortical steroids**) along with their respective relative activity, pharmacological potency *vis-a-vis* **hydrocortisone ;** anti-inflammatory, topical features, sodium retention, and various commonly employed dosage forms.

Table 24.1 : Cardinal Adrenocortical Setoids

S. No.	Classification of Drugs	Relative Activity			Dosage Forms (s) Available
		Anti-inflammatory	Toptical Features	Na Retention	
1.	**Short-to Medium Acting Glucocorticoids**				
	(*a*) Hydrocortisone	1	1	1	Oral, Inj, Top.,
	(*b*) Cortisone	0.8	0	0.8	—do—
	(*c*) Prednisone	4	0	0.3	Oral,
	(*d*) Methylprednisolone	5	5	0	Oral, Inj., Top.,
	(*e*) Prednisolone	5	4	0.3	—do—
2.	**Intermediate-Acting Glucocorticoids**				
	(*a*) Triamcinolone	5	5–100	0	—do—
	(*b*) Fluprednisolone	15	7	0	Oral,
3.	**Long-Acting Glucocorticoids**				
	(*a*) Betamethansone	25-40	10	0	Oral, Inj., Top.,
	(*b*) Dexamethasone	30	10-40	0	—do—
4.	**Mineralocorticoids**				
	(*a*) Fludrocortisone	10	10	250	—do—
	(*b*) Desoxycorticosterone Acetate	0	0	20	Inj., Pellets,

[Adapted From : Remington : The Science and Practice of Pharmacy, Vol. 2, Lippincott Williams & Wilkins, New York, 21st. edn., 2006]

Biological Activity Profile : The biological activity profile of the **glucocorticoids** and the **mineralocorticoids** shall now be discussed briefly as under :

(*a*) **Glucocordicoids :** The various **salient features** are :

(1) Affect all cells, but not all to the same extent and manner.

(2) Prime activity rests upon their anti-inflammatory and immunosupressant effects.

(3) Mainly check and prevent release of the host of **'lytic enzymes'** which cause tissue damage during all inflammation and eventually give rise to **leukotactic substances.***

(4) Minimise phagocytosis by macrophages.

(5) Decrease lipid **eicosanoid** and **prostaglandin (PG)** generation by inhibiting the production of **cytokines** which specifically induce **cycloxygenase-II** in inflammatory cells.

*__Leukotactic :__ Possessing the power of attracting leukocytes.

(6) Once it has gained entry *via* permeation across a cell membrane, it critically gets combined with a highly specific **cytosolic glucocorticoid receptor** which is **inactive** by virtue of the fact it is strategically bound to certain **particular proteins** *viz.*, some **'thermal shock proteins'** which exclusively prevent them from either having an **access to the nucleus** or **getting bound intimately to DNA.**

(*b*) **Mineralocorticoids :** The cardinal **salient features** are as enumerated under :

(1) They especially exert their action upon the **distal tubules** and **collecting ducts** of the kidney to enhance specifically the **expression of genes** which help in encoding the proteins that increase reabsorption of Na^+ from the tubular fluids.

(2) Overall effect upon the **electrolytes** are intimately linked with an appreciable increment in the number of **Na^+ and K^+ channels** strategically located in the luminal membrane tubular cells ; and hence, they in turn enhance distinctly the activity of the basolateral membrane **Na^+/K^+-activated ATPase.** The ultimate result being a region of Na^+ to the systemic circulation in exchange for K^+.

(3) Identical **'electrolyte effects'** are duly promoted by the **mineralocorticoids** in a host of other tissues *viz.*, **colon, salivary glands,** and **sweat glands.**

(4) The most predominant **'primary effects of mineralocorticoids'** are adequately observed upon the **cortical collecting tubule** cells strategically positioned in the kidneys to enhance substantially **sodium reabsorption** *vis-a-vis* **potassium secretion.** This eventually leads to an **elevated aldosterone** titer **values** that actually governs, controls, and monitors effectively **sodium retention** and **potassium depletion** thereby giving rise to **volume expansion and weight gain, metabolic alkalosis,** and **hypertension.**

2. CLASSIFICATION

The **adrenocortical steroids** may be classified judiciously according to the categorization already shown as in **Table 24.1.** The various potent compounds belonging to different groups shall now be treated individually in the sections that follows :

Glucocorticoids : The **glucocorticoids** may be categorized* into *three* sub-groups as given below :

(*a*) Short-to medium acting glucocorticoids,

(*b*) Intermediate acting glucocorticoids, and

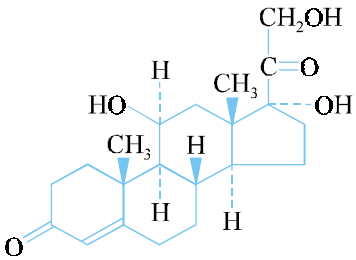
(*c*) Long-acting glucocorticoids.

2.1. Short-to Medium Acting Glucocorticoids

The various examples are as given under :

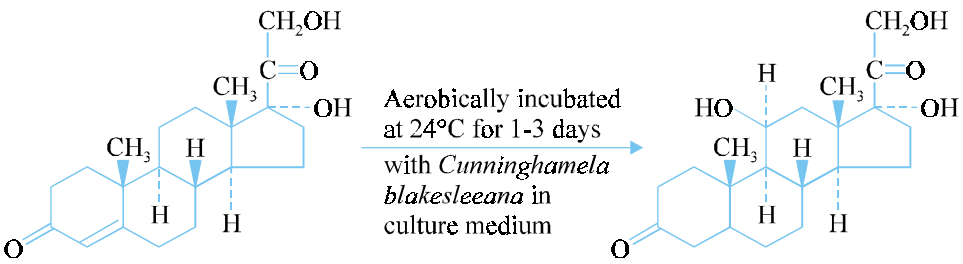
*__Glucocorticoids__ are classified according to their pharmacological activities, just like the **Sedatives and Hypnotics** *i.e.*, the **Barbiturates** (see **Chapter-6**).

2.1.1. Hydrocortisone INN, BAN, USAN



(11β)-Pregn-4-ene-3, 20-dione,11,17,21-trihydroxy- ; Compound F ; Reichstein's 'Substance M'. Hydrocortone(R) ; Cortef(R) ; Hytone(R) ; Hycoson(R) ;

Microbiological Method*



11-Desoxy-17--hydroxy-corticosterone (I)

Hydrocortisone

A sterilized culture medium is first prepared from **peptone** (0.5%), dextrose (2%), soyabean meal (0.5%), KH_2PO_4 (0.5%), NaCl (0.5%) and yeast extract (0.3%) in tap water. To 200 mL of this medium is added an inoculum of the vegetative mycella of *Cunninghamela blakesleeana*. The spores thus obtained are duly transferred from a sport slant into a broth medium carefully. The resulting broth medium is aerobically incubated at 24°C for a duration of 1 to 3 days in a **reciprocating shaker** till such time the development of the desired vegetative growth commences. The inoculated culture medium containing the added vegetative mycella of *C. blakesleeana* is incubated for 2 days at 24°C following which is added 66 mg of compound (I) in solution in a minimum quantity of ethanol, and the incubation is maintained duly for 7 hourse at 24°C. The beer comprising of steroid is diluted with 800 mL of acetone, shaken for 1 hr. on a **reciprocating shaker,** and filtered duly. The solid residue (cake) thus obtained is subsequently suspended in 500 mL of pure acetone, duly shaken for another 1 hr., and filtered again. The filtrates obtained are adequately mixed and the excess of the **acetone** is removed under reduced pressure at 50°C. A small quantum of acetone is added, if required, so as to bring the

*Murray HC and Peterson DH : US Patent NO : 2, 602, 709 (July 8, 1952) ; US Patent NO : 2, 649, 400 (August 18, 1953) ; US Patent No : 2, 649, 402 (August 18, 1953) ; and US Patent No : 2, 794, 816 (June 4, 1957) ; All assigned to the Upjohn Co., USA.

concentration to 20% acetone ; and this resulting aqueous acetone solution is successively extracted at least 5 times with 1/3rd volume of **Skelly solve B petroleum ether** in order to remove the residual fatty materials completely. These extracts are duly back-washed at least 2 times with approx. 1/10 the volume of 20% aqueous acetone and the washings are added into the main acetone extract.

Finally, the combined acetone extracts are extracted once again at least 6 litres using 1/4th volume of ethylene dichloride and the extract is carefully evaported to get rid of the solvent under vacuum to obtain **hydrocortisone.**

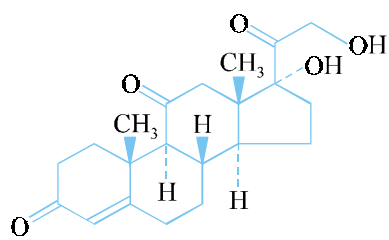
Hydrocortisone represnts the principal natural glucocorticoid in human beings ; and thus, may be regarded as the **prototype of all glucocorticoids.** It essentially bears the following characteristic features, namely :

- **Plasma half-life :** 1.5–3 hrs.
- **Biological half-life :** 8–12 hrs., and
- **Volume of distribution :** 0.3–0.5 L . kg^{-1} (but varying with doses)

Mechanism of Action : The proven pharmacological activity of this **drug** is largely mediated by **cytoplasmic glucocorticoid receptors.** After getting bound to the receptor intimately, the ensuing **steroid-receptor complex** further gets hooked onto **chromatin** and thereby stimulate the remarkable generation of **mRNA.** In turn the mRNA duly stimulates the synthesis of enzymes that eventually cause the production of various pharmacological activities.

Dose : As sodium succinate salt of hydrocortisone injection as 100 mg IM ; as hydrocortisone acetate intraarticular injection ; and as topical cream 1–2.5% for skin, ear, and eye (Wycort$^{(R)}$).

2.1.2. Cortisone INN, BAN, USAN



17, 21-Dihydroxypregn-4-ene-3, 11, 20-trione ; Cortisone Acetate USP ;

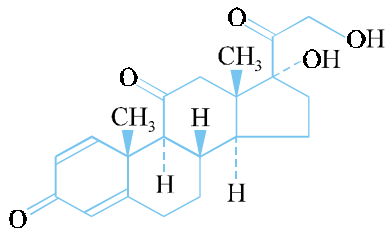
Cortogen$^{(R)}$; Cortone$^{(R)}$; Corlin$^{(R)}$; Adreson$^{(R)}$; Cortadren$^{(R)}$;

Cortisone exhibits good systemic anti-inflammatory activity and low-to-moderate salt-retention activity after its due *in vivo* conversion to **hydrocortisone acetate.** Importantly, this conversion is duly mediated by **11β-hydroxysteroid dehydrogenase.** It exerts a host of therapeutic usages *viz.*, collagen diseases, Addison's disease, allergic reactions, acute shock, and several other indications.

Cortisone has a **plasma half-life** ranging between 1.5 to 3.0 hours.

Dose : *Adult dose 20–100 mg/day oral or IM.*

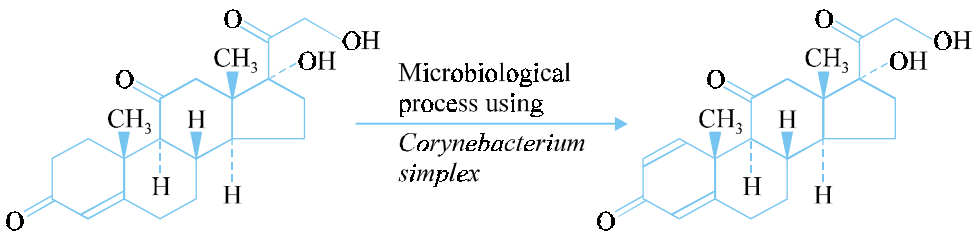
2.1.3. Prednisone INN, BAN, USAN



17, 21-Dihydroxypregna-1, 4-diene-3, 11, 20-trione ; Δ^1-Cortisone ; Deltacortisone ; Retrocortine ; IP, BP, USP,

Deltasone$^{(R)}$; Orasone$^{(R)}$;

Microbiological Method



Cortisone Prednisone

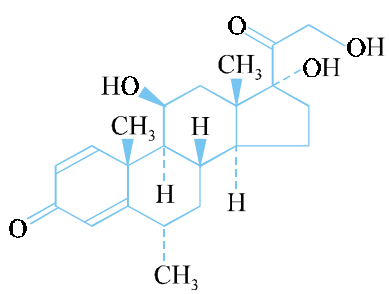
Prednisone may be prepared from **cortisone** by a highly specific microbiological process using *Corynebacterium simplex*, that selectively dehydrogenates **cortisone** at the C-1 and C-2 positions.

It is, however, pertinent to mention here that the **'active form'** of the drug is its respective metabolite, **prednisolone. Prednisone** remarkably exhibits 3 to 5 folds the **glucocorticoid activity** in comparison to **hydrocortisone,** whereas it distinctly shows somewhat reduced **mineralocorticoid activity ;** nevertheless, it may cause **noticeable sodium retention and potassium depletion.** It enjoys the reputation of being the glucocorticoid of choice for use in cancer chemo-therapy both profusely and predominantly, but preferentially along with other drugs. It also finds its abundant usage in **'pediatrics'** for the treatment of nephrosis, rheumatic carditis, neoplasms, leukemias, and tuberculosis.

It has **plasma-half life** ranging between 3 to 5 hours, and **biological-half life** varying between 12 to 36 hours.

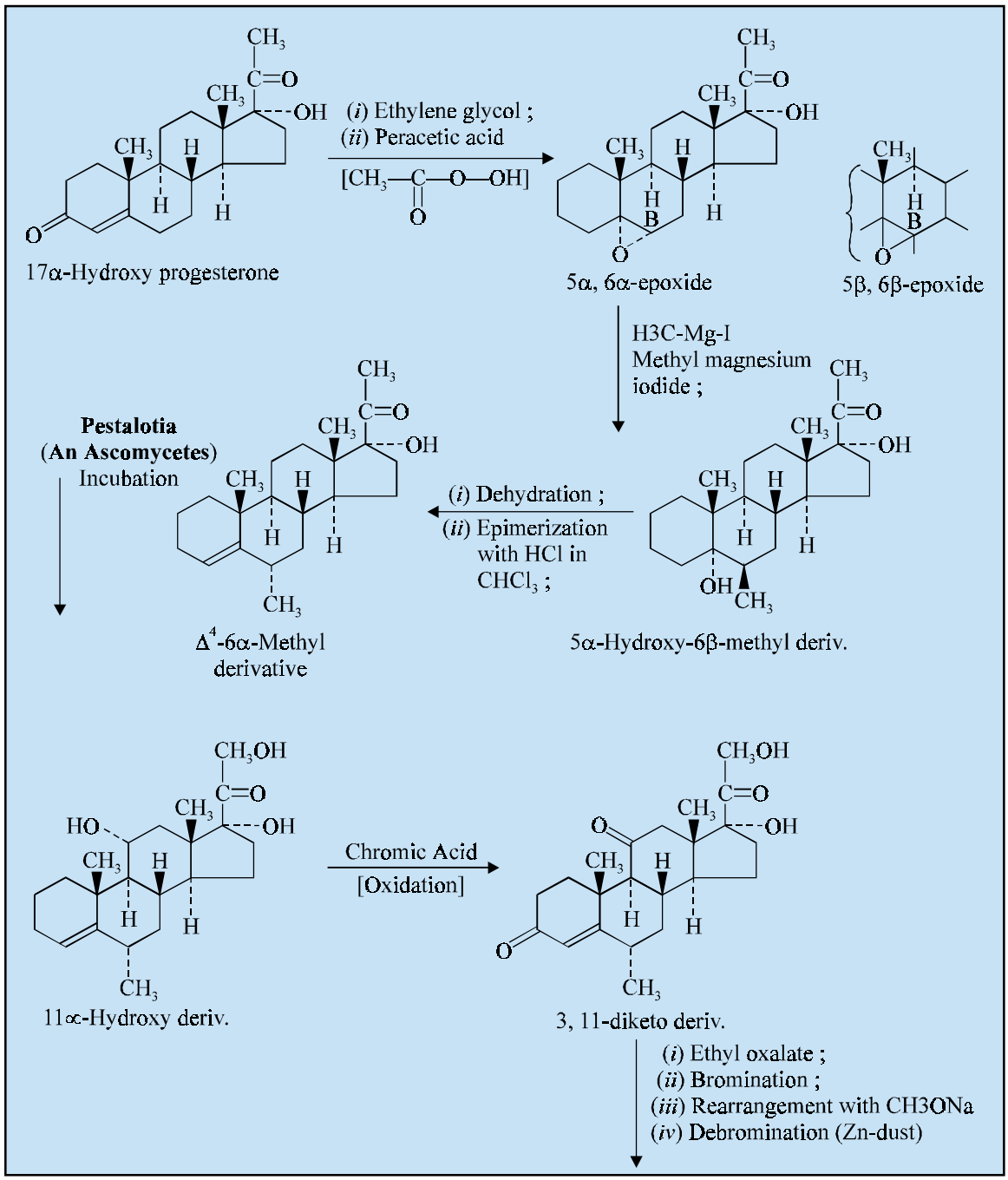
Dose : *Adult oral : 5 to 6 mg per day ; IM/IV-injection 10 to 40 mg ; and topical (as creams) for eyes and skin 0.25%.*

2.1.4. Methylprednisolone INN, BAN, USAN



(6α, 11β)-11, 17, 21-Trihydroxy-6-methylpregna-1, 4-diene-3, 20-dione ; IP, BP, USP, Medrol(R) ; Medrone(R) ; Urbason(R) ; Cytosyn(R) ;

Synthesis Methylprednisolone may be synthesized by the combination of both **synthetic** and **microbiological** procedures as given under :



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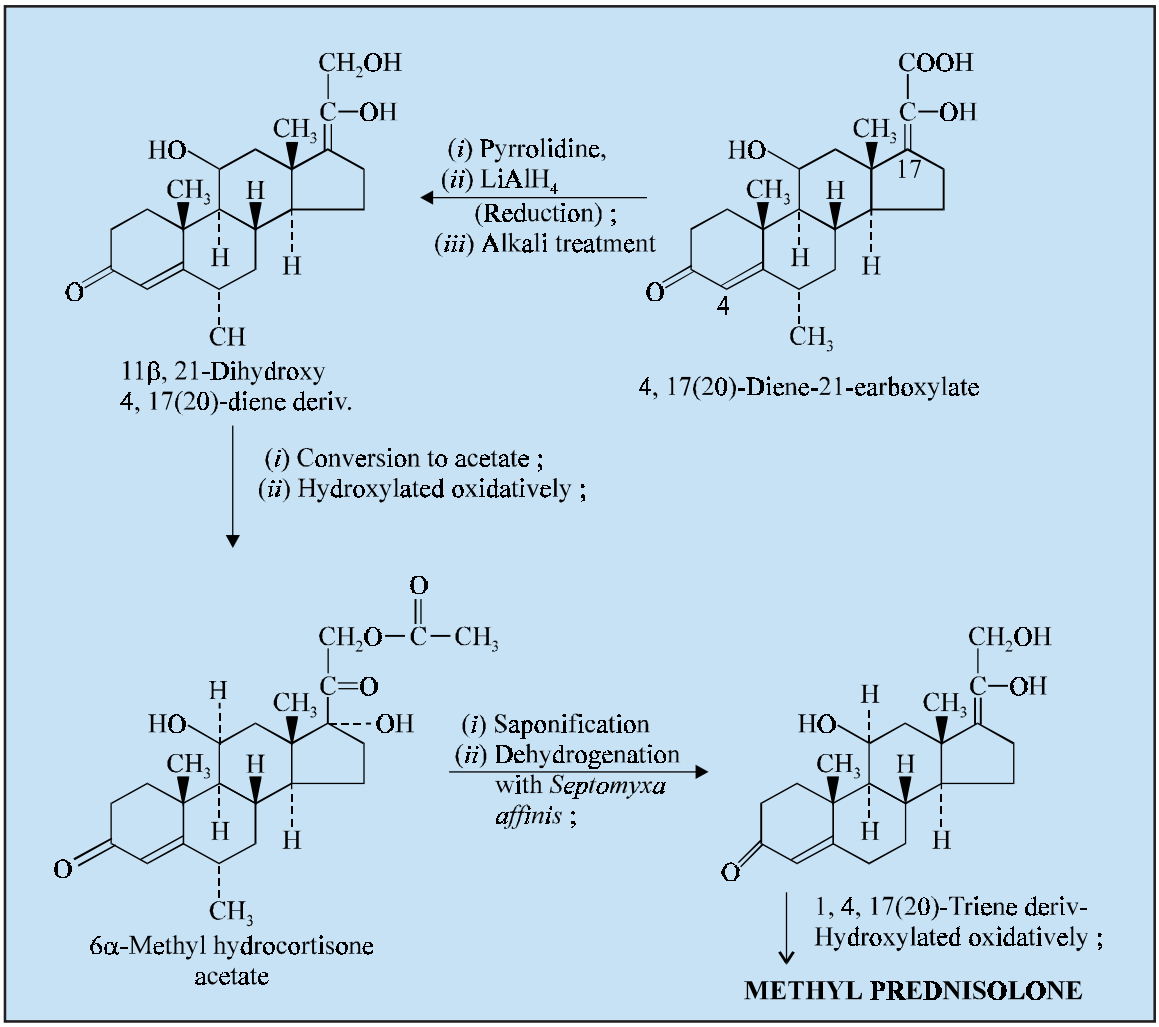


Hydroxyprogesterone on treatment with ethylene glycol and peracetic acid yields an admixture of two isomers *viz.*, 5α, 6α-epoxide and 5β, 6β-epoxide, which upon reaction with methyl-magnesium-bromide (**Grignard's reagent**) gives rise to 5α-hydroxy-6β-methyl derivative. The resulting product first on dehydration followed by epimerization with $HCl/CHCl_3$ produced Δ^4-6α-methyl derivative, which upon incubation with **pestolotia**-an **ascomycetes** gives rise to the corresponding 11α-hydroxy structural analogue. This product on oxidation with chromic acid yields 3,11-diketo derivative, which on sequential treatment with ethyl oxalate-bromination-rearrangement with sodium methoxide (CH_3ONa) and debromination with Zn-dust yields 4,17(20)-diene-21-carboxylate. Further, reaction of this with pyrrolidine–$LiAlH_4$–alkali treatment gives 11β, 21-dihydroxy-4,17(20)-diene derivative, which upon conversion to acetate and oxidative hydroxylation yields 6α-methyl hydrocortisone aetate. The resulting product on saponification and dehydrogenation with *Septomyxa affinis* gives rise to 1,4,17(20)-triene derivative, and this finally on being hydroxylated oxidatively yields the desired official compound, **methylprednisolone.**

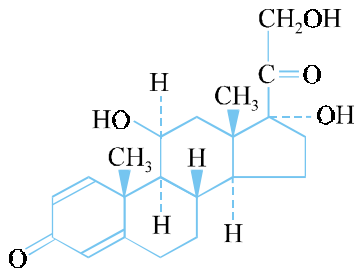
Methylprednisolone induces significantly less retention of sodium and water in comparison to the **parent prednisolone.** It is worthwhile to state here that as it inherently possesses extremely feeble **mineralocorticoid activity,** it is not used in the control and management of acute adrenal insufficiency. It essentially shows the following characteristics :

- **Plasma half-life :** 3—4 hours ;
- **Biological half-life :** 18—36 hours ; and
- **Volume of distribution :** 0.7 L . kg^{-1}.

Note : Methylprednisolone never gets bound to transcortin.

Dose : *Adult : 10–40 mg either via IM or slow IV.*

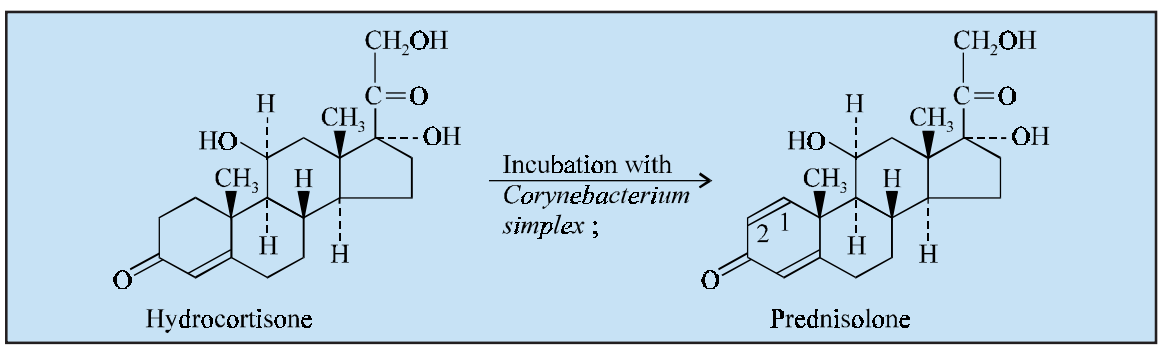
2.1.5. Prednisolone INN, BAN, USAN



(11β)-Pregna-1, 4-diene-3,20-dione, 11,17,21-trihydroxy ;

Prelone(R) ; Emsolone(R) ;

Microbiological Method : Prednisolone is prepared by the **microbiological method** starting with **hydrocortisone** using *Corynebacterium simplex,* which strategically and selectively causes dehydrogenation of the initial product at C-1 and C-2 positions.



Prednisolone-a glucocorticoid is four fold as potent as but comparatively somewhat weaker as compound to **hydrocortisone** as a **mineralocorticoid,** whereas it may lead to retention of Na^+ and depletion of K^+.

It essentially has the following characteristics :

- **Plasma half-life :** ~ 3 hours ;
- **Biological half-life :** 18–36 hours,
- **Pharmacokinetics :** dose-dependent because of non-linear protein-bondage ;

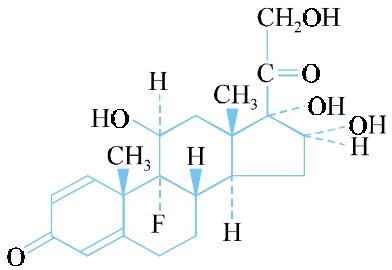
Note : Prednisolone is the biologically active metabolite of Prednisone.

Dose : *Adult : Oral : 5–60 mg per day ; IM/IV (intraarticular) injection : 10–40 mg ; topical (skin and eyes) : 0.25%.*

2.2. Intermediate Acting Glucocorticoids

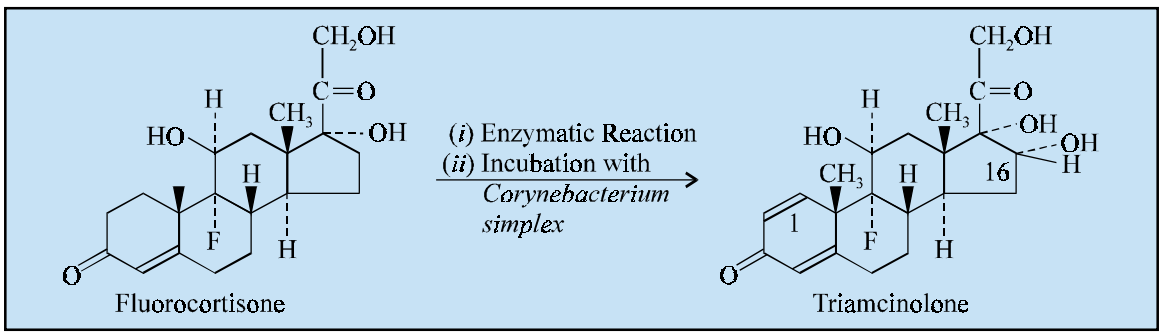
The typical examples are as enumerated below :

2.2.1. Triamcinolone INN, BAN, USAN



(11β,16α)-Pregna-1,4-diene-3,20-dione, 9-fluoro-11,16,17,21-tetrahydroxy– ; IP ; BP ; USP ; Aristocort(R) ; Kenacort(R) ; Tricort(R) ;

Enzymatic and Microbiologic Method



Triamcinolone may be prepared by a specific enzymatic reaction with **fluorocortisone** whereby the **α-hydroxy group** is strategically introduced at C-16. The resulting product is subsequently incubated with the microbe *Corynebacterium simplex* which causes the dehydrogenation between C-1 and C-2 to produce the official compound **triamcinolone.**

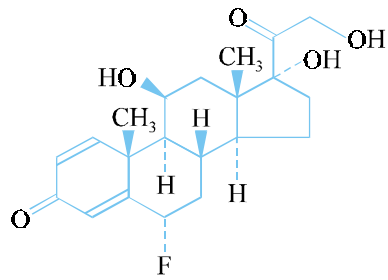
Triamcinolone is found to be 7–13 folds more potent than **hydrocortisone.** It has been established beyond any reasonable doubt that the therapeutically effective doses of this **drug** are practically devoid of **mineralocorticoid** activity together with other observed side effects of **hydrocortisone.** When administered orally, more quantum of this **drug** actually survives the first pass through the liver than does **hydrocortisone** ; and, therefore, one may predict its presence in blood level somewhat more precisely.

Triamcinolone essentially bears the following characteristic features, namely :

- **Plasma half-life** : approximately 5 hours ;
- **Biological half-life** : ranges between 18–36 hours ;
- **Volume of distribution** : varies between 1.4–2.1 L . kg^{-1} which being **dose-dependent.**

Dose : *Adult : oral : 8–32 mg per day ; intraarticular : 2.5–15 mg ; intradermal and deep intramuscular injection ; and as topical cream : 0.1% [as Acetonide : Ledercort].*

2.2.2. Fluprednisolone INN, BAN, USAN



(6α, 11β)-6-Fluoro-11, 17, 21-trihydroxypregna-1, 4-diene-3, 20-dione ; IP ; BP ; USP ;

Alphadrol(R) ; Etadrol(R) ;

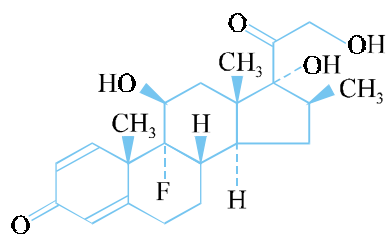
Fluprednisolone is an **intermediate acting glucocortinoid** which exerts an anti-inflammatory activity.

2.3. Long Acting Glucocorticoids

The *two* most important members belonging to this category of **long-acting barbiturates** are, namely : **Betamethasone**, **Dexamethasone**,

These two drugs shall now be treated individually in the sections that follows :

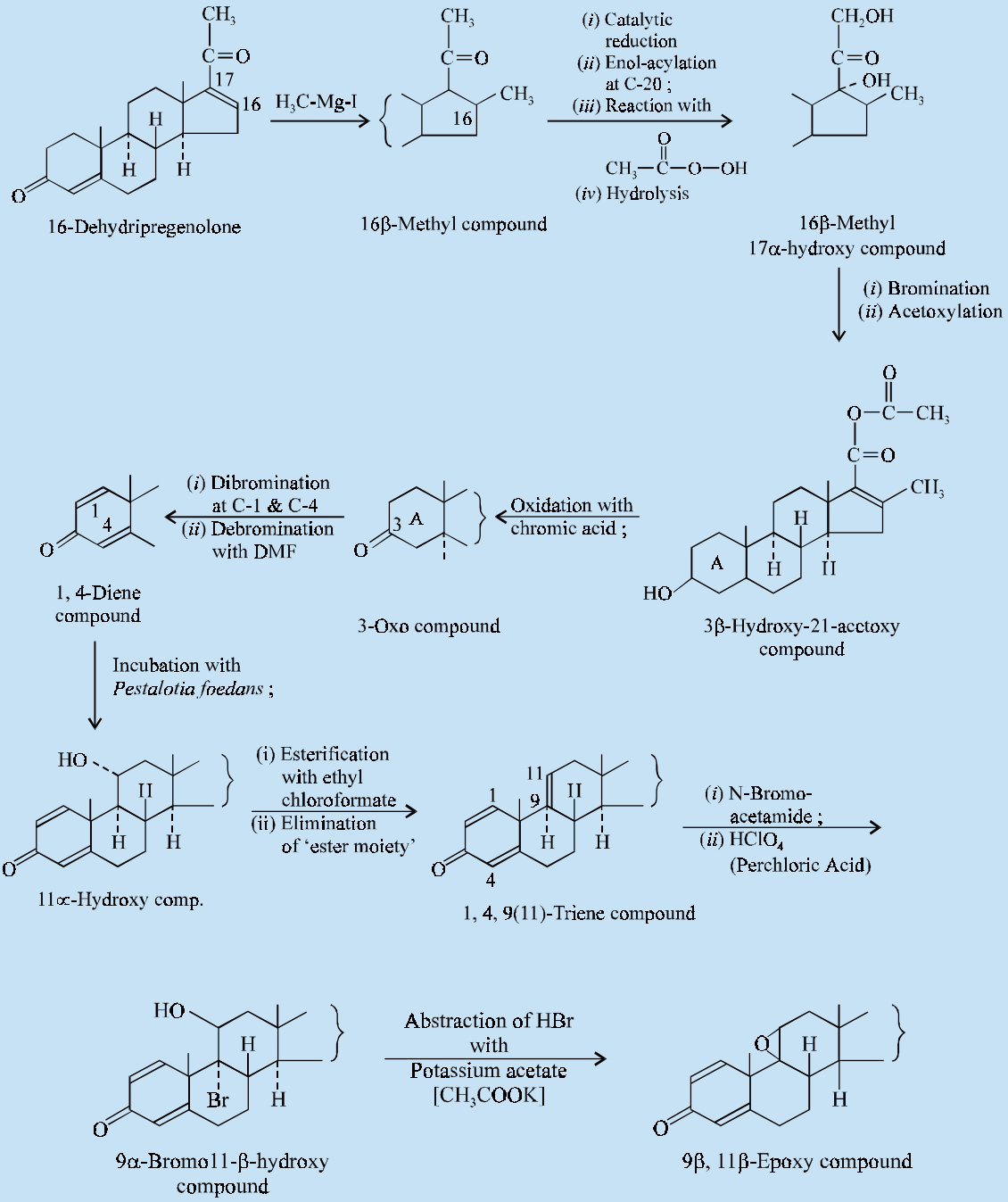
2.3.1. Betamethasone INN, BAN, USAN



(11β, 16β)-9-Fluoro-11, 17, 21-trihydroxy-16-methylpregna-1, 4-diene-3, 20-dione ; IP ; BP ; USP ; Eur. P. ;

Betadexamethasone(R) ; Flubenisolone(R) ; β-Methasone(R) ; Betnesol(R) ;

Synthesis



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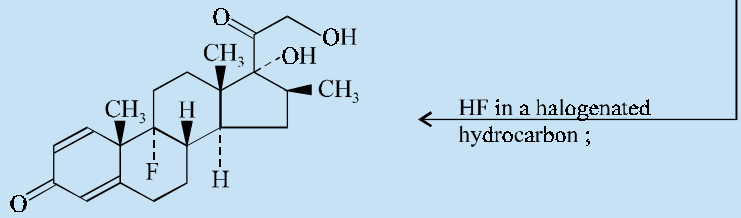


Betamethasone

16-Dehydropregenolone is first treated with methyl magnesium iodide (Grignard's reagent) to insert the 16β-methyl moiety. The resulting compound is subjected to catalytic reduction of the remaining double bond between C-4/C-5, enol-acylation at C-20, and further reaction with peracetic acid [$CH_3-\overset{\overset{O}{\|}}{C}-O-OH$] followed by hydrolysis to the 16β-methyl, 17α-hydroxy compound. This product upon careful bromination and acetoxylation give rise to the formation of 3β-hydroxy-21-acetoxy compound, which is oxidized to the corresponding 3-oxo compound with chromic acid. Dibromination at C-1 and C-4 followed by debromination with dimethyformamids (DMF) yield 1, 4-diene compound, then incubation with *Pestalotia foedans* (or some other similar organism) forms the 11α-hydroxy compound. This compound upon esterification with ethyl chloroformate followed by elimation of the ester moiety results in 1, 4, 9(11)-triene compound, which on treatment with N-bromoacetamide followed by perchloric acid [$HClO_4$] gives the 9α-bromo-β-hydroxy compound. The resulting product when treated with potassium acetate [CH_3COOK] abstracts a mole of HBr to give 9β, 11β-epoxy compound, which on reaction with hydrofluoric acid [HF] in a halogenated hydrocarbon yields the official compound, **betamethasone.**

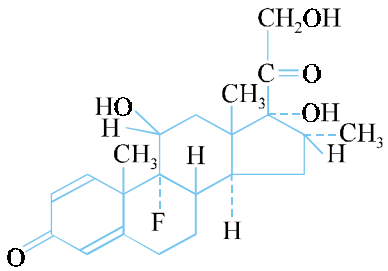
Betamethasone is an extremely potent **long-acting glucocorticoid** having a 20–30 folds enhanced activity in comparison to **cortisol.** It has been duly observed that it very rarely causes induction of sodium and water retention, and potassium depletion *viz.*, co-treatment with **cortisone** and several other **adrenal corticoids.**

It essentially has the following characteristic features :

- **Plasma half-life** : approximately 6.5 hours.
- **Biological half-life** : 36–54 hours, and
- **Volume of distribution** : 1.8 L . kg^{-1}.

Dose : *Adult : oral : 0.5–5 mg per day ; IM/IV : 4–20 mg per day ; topical : 0.1% as betamethasone benzoate cream [Topicasone$^{(R)}$], and betamethasone valerate [Betnovate$^{(R)}$].*

2.3.2. Dexamethasone INN, BAN, USAN



(11β-16α)-Pregna-1, 4-diene-3, 20-dione, 9-fluoro-11, 17, 21-trihydroxy-16 methyl– ; IP ; BP ; USP ;

Decadron(R) ; Dexona(R) ;

Dexamethasone may be prepared almost in an identical manner to that of **Betamethasone** (see Section 2.3.1) ; however, the difference refers to the **16-methyl moiety** being inserted strategically in the **α-configuration.**

Dexamethasone is specifically employed in the treatment of antiallergic and anti-inflammatory conditions. However, it finds its abundant usage in the control, management, and treatment of **glucocorticoid-responsive dermatoses.** It has a **systemic glucocorticoid potency** almost 25 times that of **cortisone.** Based on its ability to suppress distinctly the **pituitary-adrenocortical function** the drug is most frequently used for differential diagnostic purposes particularly in **Cushing's syndrome.***

The various characteristic features of **dexamethasone** are as stated under :

- **Plasma half-life** : ranges between 3–4 hours,
- **Biological half-life** : varies between 36–54 hours, and
- **Volume of distribution** : 0.75 L . kg^{-1}.

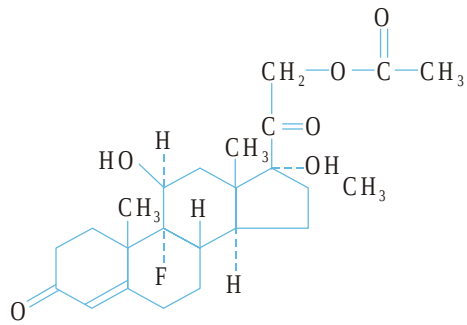
Note : Dexamethasone binds linearity to albumin but does not bind to transcortin.

2.4. Mineralocorticoids

There are several important drug substances that belong to this category *i.e.*, **mineralocorticoids,** but only *two* members from this group shall be discussed, namely : **Fludrocortisone ; Desoxycorticosterone.**

*__Cusling's Syndrome :__ A syndrome resulting from hyposecretion of the adernal cortex in which there is excessive production of **glucocorticoids**.

2.4.1. Fludrocortisone Acetate INN, BAN, USAN



(11β)-Pregn-4-ene-3, 20-diones, 21-(acetyloxy)-9-fluoro-11, 17-dihydroxy- ; IP ; BP ; USP ; Eur. P.,

Florinet Acetate(R) ; Fluoricort(R) ;

Fludrocortisone is a potent **mineralocorticoid** having substantial glucocorticoid activity.

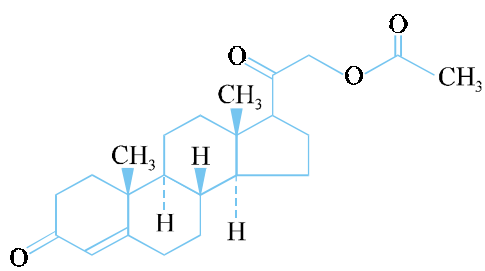
Its characteristic features are as stated below :

• **Plasma half-life** : nearly 3.5 hours, and

• **Biological half-life** : varies between 18 to 36 hours

Dose : *Adult : oral : 0.2–2 mg per day.*

2.4.2. Desoxycorticosterone Acetate INN, BAN, USAN



21-Acetyl oxypregn-4-ene-3, 20-dione, Desoxy cortone acetate ; Deoxycortone acetate ; Percorten(R) ; Dorcostrin(R) ; Syncotryl(R) ;

Desoxycorticosterone is a **mineralocorticoid** belonging to the class of **adrenocortical steroid.**

Dose : *Adult : IM : 10 to 20 mg (once or twice per week) ; sub-lingual (SL) : 0.25 mg.*

Probable Questions for B. Pharm. Examinations

1. (*a*) What are **Adrenocortical Steroids** ?

(*b*) Give examples of some **'Cardinal Adrenocortical Steroids'** along with their **relative activitiy**

with respect to **anti-inflammatory**, **topical features**, and **Na-relention**.

2. Discuss the **salient features** of **Glucocorticoids** and **Mineralocorticoids**.

3. Classify the **Glucocorticoids** on the basis of their pharmacological activities. Give the structure, chemical name, and uses of at least **ONE** potent compound from each category.

4. Discuss the **synthesis** of any **TWO** compounds belonging to the class of **Glucocorticoids** :

(*a*) Hydrocortisone,

(*b*) Methylprednisolone,

(*c*) Prednisolone,

(*d*) Triamcinolone

5. Give the structure, chemcial name, and uses of the following Mineralocosticoids :

(*i*) Fludrocortisone Acetate

(*ii*) Desoxycortisone Acetate

RECOMMENDED READINGS

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2. Hardman JG and Limbird LE (eds.) : **Goodman and Gilman's The Phamacological Basis of Therpeutics,** McGraw Hill, New York, 10th edn., 2001.

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Antibiotics

Chapter

25 Antibiotics

1. INTRODUCTION

The term **"antibiotic"** was put forward by Vuillemin in 1889, **to designate the active component involved in the process of 'antibiosis' or to the opposition of one living micro-organism to another**. According to another school of thought—'**antibiotics are nothing but the microbial metabolites which in relatively high dilution may inhibit the growth of micro-organisms**'.

Waksman proposed the widely cited definition that—***'an antibiotic or an antibiotic substance is a substance produced by the microorganisms, which has the capacity of inhibiting the growth and even of destroying other micro-organisms'***.

However, the restriction that an **antibiotic** must be a product of a micro-organism is not in keeping with common use.

Later on Benedict and Langlykke coined a more general and acceptable definition of an **antibiotic** which states that—***'a chemical compound derived from or produced by a living organism, which is capable, in small concentrations, of inhibiting the life processes of micro-organisms.'***

Therefore, a substance may be classified as an **antibiotic** provided it meets the following *four* **cardinal requirements** ; namely :

(*a*) that it is a product of metabolism

(*b*) that it is a synthetic product produced as a structural analogue of a naturally occurring antibiotic.

(*c*) that it antagonizes the growth and/or the survival of one or more species of microorganisms

(*d*) that it is effective in low concentrations.

In another latest version **'antibiotics'** may be defined as—**'microbial metabolites or synthetic structural analogues inspired by them which, in small dosage regimens, inhibit the growth and survival of microorganisms without any serious toxicity whatsoever to the parent host.'**

Importantly, selective toxicity happens to be the **'key concept'** amongst the antibiotics. There are several vital and glaring instances whereby the **'clinical utility of natural antibiotics'** has been enormously augmented *via.* critical medicinal chemical manipulation of the **mother structure** that would ultimately give rise to not only broader antimicrobial spectrum but also higher potency, lower toxicity and more convenient way of administration.

It is pertinent to state at this point in time that in the modern era the enormous and wide application of '**antibiotics**' in animal nutrition and disease has eventually resulted in the overwhelming sensitization of a comparatively huge number of the susceptible people across the globe, most of whom have developed serious reactions upon contact with such type of drug substances. In the same vein, the most frequent and wide agricultural usage have also made a significant contribution to the ever increasing '**pool of antibiotic resistant bacteria**' in a community.

2. CLASSIFICATION

In this chapter the **antibiotics** will be discussed explicitely under the following *four* main heads, namely :

(*a*) β-Lactam antibiotics,

(*b*) Aminoglycoside Antibiotics,

(*c*) Chloramphenicol, and

(*d*) Tetracyclines.

These *four* types of antibiotics shall be treated in an elaborated manner in the pages that follows :

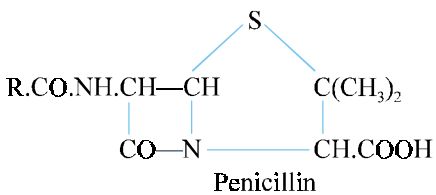
3. β-LACTAM ANTIBIOTICS

The **β-lactam antibiotics** may be further sub-divided into *two* categories, namely :

(*a*) Penicillins, and (*b*) Cephalosporins.

3.1. Penicillins

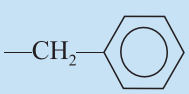
Penicillin is the name assigned to the mixture of natural compounds having the molecular formula $C_9H_{11}O_4N_2SR$, and differing only in the nature of 'R'.



Penicillin

These are mainly produced by various strains of *Penicillium notatum* and *Penicillium chrysogenum*. There are at least **six naturally occurring penicillins,** whose chemical names, other names and the nature of 'R' are given in the following table :

3.1.1. Naturally Occurring Penicillins

S. No.	Chemical Name	Other Names	—R
1	**Pent-2-enylpenicillin**	Penicillin-1 or F	$—CH_2.CH = CH.CH_2CH_3$
2	**Benzylpenicillin**	Penicillin-II or G	

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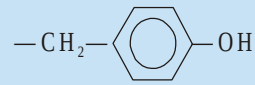
3	***p*-Hydroxybenzyl-penicillin**	Penicillin-III or X	
4	***n*-Heptylpenicillin**	Penicillin-IV or K	$—(CH_2)_6.CH_3$
5	***n*-Amylpenicillin**	Dihydro-F-penicillin	$—(CH_2)_4.CH_3$
6	**Phenoxymethyl-penicillin**	Penicillin-V	$—CH_2—O—C_6H_5$

3.1.2. Structure of the Penicillins

Following are the various salient features which ultimately determine the general structure of all the **penicillins** :

1. The **penicillins** are all strong monobasic acids, *i.e.*, they form salts.

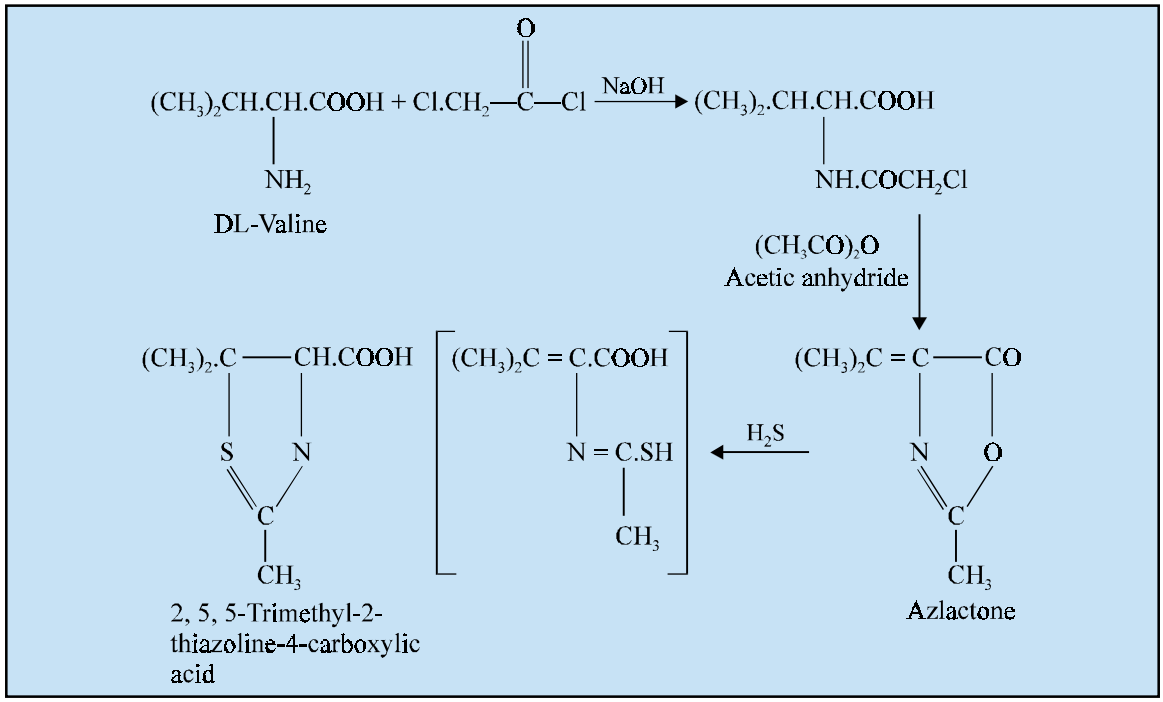
2. The **penicillins** are hydrolysed by hot dilute inorganic acids ; one carbon atom is eliminated as carbon dioxide (CO_2) and two products are obtained in equimolecular proportions, one being an **amine,** *Pencillamine* and the other an **aldehyde, Penniloaldehyde**.

$$C_9H_{11}O_4N_2SR + 2H_2O \xrightarrow{HCl} CO_2 + \underset{\text{Amine}}{C_5H_{11}O_2NS} + \underset{\text{Aldehyde}}{C_3H_4O_2NR}$$

All the **penicillins** give the same amine, but different aldehydes, because it bears the variable component 'R' in it.

3. ***D-Penicillamine* ($C_5H_{11}O_2NS$)**

Penicillamine instantly gives the indigo colour reaction with ferric chloride, a test characteristic of cysteine, thereby suggesting that the amine is probably a substituted cysteine. The structure of **penicillamine** was later on proved to be **D-β: β-dimethylcysteine** by synthesis as described below :



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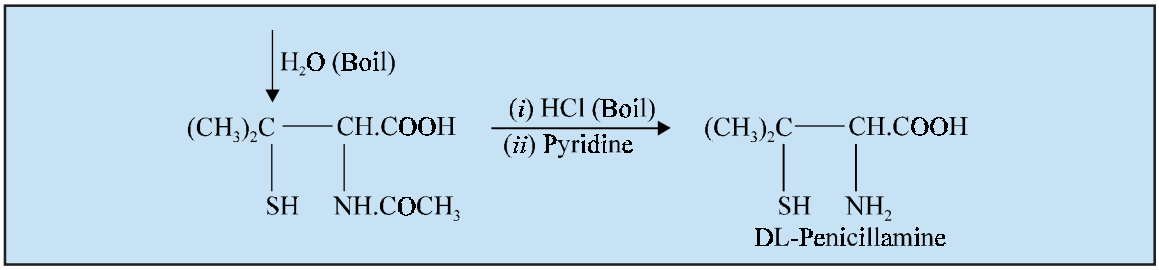
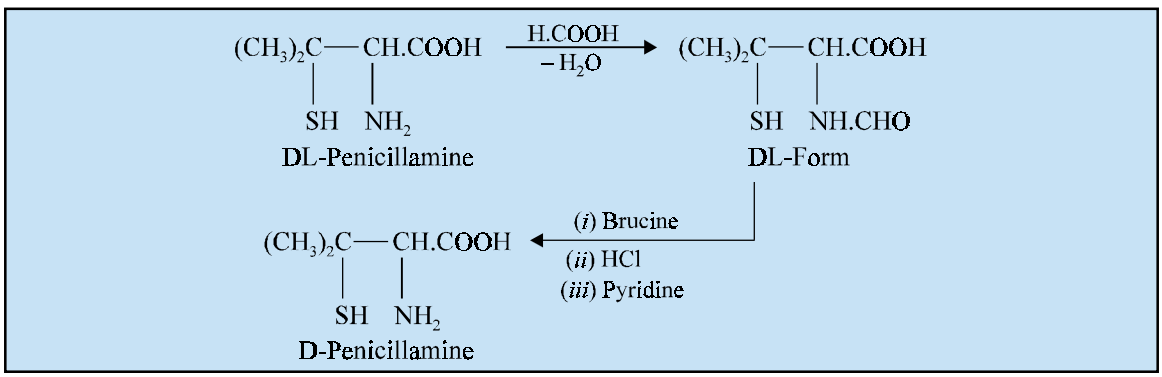


Resolution of the racemic amine

The resulting racemic mixture of **penicillamine** was *first* converted into the formyl derivative ; and *secondly* it was resolved by means of brucine and thirdly, the formyl group was removed by hydrolysis, thus :



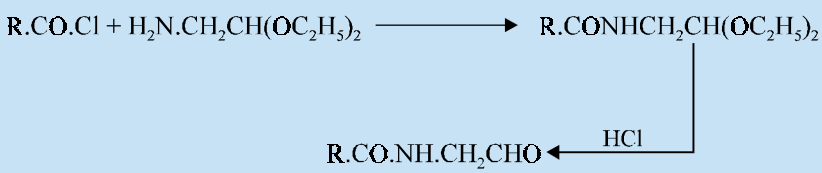
D-Penicillamine was found to be identical with the **natural penicillamine**. When treated with diazomethane ($CH_2=N^+=N^-$), **penicillin** is converted into its methyl ester and this, on treatment with an aqueous solution of mercuric chloride, gives the methyl ester of **pencillamine**, thereby proving that the carboxyl group in penicillamine is the carboxyl group present in the **penicillin** moelcule itself.

4. *Penilloaldehyde*

It has been observed that on vigorous hydrolysis, all the **penilloaldehydes** give a substituted acetic acid and an **aminoacetaldehyde**. Hence, the **penilloaldehydes** may be considered as acylated derivatives of **aminoacetaldehyde**. Thus

$$R.CO.NH.CH_2.CHO + H_2O \rightarrow R.COOH + H_2N.CH_2.CHO$$

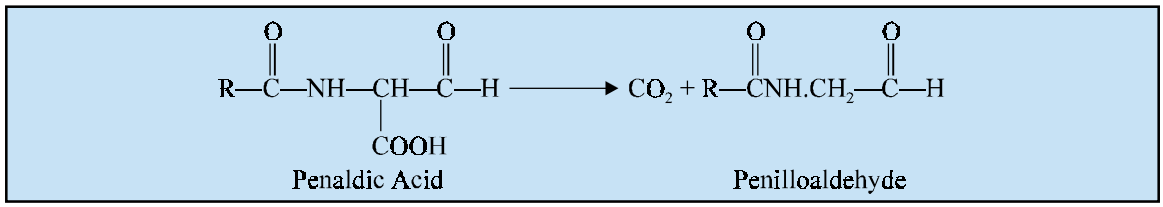
This structure has been confirmed by synthesis :



5. *Carbon Dioxide (CO_2) Molecule*

As stated earlier the acid hydrolysis of penicillin yields three products only *viz.*, **penicillamine**, **penilloaldehyde** and **carbon dioxide**. The liberation of a molecule of **carbon dioxide** gave rise to the belief that it is formed by the ready decarboxylation of an unstable acid. Such an acid is a **β-keto acid**.

Hence, a possible explanation may be put forward that perhaps a **penilloaldehyde-carboxylic acid** (**penaldic acid**) is formed as an intermediate in the hydrolysis of **penicillin**, thus

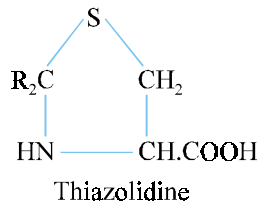


6. ***Combination of Penicillamine and Penilloaldehyde in Penicillin***

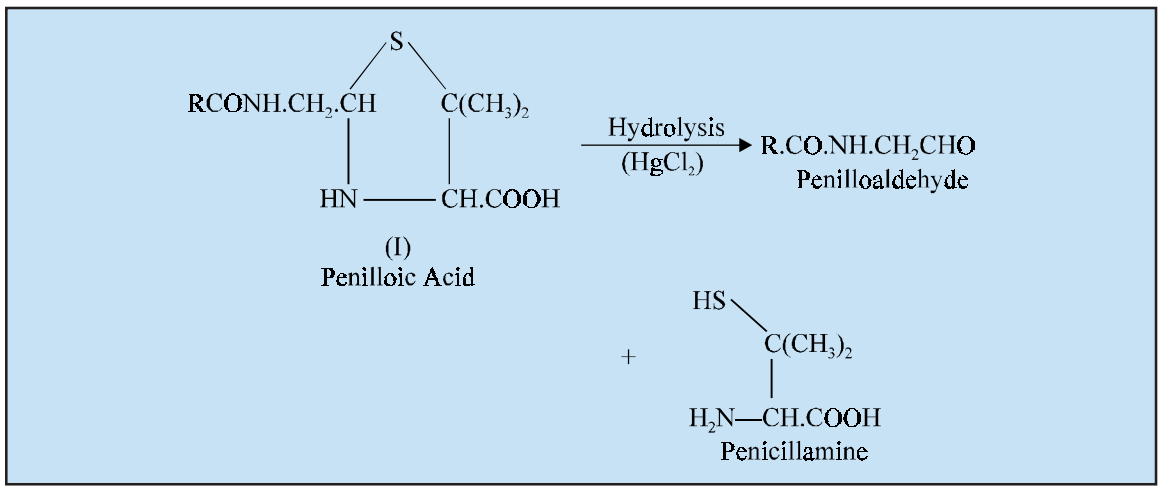
It has been observed that the hydrolysis of **penicillin** with dilute alkali or with the enzyme (***penicillinase***) yields **penicilloic acid** (a dicarboxylic acid), which readily eliminates a molecule of carbon dioxide to form **penilloic acid**, thereby suggesting that a carboxyl group is present in the β-position with regard to a negative group.

7. ***Presence of Thiazolidine Ring***

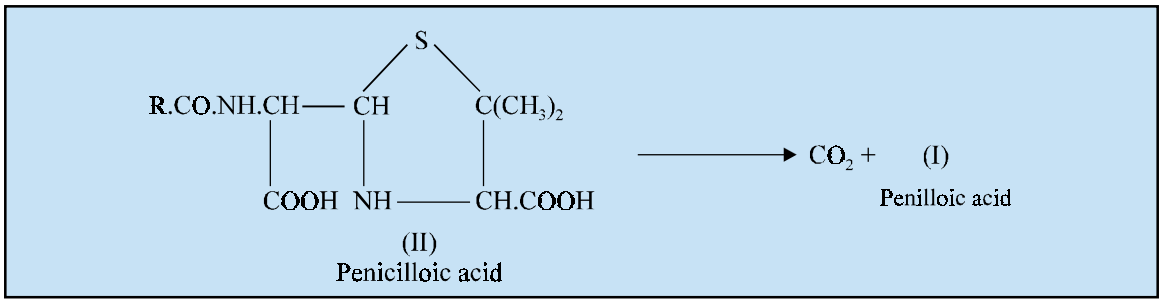
It has been established experimentally that **penilloic acid** upon hydrolysis with aqueous mercuric chloride yields **penicillamine** and **penilloaldehyde** respectively. This type of hydrolysis is characteristic of compounds containing a **thiazolidine ring**, *i.e.*,



Hence, **penilloic acid** could be (I), because this particular structure would give the above required products. Thus

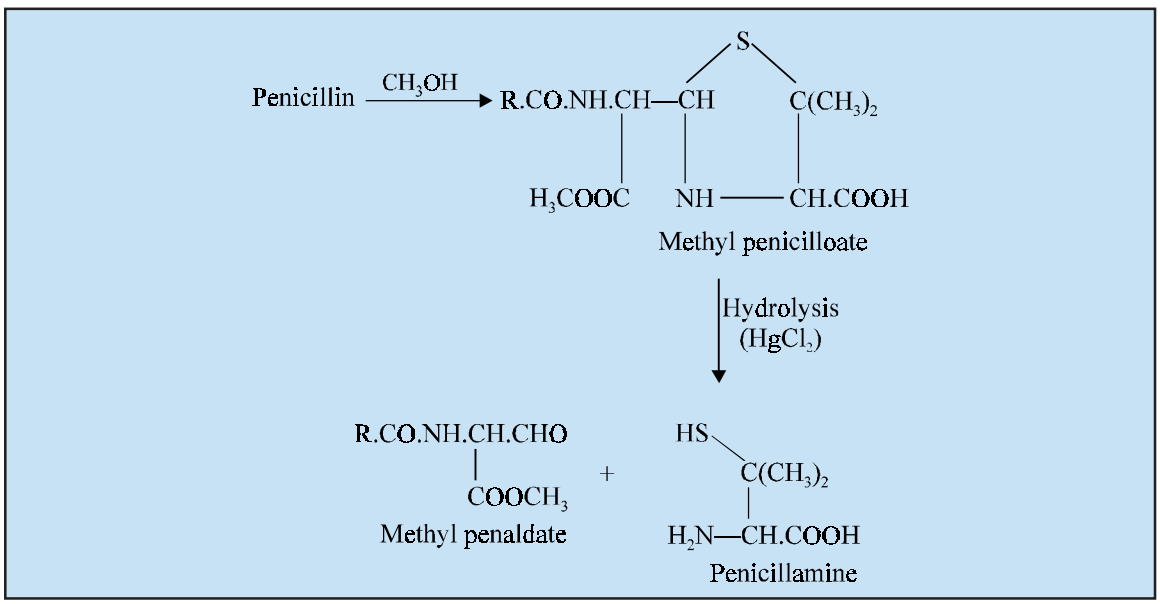


Therefore, if (I) is **penilloic acid**, then **penicilloic acid** would be (II)



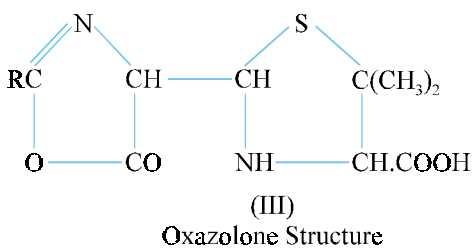
8. ***Evidence for Structure (II)***

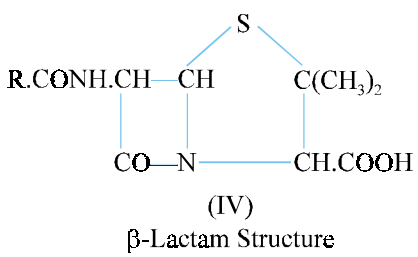
The treatment of **penicillin** with methanol yields the corresponding **ester methyl penicilloate** which, on hydrolysis with aqueous mercuric chloride, gives **methyl penaldate** and **penicillamine**. Thus



9. ***Probable Structures for Penicillin***

Based on the foregoing chemical evidences two probable structures for **penicillin** have been put forward *viz* ; (III) and (IV) ;





At this juncture, however, it was not quite possible to decide between the two structures (III) and (IV) on the ground of chemical evidence alone, since **penicillin** is prone to undergo abrupt molecular rearrangement, *e.g.*, on treatment with dilute acid, **penicillin** rearranges to **penillic acid**.

Therefore, it was absolutely necessary to examine the molecule by physical methods (thereby leaving the molecule intact). In fact, an intensive study of the **penicillins** was carried out with respect to their infra-red and X-ray diffraction analysis.

(a) Infra-Red Analysis

The infra-red (IR) spectra of many **penicillins** were examined and therefrom a correlation between various bands and functional groups was established by examining the spectra of synthetic model compounds which contained different portions of structures (III) and (IV) that had been proposed above on the basis of chemical evidence.

This fact may be further illustrated by taking into consideration the methyl ester as well as the **sodium salt of benzyl penicillin** that exhibited the following characteristic peaks of all the **penicillins** in these regions :

Penicillin (as)	**Characteristic Peaks (cm^{-1})**
Methyl Ester :	3333, 1770, 1748, 1684, 1506
Sodium Salt :	3333, 1770, 1613, 1681, 1515

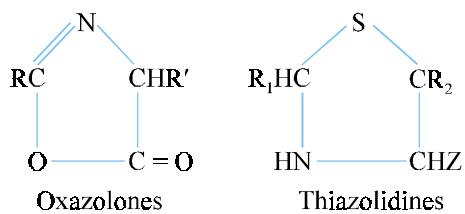
The band at 3333 cm^{-1} in both compounds was due to the NH group (str.)

The 1748 cm^{-1} band of methyl ester and the 1613 cm^{-1} band of the corresponding salt were assigned to the carbonyl group (str.) in the carboxyl group (as ester or salt respectively).

Further, model **oxazolones** were studied that showed two characteristic bands ; one at 1825 cm^{-1} for the carbonyl group and the other at 1675 cm^{-1} for the C = N moiety.

However, the absence of the first band but possible presence of the second in the benzylpenicillin derivatives would not allow an ultimate decision to be reached between (III) and (IV).

On specifically examining a large number of **thiazolidines** in the double bond region down to 1470 cm^{-1}, only the carbonyl bond was revealed to be present (~ 1748 and 1613 cm^{-1}).



[Z = – $COOCH_3$; – $COO^{\ominus}$ etc.]

A number of 1°, 2° and 3° amides were studied extensively. It was found that all the three types showed a characteristic band close to 1670 cm^{-1} which may be attributed to the carbonyl group ; but in the case of the primary amides there was an additional band at 1613 cm^{-1} and with the secondary amides the band was found to be close to 1515 cm^{-1}. These findings reveal that the penicillins possess the secondary amide structure (IV), because the secondary amide band at 1670 cm^{-1} was almost equal to 1684 and 1681 cm^{-1}, besides the band at 1515 cm^{-1} was equivalent to 1506 and 1515 cm^{-1}. Thus, in all, four out of five bands have been accounted for duly.

Finally, a large number of **β-lactams** and fused **thiazolidine-β-lactams** were studied intensively. The former category of compounds did not display a band near 1770 cm^{-1}, but all the latter were found

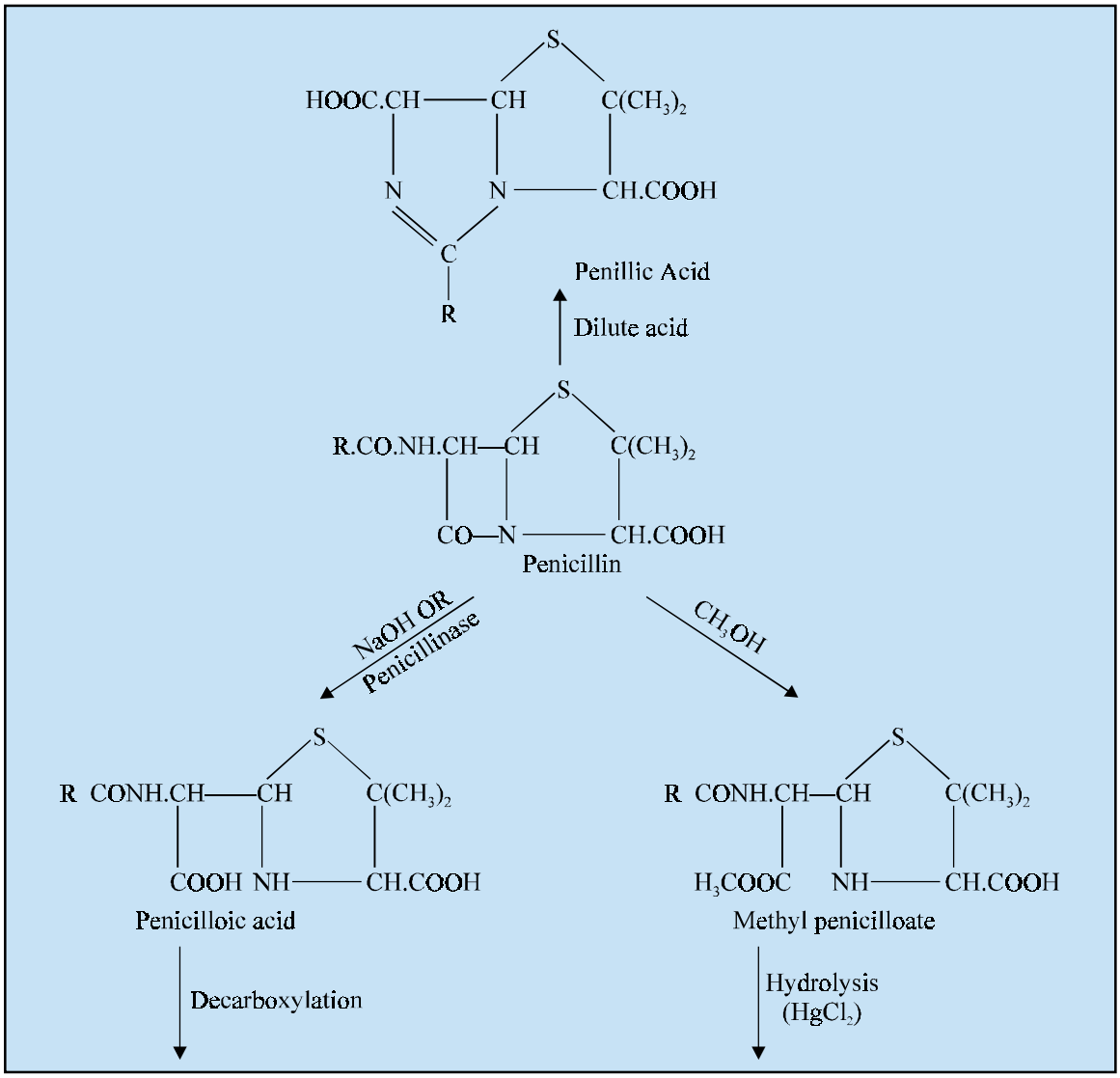
to exhibit a band at 1770 cm^{-1}. This ultimately accounts for the fifth band, and hecne it follows that (IV) is the structure of the **penicillins**.

(b) X-Ray Diffraction Analysis

The **X-ray diffraction analysis** of the sodium, potassium and the rubidium salts of the benzylpenicillin showed the presence of a **β-lactam ring**, thereby further supporting the fact that structure (IV) is the **probable structure of penicillin**.

Based on this structure (IV) and also the various chemical reactions studied so far, one may summarise them as described below :

3.1.3. Chemical Reactions of the Penicillins



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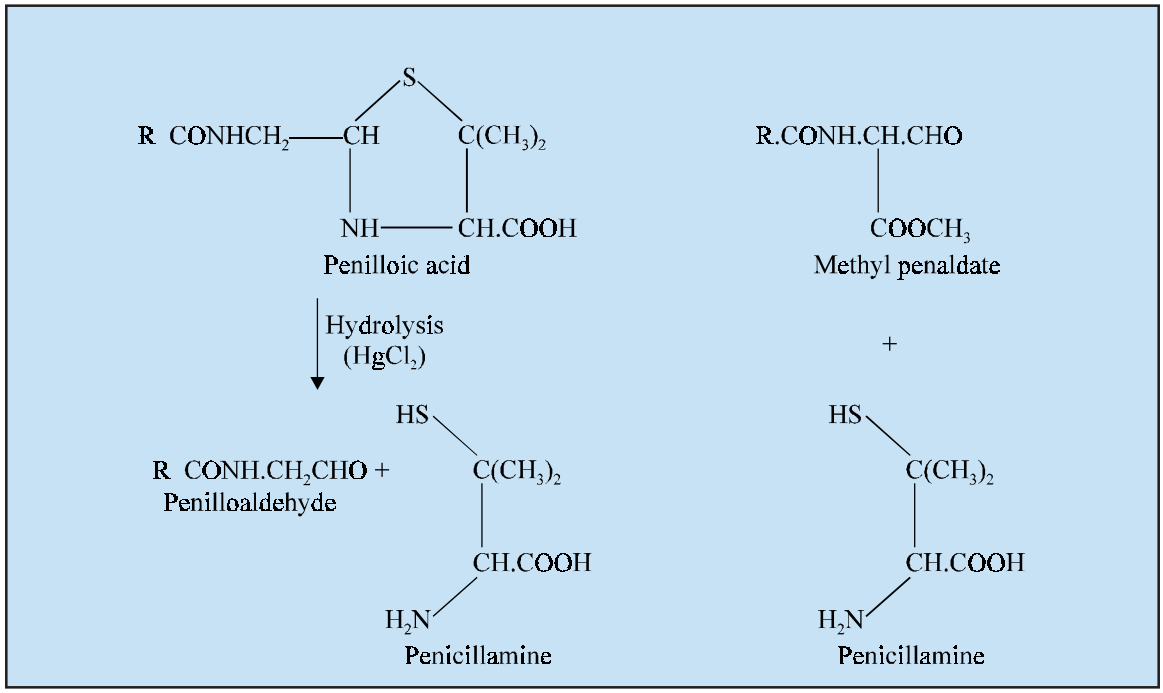


3.1.4. The Penicillin Variants

The advent of latest developments in '**medicinal chemistry**', in fact, put forward the following *five* **penicillin variants**, namely :

(*a*) Natural Penicillins (best streptococcal and narrow spectrum)

(*b*) Penicillinase-resistant Penicilins (antistaphylococcal)

(*c*) Aminopenicillins (improved Gram – ve : *H-influenzae*, *Enterococcus*, *Shigella*, *Salmonella)*,

(*d*) Extended-spectrum (antipseudomonal) penicillins, and

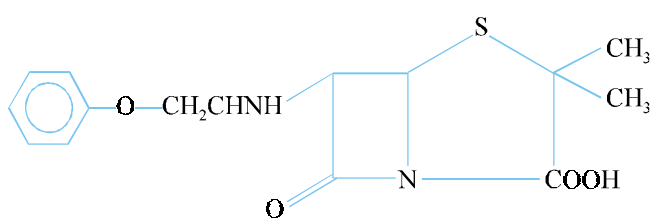
(*e*) β-Lactamase combinations (expand spectrum to staph, β-lactamase producers).

In this particular section a few typical examples from each category of **penicillin variants** shall be discussed comprehensively.

3.1.4.1. Natural Penicillins (best streptococcal and narrow spectrum)

The first successful synthesis of **penicillin** was carried out by Sheehan *et al.* (1957, 1959) who synthesized **Penicillin-V** (*i.e.*, **phenoxymethyl-penicillin**).

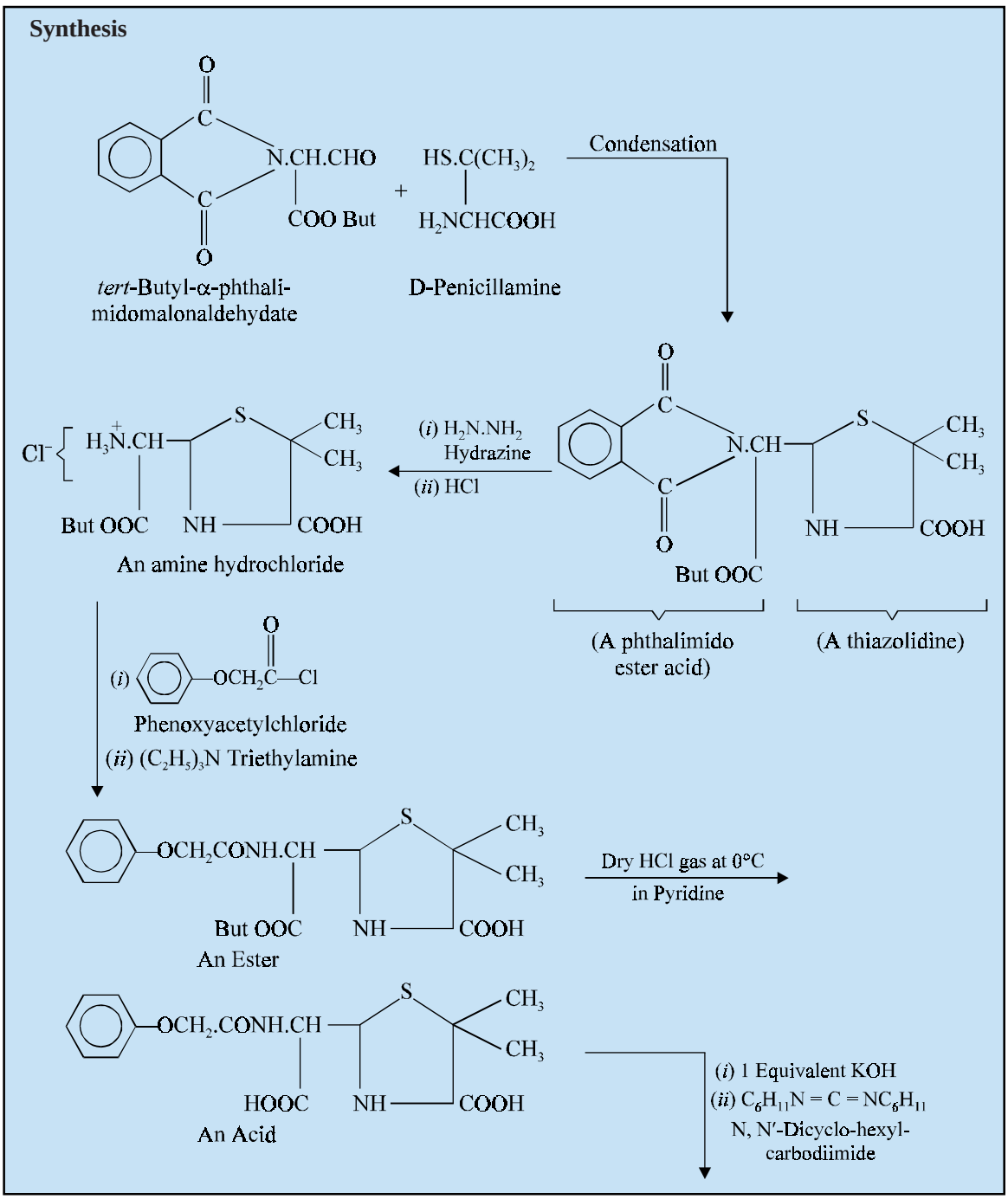
A. *Phenoxymethylpenicillin* INN, BAN *Penicillin*-V USAN,



(6R)-6-2-(-2-Phenoxyacetamido) pencillanic acid ; 4-Thia-1-azabicyclol [3,2,0]-heptane-2-carboxylic acid, 3, 3-dimethyl-7-oxo-6- [(phenoxyacetyl) amino]-, [2S-(2α, 5α, 6β)]- ; Phenoxymethylpenicillin (BP ; Eur. P ; Int. P.,) ; Penicillin-V (USP) ;

V-Cillin$^{(R)}$ (Lilly).

Synthesis



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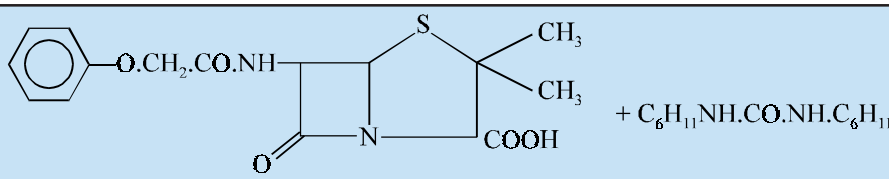


Penicillin-V

Condensation of **D-Penicillamine** and ***tert*-butyl-α-phthalimidomalonaldehydate** yields an intermediate embedded with a phthalimido ester acid and a thiazolidine ring. Treatment of this with hydrazine followed by hydrochloric acid helps the removal of phthaloyl moiety as phthalhydrazide and gives an amine hydrochloride. The resulting product on further treatment with phenoxyacetylchloride in triethylamine introduces the side chain to yield an ester. This product when subjected to a stream of hydrogen chloride gas at 0°C in pyridine helps to remove the blocking *tert*-butyl function thereby yielding **penicilloic acid**. The resulting acid undergoes cyclization to afford **penicillin-V** by stirring for 20 minutes with a solution of N, N′-dicyclohexyl carbodiimide in dioxane.

Penicillin-V is paritcularly effective in the management and control of infections caused by gram-positive bacteria, namely ; streptococcal, staphylococcal, pneumococcal and clostridial infections. It is also the drug of choice in the treatment of a number of gram-positive bacteria *viz.*, gonococcal and meningococcal infections. Besides, it is also used exclusively in the *treatment of pneumonia and other respiratory tract infections caused by Staphylococcus aureus, B. anthracis and Streptomyces pyrogenes. It is now solely used in the treatment of both syphillis and gonorrhea.*

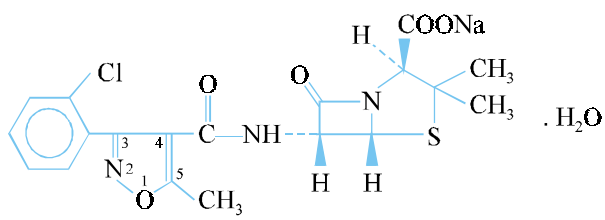
Dose. *Oral, adults and children over 12 years of age or older, usually 125 to 500 mg 3 to 4 times a day.*

Mechanism of Action. The mechanism of action of **penicillin-V** shall be discussed as under : The '**drug**' gets inactivated to a relatively lesser extent by gastric juice in comparison to penicillin G. It is, however, **the *most preferred oral penicillin* for *less serious infections*** due to the fact that serum-levels are found to be 2-5 times higher than matching doses of penicillin G ; besides, there exists relatively less variability in its degree of absorption. The oral bioavailability is about 60% at the most. It gets bound to plasma proteins between 75-80%. The volume of distribution v_d^{ss} stands at 0.73 mL. g^{-1}, which is significantly much higher than that of **penicillin G**. The '**drug**' gets excreted unchanged in the urine between 20-40% . The plasma half-life is nearly 0.5 to 1. hour.

3.1.4.2. Penicillinase-resistant Penicillins (antistaphylococcal)

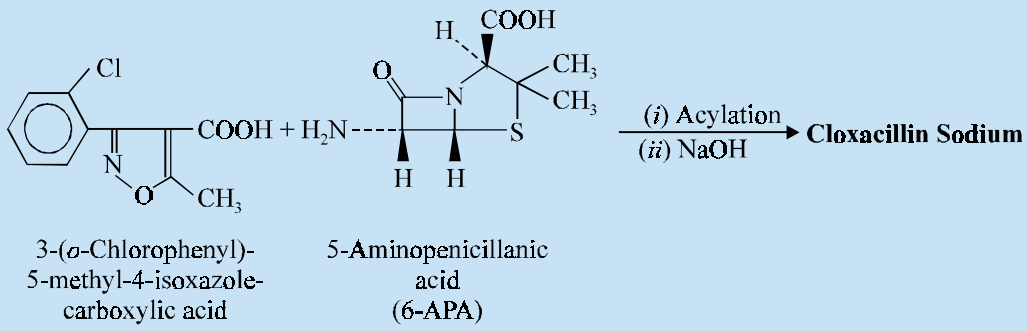
A few typical examples belonging to this class of penicillins are, namely : **cloxacillin**, **methicillin**, **oxacillin** etc., which would be treated individually in the sections that follows :

3.1.4.2.1. Cloxacillin Sodium USAN, INN



[2S-(2α, 5α, 6β)-4-Thia-1-azabicyclo [3, 2, 0] heptane-2-carboxylic acid 6-[[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl] carbonyl] amino]- 3, 3-dimethyl-7-oxo-, monosodium salt, monohydrate ; Tegopen(R); Cloxapen(R); USP ;

Synthesis



3-(*o*-Chlorophenyl)-5-methyl-4-isoxazole-carboxylic acid

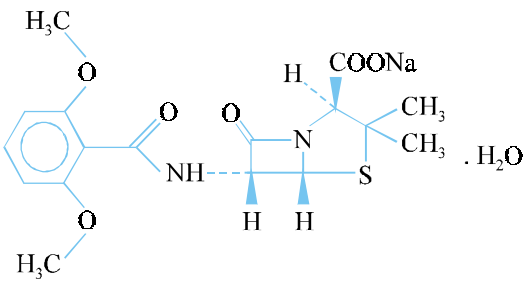
5-Aminopenicillanic acid (6-APA)

6-APA is duly acylated with 3-(*o*-chlorophenyl)-5-methyl-4-isoxazole carboxylic acid and the resulting cloxacillin base is adequately purified by recrystallization. The base thus obtained is converted to the corresponding sodium salt by treating with an equimolar concentration of NaOH.

It is a pencillinase-resistant penicillin (antistaphylococcal) usually administered orally.

Potency : Equivalent of not less than 825 mcg of cloxacillin per mg.

3.1.4.2.2. Methicillin Sodium. USAN, INN



4-Thia-1-azabicyclo [3, 2, 0] heptane-2-carboxylic acid, 6-[(2, 6-dimethoxybenzoyl) amino]-3, 3-dimethyl-7-oxo-, monosodium salt, monohydrate, [2S-(2α, 5α, 6β)]- ; USP ;
Staphcillin(R) ;

The official compound may be prepared by condensing the fermentation-produced 6-APA in an appropriate solvent with 2, 6-dimethoxybenzoyl chloride ; and the resulting methicillin is subsequently precipitated as its corresponding sodium salt by the addition of sodium acetate.

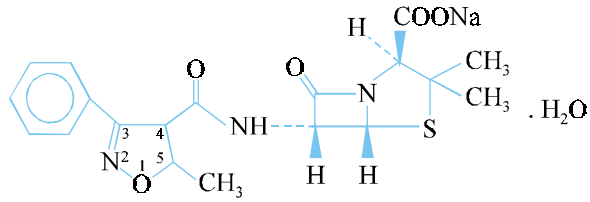
It is usually indicated in the treatment of staphylococcal infections caused by strains resistant to other penicillins. As a precautionary note it is recommended that this '**drug**' should **not** be used in general treatment and therapy so as to avoid the possibility of widespread development of organisms resistant to it.

Mechansim of Action. The '**drug**' is specifically resistant to inactivation by the presence of the enzyme **penicillinase** found in staphylococci. It has been observed to induce penicillinase formation which specifically restrains its usage in the control, management and treatment of **penicillin G-sensitive infections**.

SAR of Methicillin. The **steric hindrence** afforded by the presence of 2, 6-dimelthoxy moieties categorically renders the '**drug**' resistant to enzymatic hydrolysis.

Note. Methicillin gives rise to higher incidence of intestinal nephritis which is reportedly higher in comparison to other penicillins.

3.1.4.2.3. Oxacillin Sodium USAN, INN



[2S-(2α, 5α, 6β)]-4-Thia-1-azabicyclo [3, 2, 0] heptane-2-carboxylic acid, 3, 3-dimethyl-6-[[5-methyl-3-methyl-4-isoxazolyl) carbonyl]-amino]-7-oxo-, monosodium salt, monohydrate ; USP ; Bactocill$^{(R)}$; Prostaphlin$^{(R)}$;

6-APA produced by fermentation is subjected to condensation with 5-methyl-3-phenyl-4-isoxazolyl chloride in an appropriate organic solvent. The resulting **oxacillin** base is precipitated as the sodium salt by the addition of requisite quantum of pure sodium acetate.

The use of the '**drug**' must be restricted to the treatment of infections produced by staphylococci resistant to **penicillin G**.

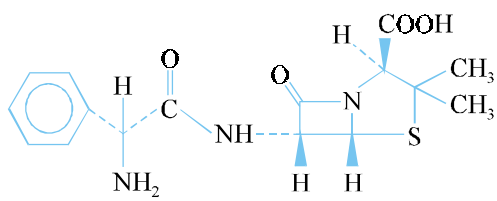
Mechanism of Action. The '**drug**' is reasonably well absorbed from the GI-tract, specifically in fasting subjects. It has been observed that the effective plasma levels of it are easily achievable in about 1 hour ; however, its extensive plasma-protein binding, it gets excreted quickly through the kidneys. The '**drug**' affords first pass metabolism in the liver to the corresponding 5-hydroxymethyl derivative. Interestingly, the resulting '**metabolite**' exhibits antibacterial activity fairly comparable to that of oxacillin, but observed to be less intimately protein bound and more readily excreted.

SAR of Oxacillin. Evidently, the steric effects due to the presence of inherent 3-phenyl and 5-methyl moieties of the isoxazolyl ring not only prevent the binding of this penicillin to the **β-lactamase active site** but also afford protection to the ensuing lactam ring from degradation. The '**drug**' is found to be comparatively resistant to acid hydrolysis and ; hence, could be given orally with good pharmacologic effect.

3.1.4.3. Aminopenicillins (improved Gram –ve : H. influenzae, Enterococcus, Shigella, Salmonella)

The medicinal compounds that are to be discussed in this category are, namely : **ampicillin** and **bacampicillin**.

3.1.4.3.1. Ampicillin USAN, BAN, INN



[2S-[2α, 5α, 6β (S*)]]-4-Thia-1-azabicyclo [3,2,0] heptane-2-carboxylic acid, 6[(aminophenylacetyl) amino]-3, 3-dimethyl-7-oxo ; USP ;

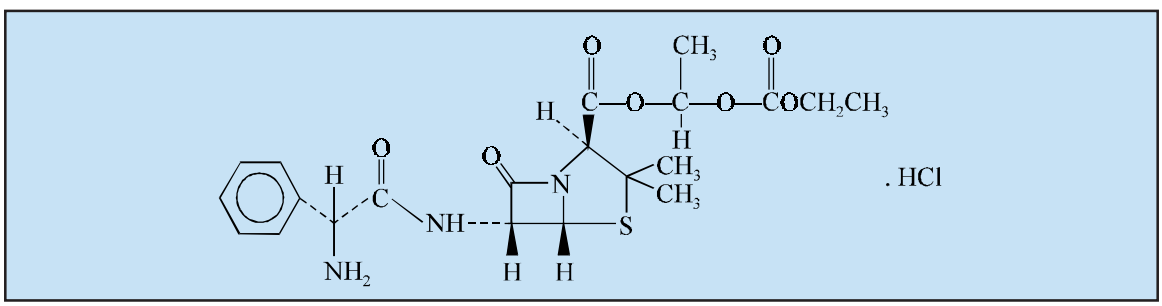
Penbriten(R) ; Polycillin(R) ; Omnipen(R) ; Amcill(R) ; Principen(R) ;

Ampicillin has an antibacterial spectrum broader than that of **pencillin G**. It is active against the same Gram-positive organisms which are susceptible to other penicillins. Besides, it is also found to be more active against certain Gram-negative organisms and enterococci than are other penicillins.

Mechanism of Action. The '**drug**' exerts its action against the same species of Gram-positive organisms which are not only susceptible to other penicillins but also is more active against certain Gram-negative organisms and enterococci than are other penicillins. It is, however, quite evident that the α-amino functional moiety does play a pivotal and vital role in affording its wider activity, but unfortunately the exact mechanism for its action is not yet established. It has been proposed that the α-amino moiety inducts its ability to cross cell wall barriers which are otherwise believed to be impenetrable to other penicillins.

SAR of Ampicillin. The **D-(–) ampicillin** is found to be more active appreciably in comparison to its isomer **L-(+) ampicillin**. The α-amino function in **ampicillin** gets protonated extensively in an acidic media that perhaps legitimately offers a satisfactory explanation with respect to ampicillin's stability to acid hydrolysis, and observed instability to alkaline hydrolysis.

3.1.4.3.2. Bacampicillin Hydrochloride USAN, BAN, INN



4-Thia-1-azabicyclo [3,2,0] heptane-2-carboxylic acid, [2S-[2α, 5α, 6β(S*)]]-6-[(aminophenylacetyl) amino]-3,3-dimethyl-7-oxo-,1-[(ethoxycarbonyl) oxyethyl] ester, monohydrochloride ; USP ;

Spectrobid(R) ;

It is an **aminopenicillin oral prodrug** which gets converted to **ampicillin** *in vivo* after undergoing hydrolysis rapidly in the presence of esterases in plasma.

It has been established beyond any reasonable doubt that its oral absorption is both rapid and virtually complete in comparison to **ampicillin** and also less affected by food.

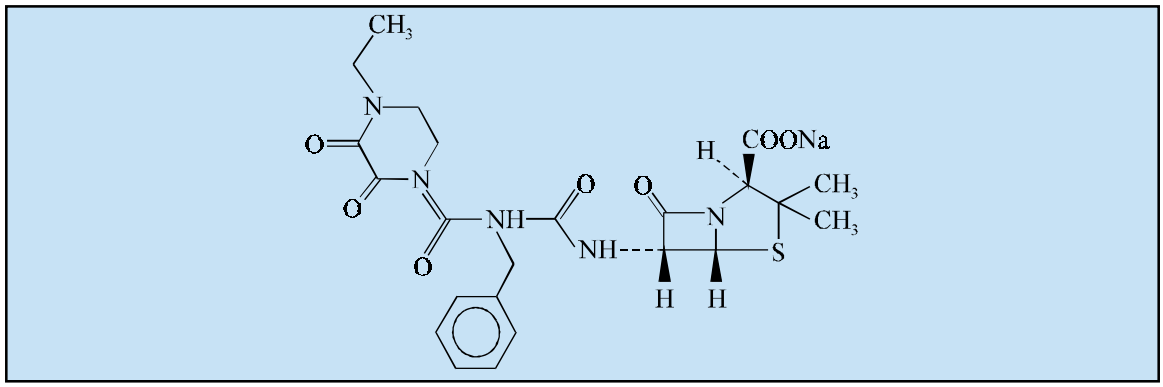
Interestingly, the plasma concentrations of **ampicillin** released from **bacampicillin** evidently exceed from those of oral pure **ampicillin** or **amoxicillin** for the first 2 to 5 hours but after that the pattern remains the same as for **ampicillin** and **amoxicillin**.*

*Neu HC : *Rev. Infect. Dis.* **3** *: 110, 1981.*

3.1.4.4. Extended-Spectrum (Antipseudomonal) Penicillins

The potent penicillin variants belonging to this category which shall be described here are : piperacillin and ticarcillin.

3.1.4.4.1. Piperacillin Sodium. USAN, INN



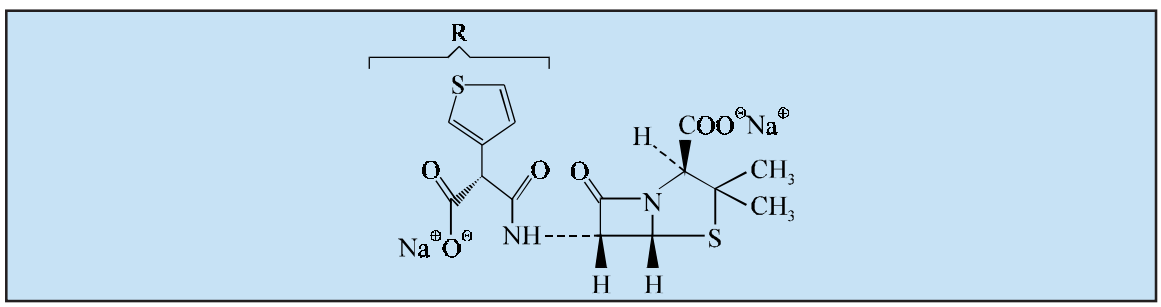
[2S-[2α, 5α, 6β (S*)]]-4-Thia-1-azabicyclo [3,2,0] heptane-2-carboxylic acid, 6-[[[[4-ethyl-2, 3-dioxo-1-piperazinyl) carbonyl] amino]-phenylacetyl] amino]-3, 3-dimethyl-7-oxo-, monosodium salt ;

Pipracil(R);

It is found to be the most active penicillin variant against *Ps aeruginosa*, having a potency almost matching to that of gentamycin. It distinctly shows more activity against *Klebsiella* and several other enteric bacilli in comparison to either *carbenicillin* or *ticarcillin.* It is invariably employed as an '*alternative drug*' for specific use against infections caused by various pathogenic strains, such as : *Acinetobacter, Bacteroides fragilis* (GI-strains), Enterobacter, *E. coli, Kl pneumoniae, Morganella morganii, Pr mirabilis or vulgaris, Providencia rettgeri* or *stuartii, Ps aeruginosa* (UTIs) or *Serratia*. Besides, it exhibits a low efficacy against penicillinase—and other members of the β-lactamase-producing organisms.

Mechanism of Action. The '**drug**' gets destroyed readily by stomach acid (HCl) ; and, therefore, it is only active when adminstered either by IM or IV. It has been observed that the β-lactamase susceptibility of this '**drug**' is not '*absolute'* by virtue of the fact that β-lactamase producing ampicillin-resistant strains of *N-gonorrhoeae* and *H. influenzae* are found to be susceptible to it.

3.1.4.4.2. Ticarcillin Disodium. USAN, BAN, INN



[2S-[2α, 5α, 6β (S*)]]-4-Thia-1-azabicyclo [3,2,0] heptane-2-carboxylic acid, 6-[(carboxy-3-thienylacetyl) amino]-3, 3-dimethyl-7-oxo-, disodium salt ; USP ;

Ticar(R) ;

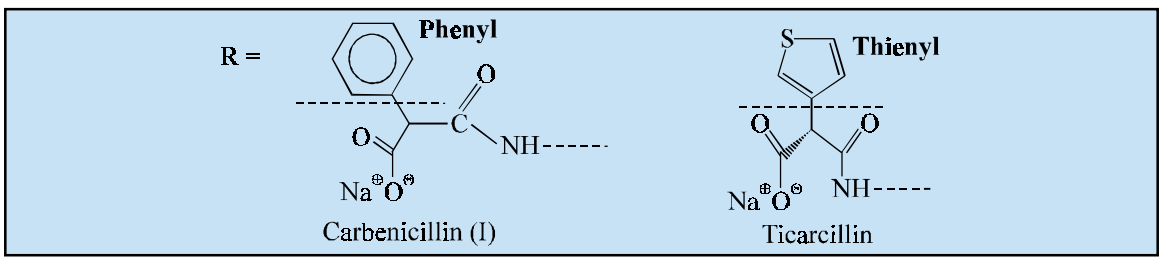
The '**drug**' possesses an almost similar antibacterial profile and pharmacokinetic characteristics as those of **carbenicillin (I)**. Because, of the following *two* positive advantages ticarcillin proved to be highly crucial and vital in the treatment of serious infections (*e.g., Ps aeruginosa)* that may require a *high-dose therapy* :

(*a*) Possesses a slightly better pharmacokinetic characteristics *viz.,* elevated serum levels, and longer duration of action.

(*b*) Higher *in vitro* antibacterial activity against a good number of species belonging to Gram-negative bacilli *e.g., Ps aeruginosa* and *Bacteroides fragilis.*

In plasma, the '**drug**' is protein-bound to the extent of 55-65%. The volume of distribution is 0.22 mL. g^{-1}. It is eliminated by renal excretion. The half-life is found to be 0.5 to 1 hr, except 15 hour in the instance of complete renal failure.

SAR of Ticarcillin. The '**drug**' happens to be an '**isostere**' of carbenicillin wherein the '**phenyl**' moiety has been duly replaced by a '**thienyl**' group as shown below :

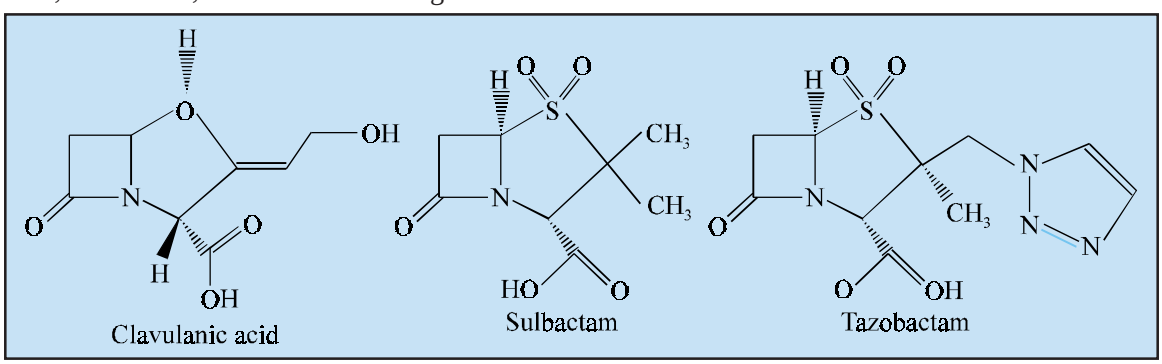


3.1.4.5. β-Lactamase Combinations (expand spectrum to staph-, β-lactamase producers)

In general, it has been observed that the **penicillinase-resistant penicillins** normally get bound to the *penicillinases* ; however, the actual dissociation of the **'drug-enzyme complex'** is rather quite rapid. In actual practice, they have been successfully supplanted by *three* substances, namely : **clavulanic acid**, **sulbactam** and **tazobactam**. In fact, all these are regarded as newer breeds of β-lactamase inhibitors that specifically acylate the enzymes by creation of a '*double-bond*' (greater electronic bondage) and consequently afford dissociation very slowly, thereby significantly enhancing the potency of the penicillins against certain organisms and ultimately increase their therapeutic efficacy*.

The combination of **β-lactam inhibitors** with other **antibiotics** helps to expand the spectrum of the antibiotic to a significant extent which may be observed evidently by carrying out the *in vitro* studies.

However, there are *three* important **β-lactamase inhibitors** duly recognized, namely : **clauvulanic acid**, **sulbactam**, and **tazobactam** as given under : **Clavulanic acid Sulbactam Tazobactam**



*Kar A., *Pharmacognosy and Pharmacobiotechnology,* New Age International, New Delhi, 2nd edn., 2006.

A few typical examples of these β-*lactamase inhibitors* in combination with other '*antibiotics*', which are available commercially, shall now be discussed individually as under :

3.1.4.5.1. Clavulanate-Amoxicillin

A combination administered orally. It causes *more diarrhea than amoxicillin.*

3.1.4.5.2. Clavulanate-Ticarcillin

A combination given IV. It is active versus *more Gram-negative bacilli*.

3.1.4.5.3. Sulbactam-Ampicillin

A combination given IV. It is active versus *Staphylococcus* and β-lactamase producing *H. influenzae* and *Streptococcus pneumoniae.*

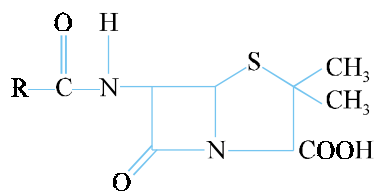
3.1.4.5.4. Tazobactam-Piperacillin

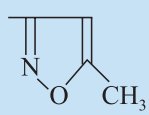
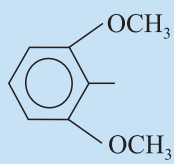
A combination given IV. It is active versus *more Gram-negative bacilli.*

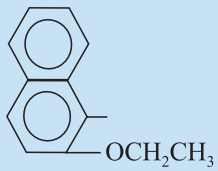
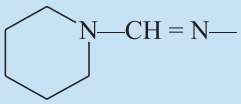
3.1.5. Other Clinically Useful Derivatives of Penicillin-V

Name	Official Status	Brand Name(s)	Dose
Penicillin V Benzathine	USP,	—	—
Phenoxymethylpenicillin Calcium	BP, Int. P.;	—	—
Penicillin V Hydrabamine	USP (XX)	—	—
	BP, USP, Int Int. P., Ind. P.;	Pfizerpen(R) (Pfizer) ; Panapar VK(R) (Parke-Davis)	—

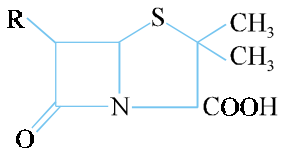
3.1.6. Structures of Some Cinically Useful Penicillins

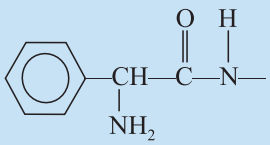
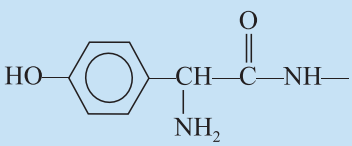
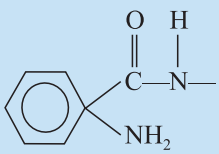


Name	R	Official Status	Brand Name(s)	Dose
Cloxaxillin Sodium		BP; USP; Int. P.;	Cloxapen(R) (Beecham)	500 mg 4 times daily
Methicillin Sodium		B.P. (1973), USP ; Int. P.	Azapen(R) (Pfizer) ; Celbenin (Beecham) ;	1g IM every 4 to 6 hours

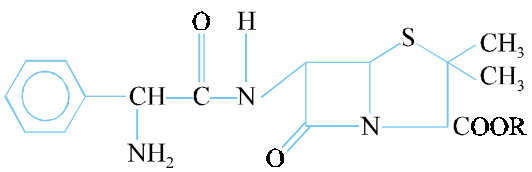
Nafcillin Sodium		USP ; Int. P. ;	Unipen(R) (Wyeth)	500 mg (*e.g.*, of Nafcillin) every 4 to 6 hours
Mecillinam Sodium			Coactin(R) (Hoffmann-La Roche)	12.5 to 15 mg per kg every 6 hours

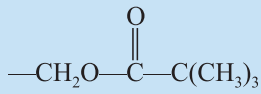
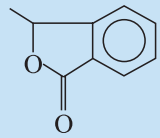
3.1.7. Structures of Some Clinically Useful Penicillins Related to Ampicillin



Name	R	Official Status	Brand Name(s)	Dose
Ampicillin		BP; USP; Int. P.;	Amcil(R) (Parke-Davis) ; Omnipen(R) (Wyeth) ; Pfizerpen A(R) (Pfizer)	150-300 mg per kg body weight daily
Amoxicillin Trihydrate		BP ; USP ;	Amoxil(R) (Bencard, U.K.) ; Utimox(R) (Parker-Davis)	500 mg IM every 8 hours
Ciclacillin		USP ;	Cyclapen-W(R) (Wyeth)	150-500 mg 4 times daily

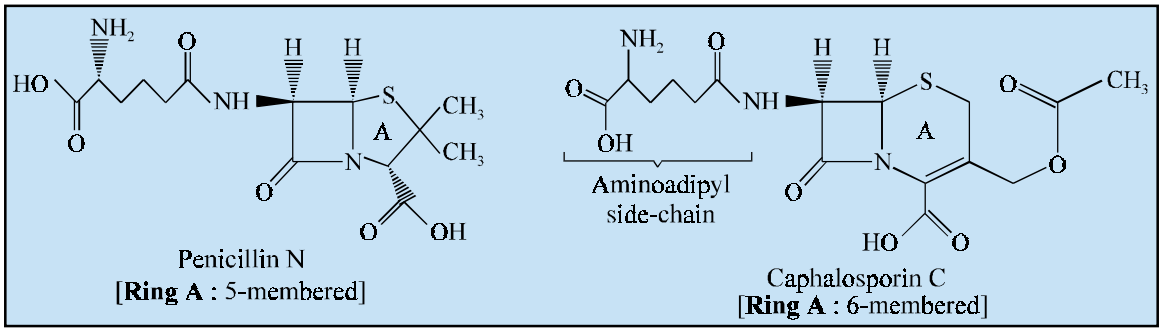
3.1.8. Structures of some Cinically Useful Ester of Ampicillin



Name	R	Official Status	Brand Name(s)	Dose
Pivampicillin Hydrochloride		—	Pondocillin(R) (Burgers, U.K.) ;	500 mg 3 to 4 times daily
Talampicillin Napsylate		—	Talpen(R) (Beecham Research, U.K.)	250 to 500 mg 3 times daily

3.2. Cephalosporins

After the spectacular world-wide recognition and tremendous success of the **penicillins**, the best known family of **β-lactams** are termed as the **cephalosporins**, wherein the **β-lactam ring** is strategically fused to a **6-membered dihydrothiazine ring system** as shown below : Caphalosporin C



Giuseppe Brotzu's epoch making discovery, in 1945, in the species **cephalosporium** fungi obtained from *C. acremonium* showed a remarkable inhibition in the growth of a rather wide spectrum of both Gram-positive and Gram-negative organisms. Abraham and Newton (1961) at Oxford for the first time not only isolated successfully but also characterized **cephalosporin C**.* However, the confirmation of its structure was ascertained by X-ray crystallography.**

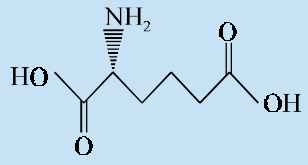
Inspite of the glaring evidence that **cephalosporin C** was resistant to *S. aureus* β-lactamase, besides its prevailing antibacterial activity was inferior in comparison to **penicillin N** and other penicillin structural analogues.

It has been observed critically that the **natural products** usually exhibit a relatively **lower level of antibacterial activity**. Therefore, the articulate and judicial '*cleavage*' of the amide bond of the **aminoadipyl side-chain** present in **cephalosporin C** provides **7-amino-cephalosporanic acid (7-ACA),** which is most ideally suitable for the synthesis of a wide range of semisynthetic cephalosporins *via* acylation of the C(7)-amino functional moiety*** as depicted under :

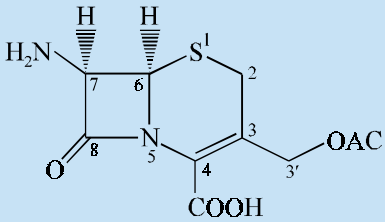
*EP Abraham and GGF Newton, *Biochem J.*, **79**, 377, (1961)

DC Hodgkin and EN Masien, *Biochem. J.*, **79, 393, (1961).

***Fechtig B *et al., Helv. Chim Acta.* **51,** 1108, 1968.



Aminoadipyl Side-chain
(Present in Cephalosporin C)



7-ACA

3.2.1. Classification

The '**cephalosporins**' may be classified under the following *four* categories : Aminoadipyl

(*a*) First generation (staph, some enteric Gram-negative, bacilli)

(*b*) Second generation (more active *Vs* Gram-negative, some active *Vs H. influenzae* and anaerobes)

(*c*) Third generation (best Gram-negative spectrum, β-lactamase resistant, poor *Vs* staph.)

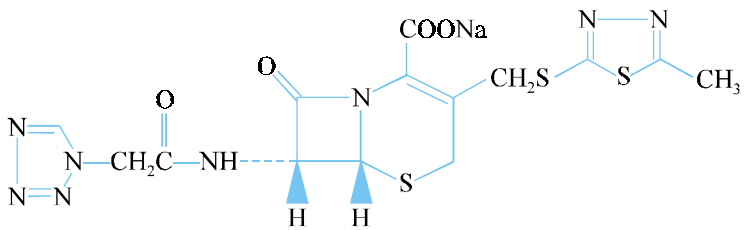
(*d*) Fourth generation.

A few typical examples of '**cephalosporins**' belonging to each of the above *four* generation shall now be discussed more explicitely in the sections that follows :

3.2.1.1. First generation cephalosporins

The following *three* drugs belonging to this class of compounds shall be treated in an elaborated manner, namely : **cefazolin**, **cephalexin**, and **cephradine**.

3.2.1.1.1. Cefazolin Sodium USAN



5-Thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, 3-[[(5-methyl-(6R-*trans*)-1,3,4-thiadiazol-2-yl) thio] methyl]-8-oxo-7-[[(1H-tetrazol-1 yl) acetyl]-amino]-, monosodium salt, USP ;

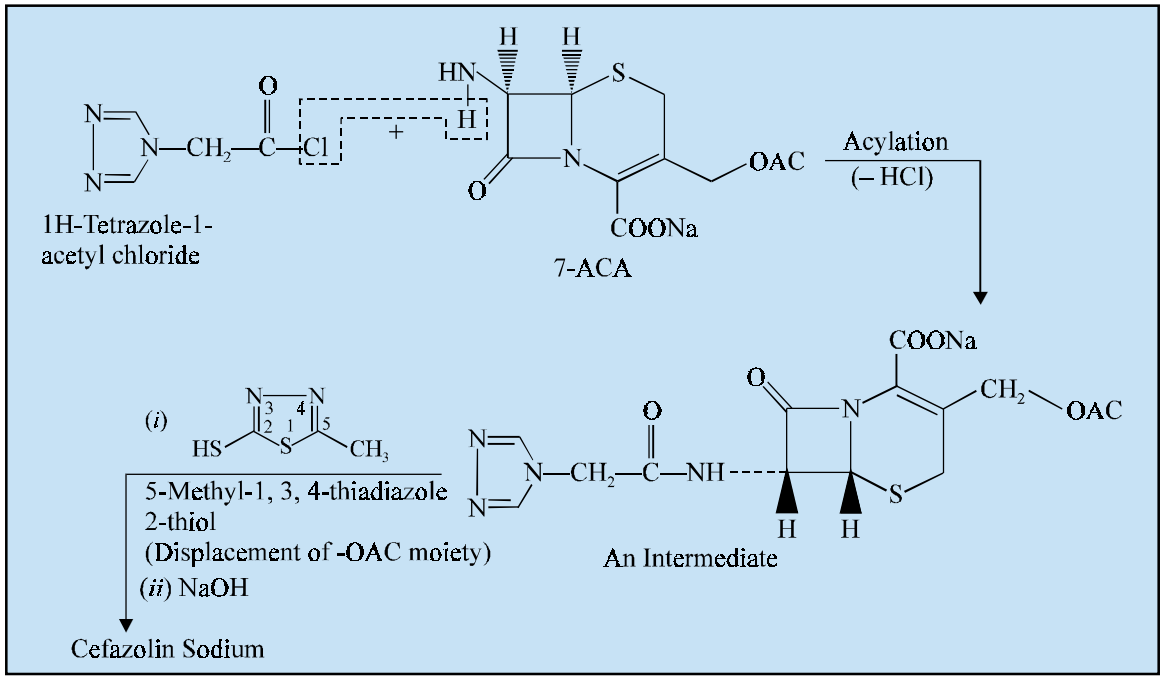
Ancef(R); Kefzol(R);

Synthesis

The acylation of the sodium salt of **7-aminocephalosporanic acid** (*i.e.*, **7-ACA**) with 1H-tetrazole-1-acetyl chloride gives rise to the formation of an intermediate with the elimination of a mole of HCl. The resulting product on being treated with 5-methyl-1, 3, 4-thiadiazole-2-thiol affords the displacement of the acetoxy moiety which upon treatment with an equimolar concentration of NaOH yields the official compound.

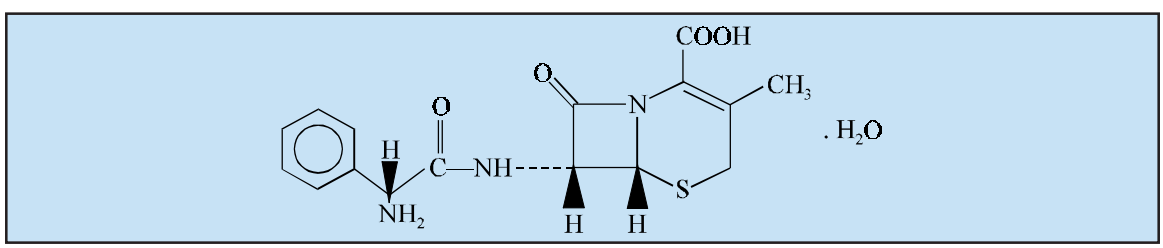
It is a **first-generation cephalosporins** given IM or IV. The '**drug**' may be employed to treat infections of the skin, bone, soft tissues, respiratory tract, urinary tract, and endocarditis and septicemia caused by susceptible organisms. It has been observed that amongst UTIs, cystitis responds much predominantly and better in comparison to *pyelonephritis.** It is regarded to be the preferred cephalosporin for most surgical prophylaxis due to its inherent long half-life.

*Inflammation of kidney and renal pelvis.



Mechanism of Action. The '**drug**' possesses activity against Gram-positive organism, but exhibits a relatively narrow spectrum against Gram-negative strains due in part to their susceptibility to the β-lactamases. However, the Gram-negative activity essentially confined to *E. coli, Klebsiella* and *Pr mirabilis.* It has also been observed that certain *Gram-negative organisms* and *penicillinase-producing staphylococci* which are resistant to both **penicillin G** and **ampicillin** are evidently sensitive to **cefazolin**.

3.2.1.1.2. Cephalexin USAN, INN, BAN



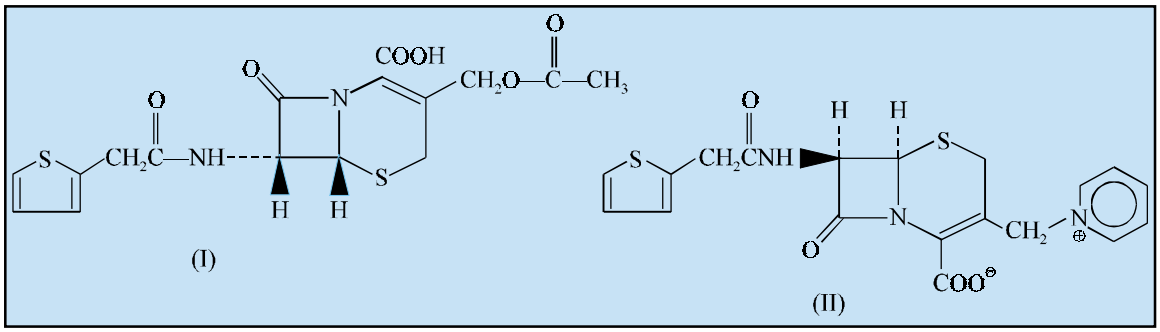
5-Thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, [6R-(6α,7β (R*)]]-7-[(aminophenylacetyl) amino]-3-methyl-8-oxo-, monohydrate ;

Keflex$^{(R)}$;

It is approved and recommended for use against respiratory infections caused by pneumococcus together with β-hemolytic streptococci ; otitis media by *H. influenzae, Branhamella catarrhalis,* pneumococcus, staphylococci ; skin and soft tissue infections by staphylococci and streptococci ; bone and joint infections by *Pr mirabilis* and staphylococci ; and above all the UTIs produced by *E. coli, Klebsiella* and *Pr mirabilis.*

Mechanism of Action. The '**drug**' has been specifically designed as an **orally active semisynthetic cephalosporin**. Importantly, there are *two* vital reasons that are solely responsible for the oral inactivation of cephalosporins, namely :

(*a*) **β-Lactam ring's instability to acid hydrolysis**, such as : **cephalothin** (I) and **cephaloridine** (II) :

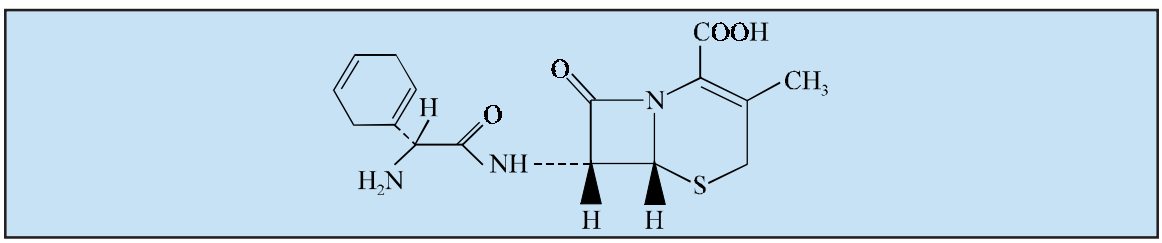


(*b*) **Solvolysis or microbial transformation of the 3-methylacetoxy moiety**, for instance : **cephalothin** and **cephaloglycin.**

The presence of α-amino moiety of **cephalexin** makes the drug '**acid stable**', whereas the reduction of the 3-acetoxymethyl to the methyl function helps in a big way to circumvent profusely the reaction taking place at the specific desired site.

Note. The '*drug*' is significantly much less potent than cephalothin and cephaloridine ; and, hence, is grossly inferior to both of them for the treatment of systemic infections of a vary serious nature.

3.2.1.1.3. Ceptradine USAN BAN, INN



5-Thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, [6R-[6α, 7β-(R*)]]-[(amino-1,4-cyclohexadien-1-ylacetyl] amino]-3-methyl-8-oxo-; USP ;

Anspor(R) ; Velosef(R) ;

A is recognized as a short-acting first-generation **cephalosporin** administered IM or IV. Recommended usually for the treatment of UTIs and respiratory tract infections.

Mechanism of Action. The '**drug**' is minimally protein bound and gets excreted almost 100% after oral administration *via* the kidneys. It is, however, found to be fairly stable in an acidic media (*e.g.*, gastric juice).

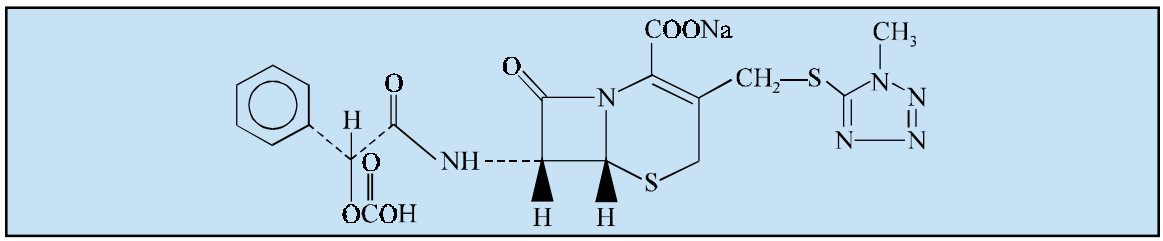
SAR of Cephradine. The '**drug**' has a close resemblance to '**cephalexin molecule**' chemically ; and the former may be viewed as a partially hydrogenated derivative of the latter. Therefore, perhaps cephradine possesses quite similar antibacterial as well as pharmacokinetic characteristics.

Note. The secondary pharmaceutical products (*i.e.*, dosage forms) contain usually a non-stoichiometric hydrate essentially containing upto 16% water ; and, therefore, all such products must indicate explicitely by the labeling on the package itself.

3.2.1.2. Second Generation Cephalosporins

In this particular class of compounds the following typical examples shall be treated individually in delails *e.g.*, **cefamandole**, **cefoxitin**, and **cefuroxime**.

3.2.1.2.1. Cefamandole Nafate USAN, INN



5-Thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, [6R- [6α, 7β(R*)]]-7-[[(formyloxy) phenylacetyl] amino]-3-[[(1-methyl 1H-tetrazol-5-yl)-thio]methyl]-8-oxo-, monosodium salt ; Mandol$^{(R)}$;

It is a short-acting **second-generation cephalosporins** normally administered IM or IV. It exhibits a broader spectrum of activity with an increased activity against *Haemophilus influenzae*, besides the *enterobacteriaceae* produced as a result from a overwhelmingly enhanced stability to the **β-lactamases**.

SAR of Cefamandole. The various salient features are :

(*i*) A formate ester of **cefamandole**, a semisynthetic **cephalosporin** which essentially inducts D-mandelic acid as the '**acyl portion**' ; and a sulphur-containing heterocycle (*e.g.*, 5-thio-1,2,3,4-tetrazole) instead of the acetoxy moiety positioned on the C-3 methylene C-atom.

(*ii*) Esterification of the α-hydroxyl function of the D-mandeloyl moiety eventually circumvents the instability function of this '***drug***' particularly in solid-state dosage forms.* This important salient feature caters for the satisfactory concentrations of the **parent antibiotic** *in vivo via* spontaneous hydrolysis of the prevailing ester between a neutral to alkaline pH range.

(*iii*) D-Mandeloyl functional group present in this '**drug**' seems to afford noticeable resistance to a few **β-lactamases**, by virtue of the fact that **certain β-lactamase-producing Gram-negative organisms** (specifically **Enterobacteriaceae**) which display obvious resistance of **cefazolin** and other **first generation cephalosporins** (see Section 3.2.1.1.) are found to be sensitive to **cefamandole**,

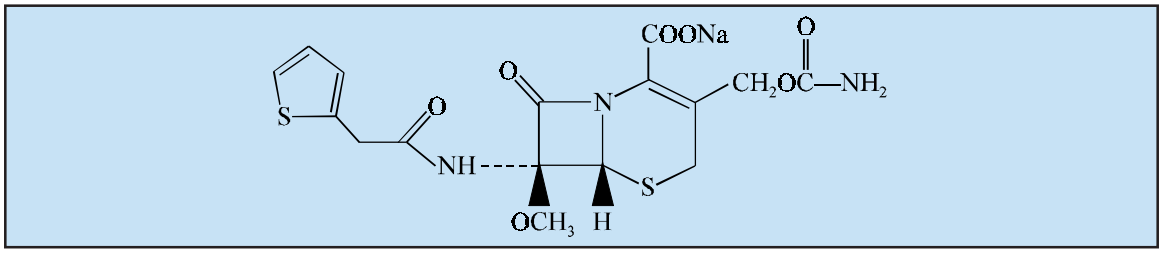
(*iv*) Besides, the '**drug**' is also active against a few **ampicillin-resistant strains** of *Neisseria* and *Haemophillus* species ; and

(*v*) Permeability and intrinsic acitivity along with viable resistance to the **β-lactamases** are the glaring factors that establishes the ensuing sensitivity of individual bacterial strains to this '**drug**'**.

*Indelicato JM *et al. J Pharm Sci,* **65** : 1175, 1976.

Ott JL *et al. Antimicrob Agents Chemother,* **15 : 14, 1979.

3.2.1.2.2. Cefoxitin Sodium USAN, INN



(6R-*cis*)-5-Thia-1-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, 3-[[(aminocarbonyl) oxy] methyl]-7-methoxy-8-oxo-7-[(2-thienylacetyl) amino]-, sodium salt ; USP ;

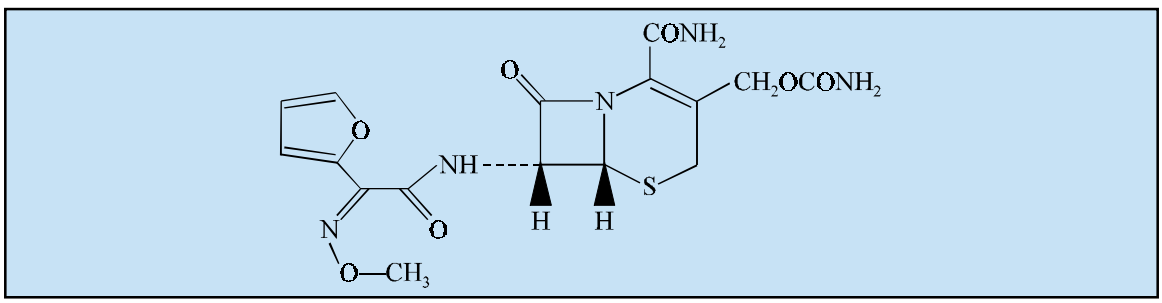
Mefoxin$^{(R)}$;

It is invariably used as an '**alternative drug**' for the treatment of intra-abdominal infections, colorectal surgery or appendectomy and ruptured viscus because it is active against most enteric anaerobes including the organism *Bacteroides fragilis.* It is also indicated in the management and treatment of bone and joint infections produced by *S. aureus,* gynecological and intra-abdominal infections caused by *Bacteroides* species together with other common enteric anaerobes and Gram-negative bacilli ; lower respiratory tract infections produced by *Bacteroides* species, *E.coli, H. influenzae, Klebsiella* spp. *S. aureus* or *Streptococcus* spp. (except enterococci) ; septicemia caused by *Bacteroides* spp., *E. coli, Klebsiella* spp., *S. aureus* or *Strep pneumoniae* ; skin infections produced by *Bacteroides* spp., *E. coli,* Klebsiella spp., *S. aureus* or *epidermidis* or *Streptococcus* spp. (except enterococci) or UTIs by *E. coli, Klebsiella* spp. or indole positive *Proteus,* and for preoperative prophylaxis.

Mechanism of Action. The '**drug**' is found to be resistant to certain **β-lactamases** which are responsible for the hydrolysis of **cephalosporins**. It has been duly observed that cefoxitin helps to antagonize the action of **cefamandole** (see Section 3.2.1.2.1) against *E. cloacae* and also that of carbenicillin against *P. aeruginosa.* As the half-life is comparatively of shorter duration ; therefore, the drug must be administered 3 to 4 times per day.

Note. Solutions of its sodium salt stable for 24 hours at an ambient temperature and lasts upto 1 week when refrigerated (0-10°C). However, 7α-methoxyl solutions helps to stabilize the β-lactam to alkaline hydrolysis to a certain extent.

3.2.1.2.3. Cefuroxime Sodium USAN, BAN, INN



5-Thia-1-azabicyclo [4, 2, 0] oct-2-ene-2-carboxylic acid, (6R, 7R)-7-[2-(2-furyl) glyoxylamido]-3-(hydroxymethyl)-8-oxo-, 7-(Z)-mono (*o*-methyloxime) carbamate (ester) ;

Kefurox$^{(R)}$; Zinacef$^{(R)}$;

It is approved for the treatment of meningitis caused by *Streptococcus pneumoniae, N. meningitidis* and *S. aureus.* It exhibits an excellent activity against all gonococci, hence is also employed to treat gonorrhea. It also finds its usage in the treatment of lower respiratory tract infections invariably caused by *H. influenzae* and *parainfluenzae, Klebsiella spp., E. coli, Strep pneumoniae,* and pyrogens and *Staph aureus.* It is also recommended for use against UTIs produced by *E. coli* and *Klebsiella*, which designates a more limited approval in comparison to other second generation cephalosporins. It is also indicated in bone infections, septicemias, and above all the surgical prophylaxis.

Mechanism of Action. The '**drug**' exerts its activity against *H. influenzae*, besides its inherent ability to penetrate directly into the **cerebrospinal fluid (CSF)** which renders it specifically beneficial for the treatment of *meningitis* caused by that susceptible organism. **Cefuroxime** gets distributed evenly throughout the entire body segments. It has been observed that almost 85% of the '**drug**' gets eliminated in the urine. Its half-life range between 1.3—1.7 hour but may get extended upto even 24 hours in the specific instance of renal failure.

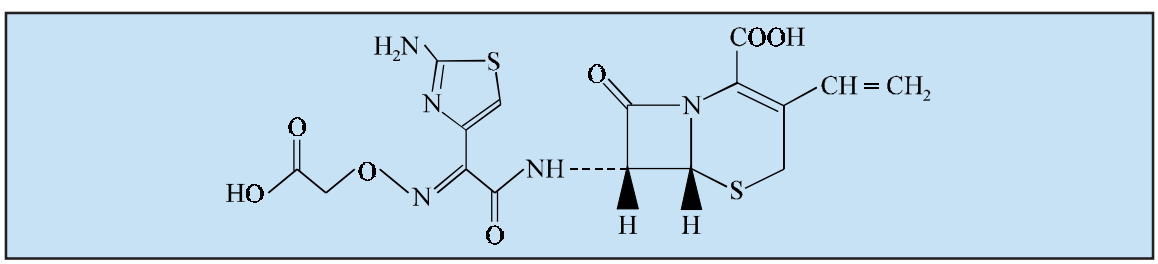
Note. The sodium salt of cefuroxime is poorly absorbed by the oral route. However, its corresponding 'axetil ester' is also available for the oral administration of otitis media, pneumonia and UTIs.

SAR of Cefuroxime. The presence of a *syn*-alkoxiamino substituent in the drug molecule is closely associated with the prevalent β-**lactamase activity** in these cephalosporins. Perhaps its inclusion into the '**second generation cephalosporins**' is duly justified due to the fact that its antibacterial spectrum bears a close similarity to that of **cefamandole** (see Section 3.2.1.2.1).

3.2.1.3. Third Generation Cephalosporins

Though there are several drugs that are approved and marketed belonging to the '**third generation cephalosporins**', but only *three* such compounds shall be discussed in this particular section, namely : **cefixime**, **ceftazidime**, and **ceftibuten**.

3.2.1.3.1. Cefixime USAN, INN



5-Thia-azabicyclo [4,2,0] oct-2-ene-2-carboxylic acid, [6R-[6α, 7β(Z)]]-7-[[(2-amino-4-thiazolyl) [(carboxymethyoxy) imino] acetyl] amino]-3-ethenyl-8-oxo- ; USP ;

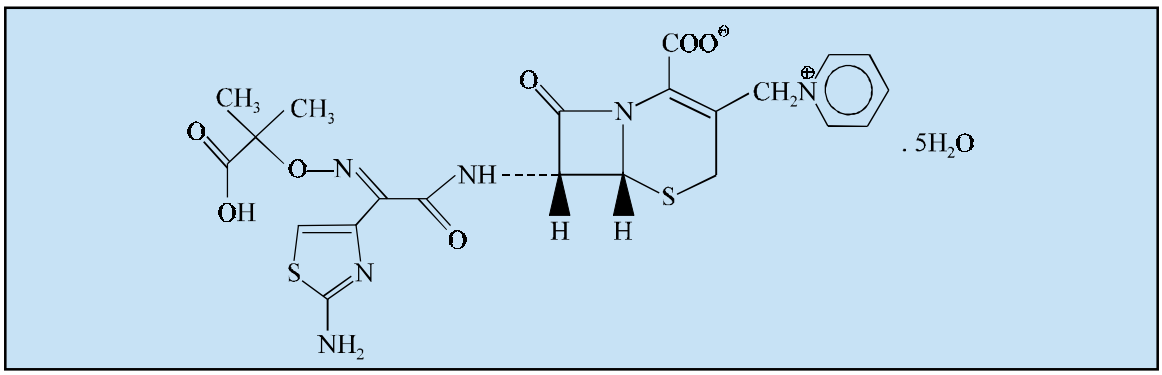
Suprax$^{(R)}$;

It is a well-known orally active **third generation cephalosporin** having superb and excellent therapeutic profile against a plethora of *E. coli, Klebsiella, H. influenzae, Branhametla catarrhalis, N. gonorrhoeae* and *meningitidis,* besides including β-*lactamase producing strains*. It is found to be active against certain common **streptococci** spp. whereas **staphylococci** are genuinely resistant. It is invariably recommended for the respiratory infections*, otitis media and uncomplicated UTIs; however, its actual therapeutic role is yet to be understood exhaustively.

*Infections due to acute bronchitis, pharyngitis, and tonsititis.

Mechanism of Action. The '**drug**' gets absorbed gradually and rather incompletely from the GI tract and exhibits a bioavailability ranging between 40-50%. Importantly, the apparent appreciable good oral absorption of this drug substance is due to its facilitated and augmented transport across the **intestinal brush-border membranes** that essentially implicate the ensuing carrier system for the **'dipeptides'**.* However, this result was not quite expected by virtue of the fact that the prevailing '**drug**' predominantly is devoid of the **ionizable α-amino moiety** either present in the '**dipeptides**' or the '**β-lactams**'previously known to be transported by the aforesaid carrier system.**

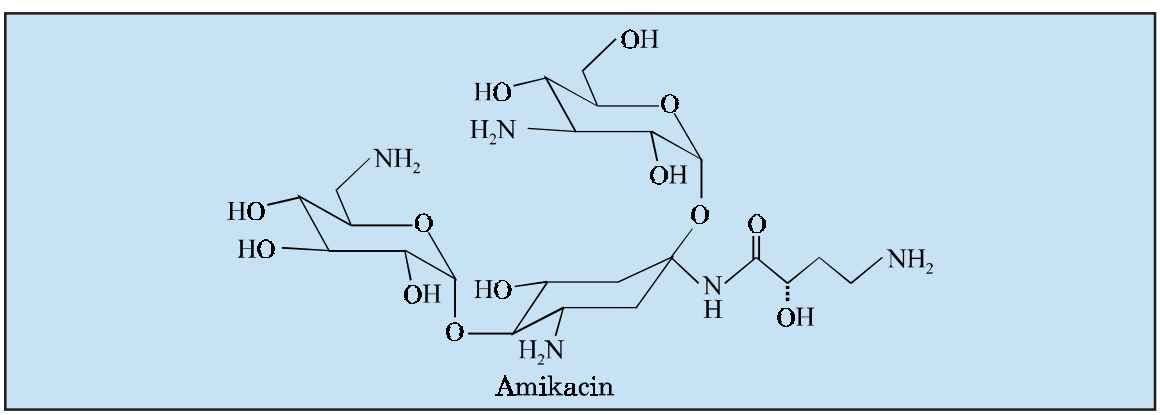
3.2.1.3.2. Ceftazidime Sodium USAN, INN



Pyridinium, [6R (6α, 7β(Z)]]-1-[[7-[[(2-amino)-4-thiazolyl)-I(1-carboxy-1-methylethoxy) imino] acetyl] amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo [4,2,0] oct-2-ene-3yl]-, hydroxide, inner salt ; USP ;

Fortaz(R) ; Tazicef(R) ; Tazidime(R) ;

The '**drug**' displays its special interest due to its inherent high activity against the *Pseudomonas* and *Enterobacteriaceae* but fails to do so for *enterococci.* It is well recognized widely as an '**alternative drug**' specifically for the management and treatment of hospital-acquired Gram-negative infections. However, a combination with **amikacin** in the treatment of infections in immunocompromised patients when *Ps aeruginosa* happens to be a causative organism.



*Tsuji A *et al. J. Pharm. Pharmacol.*, **39,** 272, 1987.

Westphal JP *et al. Clin. Pharmacol. Ther.* **57, 257, 1995.

Ceftazidime is profusely recommended for use in the treatment of bone and joint infections, CNS-infections, gynecological infections, lower respiratory tract infections, septicemia, skin and UTIs.

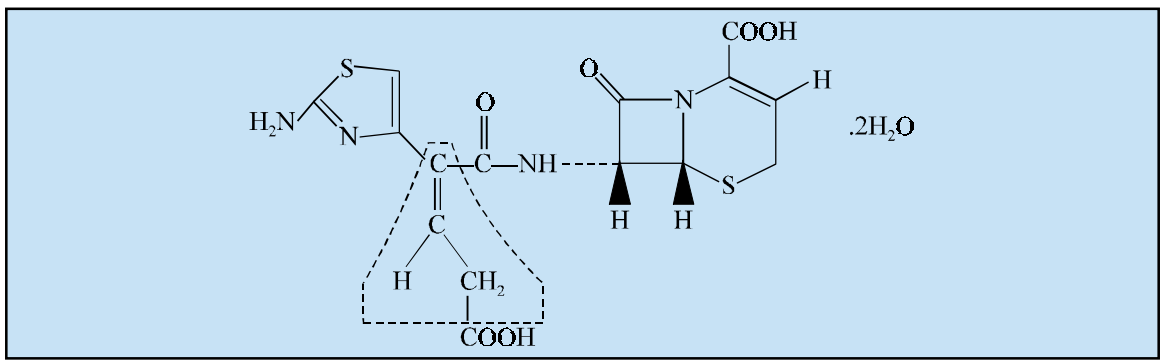
Mechanism of Action. The mechanism of action of this '**drug**' is solely attributed by the presence of *two* characteristic structural features, namely :

(*i*) a **2-methylpropionicoxaminoacyl moiety** which exclusively confers the β-lactamase resistance and, perhaps is responsible for an enhanced permeability *via* the porin channels of the cell envelope, and

(*ii*) a **pyridinium functional moiety** strategically positioned at the 3′-position which essentially affords the **Zwitterionic characteristic features** on the '**drug molecule**'.

The '**drug**' is eliminated upto 80-90% in the urine. The plasma half-life in normal healthy persons is almost 2 hours, but may be prolonged in patients having renal failure.

3.2.1.3.3. Ceftibuten INN



5-Thia-1-azabicyclo [4, 2, 0] oct-2-ene-2-carboxylic acid, [6R-[6α, 7β (Z)]]-7-[[2-(2-amino-4-thiazolyl)-4-carboxy-1-oxo-2-butenyl] amino]-8-oxo-, dihydrate ;

Cedax$^{(R)}$;

The '**drug**' has excellent potency against a majority of the members of the *Enterobacteriaceae* family, *H. influenzae, Neisseria* spp., and *M. catarrhalis.* It is found to be **not** active against *S. aureus* or *P. aeruginosa* and exhibits mild streptococcal activity. It is invariably indicated in the management and control of community-acquired respiratory tract, urinary tract and above all the gynecological infections.

SAR of Ceftibuten. It is one of the most recent chemically novel structural analogues of the **oximinocephalosporins** wherein an **olefinic methylene moiety** (C = $CHCH_2$—), as shown by the dotted line in the above structure,with **Z stereochemistry** has virtually replaced the *syn*-oximino (C = NO—) moiety (which is present in **ceftazidime***).*

Mechanism of Action. The aforesaid isosteric replacement gives rise to a compound which predominantly retains the resistance to hydrolysis catalyzed by a host of **β-lactamases**, has not only increased chemical stability but also rendered the '**drug**' orally active. It enjoys the glory of being the '*drug'* having the highest oral bioavailability amongst the prevailing third-generation cephalosporins.*

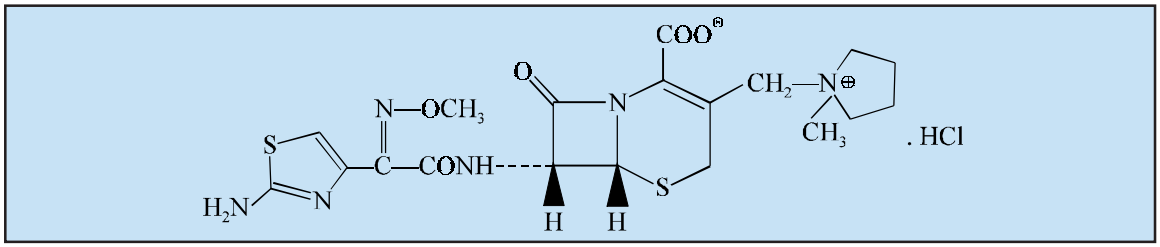
The '**drug**' gets excreted mostly unchanged in the urine. It possesses a half-life of about 2.5 hours. **Ceftibuten** has been found to have the plasma protein binding upto 63%.

3.2.1.4. Fourth Generation Cephalosporins

The only approved drug substance that belongs to the **fourth generation cephalosporins** is **cefepime** which will be discussed as under :

*Fassbenden M *et al. Clin. Infect. Dis.*, **16,** 646, 1993.

3.2.1.4.1. Cefepime Hydrochloride USAN, INN



Pyrrolidinium, [6R-(6α, 7β(Z)]]-1-[[7-(2-amino-4-thiazolyl) (methoxy-imino) acetyl] amino-2-carboxy-8-oxo-5-thia-1-azabicyclo [4,2,0] oct-2-ene-3yl]-methyl]-1-methyl-, hydroxide, inner salt hydrochloride ;

Maxipime$^{(R)}$; Axepin$^{(R)}$;

It is profoundly recognized as an altogether new approved **fourth-generation cephalosporin** which essentially possesses an extended Gram-negative spectrum against Gram-negative aerobic bacilli usually covered by **cefotaxime** and **ceftazidime** including certain strains that are found to be resistant to these **third-generation cephalosporins**.

It is, however, pertinent to state here that **cefepime** definitely exhibits an improved antibacterial profile against *Streptococcus pneumoniae* and *Staphylococcus aureus* in comparison to the **third-generation cephalosporins**. Interestingly, its specific activity against *P. aeruginosa* is found to be variable just like other antibiotics ; and the profile of activity resides between that of **ceftazidime** and **ceftoxime**.

The '**drug**' gets excreted mostly in the urine having a half-life of 2.1 hours. It is found to be bound almost minimally to the plasma proteins.

Note. Cefepime HCl may be administered IVor IM for the treatment of UTIs, pneumonias and skin infections.

4. AMINOGLYCOSIDE ANTIBIOTICS

The aminoglycoside antibiotics constitute an important category of antibacterial agents in the therapeutic armamentarium, *e.g.*, streptomycins, neomycins, paramomycins, kanamycins, gentamycins and the corresponding derivatives of these antibiotics.

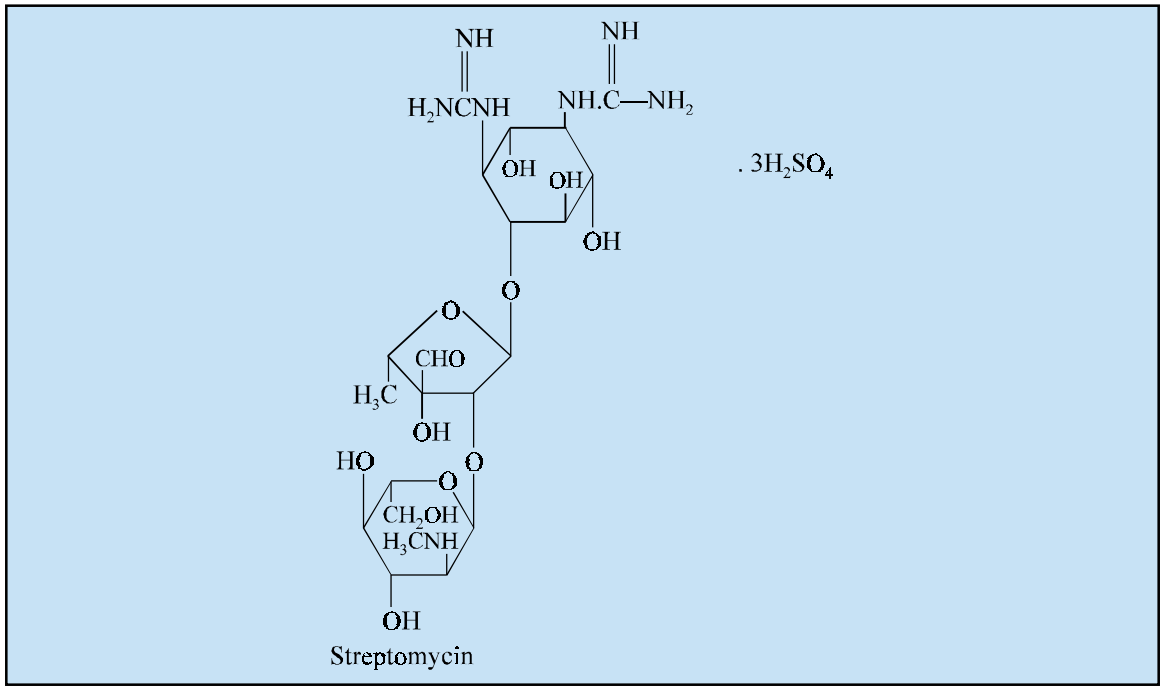
These are a bunch of closely related chemically basic carbohydrates that are mostly water-soluble. Their respective hydrochlorides and sulphates are crystalline in nature. They are found to be effective in inhibiting the growth of gram-positive as well as gram-negative bacteria. They are also effective to a great extent against mycobacteria.

In general, *they are prepared biosynthetically exclusively from an admixture of carbohydrate components of the fermentation media.*

They usually act by causing interference with the **'reading'** of the genetic code.

A few typical examples cited earlier shall be discussed below :

A. *Streptomycin* INN, *Streptomycin Sulphate* BAN, *Streptomycin Sulfate* USAN,



Streptomycin

BP ; USP ; Eur. P ; Int. P ; Ind. P ; Isoject Streptomycin Injection(R) (Pfizer) ;

Streptomycin Sulphate(R) (Glaxo, U.K.).

Streptomycin is chiefly employed in the *treatment of tuberculosis in conjunction with other drugs such as* **isoniazid and rifampicin**.

Streptomycin and **penicillin** exert a synergistic action against bacteria and are usually employed together in the treatment of subacute bacterial endocarditis caused by *Streptococcus faecalis*

It exerts **bacteriostatic action** in low concentrations and **bactericidal** in high concentrations against a plethora of Gram-negative and Gram-positive organisms. The only infection wherein this '*drug*' alone is the '**drug of choice**' are **tularemia*** and **bubonic plague**.** A combination with a *tetracycline* it may be employed in the treatment of **brucellosis***** and infections produced by *Pseudomonas mallei*. It is also an alternative drug of choice in the treatment of chancroid, rat-bite fever and tuberculosis.

Dose. *For non-tuberculosis infections, usual, 1g per day up to 5 to 10 days.*

Mechansim of Action. The '**drug**' exerts its maximum effectiveness against the organism *Mycobacterium tuberculosis*. Interestingly, the antibiotic is not a cure itself but has proved to be an excellent and valuable adjunct to other modalities of therapeutic treatment for tuberculosis. It acquires a rapid development with respect to certain strains of microorganisms. The combined administration of *streptomycin* and **penicillin** has been suggested to combat infections which may be due to organisms that are sensitive to both these antibiotics. The **'drug'** is neither absorbed nor destroyed appreciably in the GI tract.

---

* An acute plaguelike infectious disease caused by *Francisella tularensis.*

**It is caused by *Yersinia pestis* usually found in infected rats, ground squirrels and gets transmitted to humans by the bite of rat flies.

***A widespread infectious febrile disease affectiing humans and cattle (also called **Malta Fever).**

SAR of Streptomycin. The '**drug**' serves as a **triacidic base** due to the presence of *two* characteristic chemical entities, namely : (*a*) two strongly basic guanido moieties ; and (*b*) rather weakly basic methylamino function. Furthermore, hydroxy-streptomycin differs from streptomycin in essentially having a strategically positioned OH moiety in place of one of the H-atoms of the streptose methyl function. Besides, streptomycin B(*i.e.*, mannisido streptomycin) possesses a **mannose residue** attached to a glycosidic linkage *via* a OH moiety at C-4 of the N-methyl-L-glucosamine functional group. The designated stereochemical structure of the '**drug**' has been reconfirmed *via* the total synthesis.*

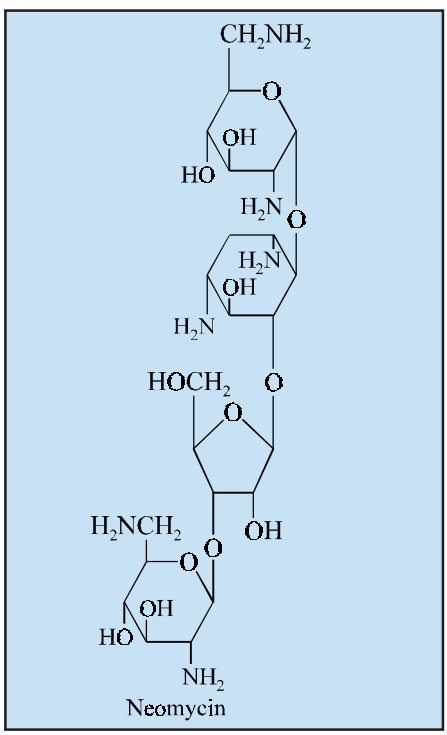
B. *Neomycin* INN, *Neomycin Sulphate* BAN, *Neomycin Sulphate* USAN,

Fradiomycin Sulphate; BP ; USP ; Eur. P ; Int. P ; Ind. P ;

Neobiotic(R) (Pfizer) ; Mycifradin(R) (Upjohn).

Neomycin *is mostly used in a wide variety of local infection such as burns, ulcers, wounds, impetigo, infected dermatoses, furunculosis, conjunctivitis, etc. It is also employed as an adjuvant in topical steroid preparations to control secondary infections in the case of inflammatory disorders.*

The '**drug**' is employed to produce intestinal antisepsis prior to large bowel surgery, for the treatment of gastroenteritis produced by toxigenic *E. coli*, and also to afford suppression of ammonia producing bowel flora in the management of hepatic coma. As it causes a rapid overgrowth of nonsusceptible organism, including staphylococci, oral therapy must not be prolonged in any case for more than 3 days. It displays broad-spectrum activity against a good number of pathogenic organisms. Besides, it demostrates a low incidence of toxic and hypersensitivity reactions.



Neomycin

Dose: *Topical, to the skin, as 5% solution, aerosol or ointment 2 to 3 times a day.*

*Umezawa S *et. al. J. Antibiotic* (Tokyo) **27,** 997, 1974.

Mechanism of Action. The '**drug**' usually gets absorbed very rarely from the digestive system ; therefore, its oral administration primarily fails to produce any substantial systemic effect.

SAR of Neomycin. The structures of **neomycin A**(**neamine**), **neomycin B** and **neomycin C** have been established ; besides, the absolute configurational structures of *neomycin* and *neamine* have been reported. It has been demonstrated that neamine could be obtained by the methanolysis of **neomycin B and C** respectively, whereby the glycosidic linkage existing between D-ribose and deoxystreptamine undergoes cessation.

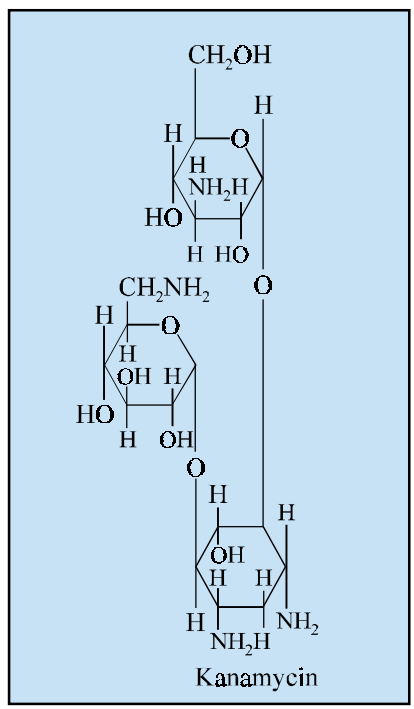
Note. The '*drug*' is invariably combined with other '*antibiotics*' namely : *gramicidin, polymyxin B sulfate* and *bacitracin*.

C. *Kanamycin* INN, *Kanamycin Sulphate* BAN, *Kanamycin Sulfate* USAN,

D-streptamine, *o*-3-amino-3-deoxy-α-D-glucopyranosyl (1 → 6)-*o*- [6-amino-6-deoxy-α-D-glucopyranosyl (1 → 4)]-2-deoxy-, sulphate ; BP ; USP ;

Kelbcil(R) (Beecham) ; Kantrex(R) (Bristol).

It is effective against some Mycoplasma and gram-positive bacteria, for instance, *Staphylococcus Pyogenes* and *Staphylococcus epidermidis*. Along with **penicillin** it is found to be effective against *Streptomyces fecalis*. It is used invariably either alone or in combination with other drugs for a variety of disorders, namely : *acute staphylococcal infections, gonorrhea, tuberculosis, acute urinary tract infections, for bowl sterilization in hepatic coma and also prior to bowl surgery.*



Kanamycin

In US the use of **kanamycin** is normally restricted to the infections related to the *intestinal tract*, such as : bacillary dysentry; *systemic infections* caused due to Gram-negative bacili, such as : *Klebsiella, Proteus, Enterobacter*, and *Serratia spp.*, which have developed resistance to some other antibiotics. It

has also been indicated for preoperative antisepsis of the bowel. However, this '*drug*' could not be useful in tuberculosis perhaps due to the fact that it develops resistance to mycoorganism rather rapidly.

Dose : *(Base equivalent)-Oral, adult, for intestinal infection, 1g after every 8 hours for 5 to 7 days ; For preparative preparations, 1g every hour for 4 doses followed by 1g every 6 hours for 36 to 72 hours.*

Mechanism of Action. Based on both clinical experience and experimental demonstration* it has been duly observed that the '*drug*' develops cross-resistance overwhelmingly in the **tubercle bacilli** specifically along with some other medicinal entities, such as : vincomycin, dihydrostreptomycin and antitubercular drug substances.

SAR of Kanamycin. Kanamycins A, B and C *i.e.*, the three closely related analogues of kanamycin have been duly established by the aid of chromatography. Kanamycin A is the '*drug*' available for therapeutic usage. It has been proved that the vital point of difference amongst the kanamycins resides solely in the **sugar residuces** strategically linked to the *glycosidic oxygen* at the C-4 position of the central deoxystreptamine. Interestingly, the kanamycins do **not** essentially possess the **D-ribose residue** as is present in *neomycins* and *paromomycins.* In all the three structural variants of kanamycin the presence of **kanosamine entity** is found to be attached glycosidically at the C-6 position of deoxystreptamine *i.e.*, 3-D-glucosamine. They also differ in the substituted D-glucoses which are observed to be attached glycosidically at the C-4 position of the inherent deoxystreptamine ring.

CAUTION : (1) ***The 'drug' causes either retarded or impaired loss of hearing.***

(2) ***Kanamycin and penicillin salts must not be combined in the same solution perhaps due to the possible inactivation of either agents significantly.***

5. CHLORAMPHENICOL

Chloramphenicol (**chloromycetin**) is a levorotatory broadspectrum antibiotic originally produced from several streptomycetes, namely : *S. venezualae, S. omiyamensis* and *S. phacochromogenes var.* chloromyceticus. It has been reported to be the drug of choice for the treatment of typhus and typhoid fever.

However, **chloramphenicol** is of paramount interest owing to the following *three* reasons :

(*a*) It is a naturally occurring aromatic nitro compound of which there is only one previously recorded example of *hiptagin*, obtain from the root bark of *Hiptage madablota* Gaertn is noteworthy.

(*b*) It is capable of exerting its effect against viral diseases as well as those due to bacterial invasion and opens up the whole field of the chemotherapy of *virus* and *rickettsial* infections in man including typhus, undulant fever, *Salmonella septicaemia*, whooping cough, gastroenteritis, *lymphogranuloma inguinale*, typhoid and paratyphoid. So far, chloramphenicol-fast strains have not been isolated.

(*c*) It is amenable to synthesis on an industrial scale.

5.1. Structure of Chloramphenicol

The structure of **chloramphenicol** has been established on the basis of the following vital chemical evidences. They are :

*Morikubo Y, *J Antibiot* [A] : **12,** 90, 1959.

(*i*) The molecular formula of chloramphenicol is $C_{11}H_{12}O_5N_2Cl_2$.

(*ii*) Its absorption spectrum is similar to that of nitrobenzene.

(*iii*) The presence of a nitro group was revealed by the reduction of chloramphenicol with tin (Sn) and hydrochloric acid, followed by diazotization and then coupling to yield an orange precipitate with β-naphthol (Rebstock *et al.* 1949).

(*iv*) When reduced catalytically (with palladium, Pd) it gives a prodcut which has an absorption spectrum very similar to that of *para*-toluidine and the resulting solution gives a positive test for ionic chlorine.

(*v*) Hydrolysis of **chloramphenicol** with either acid or alkali produces dichloroacetic acid together with an optically active base $C_9H_{12}O_4N_2$. Thus :

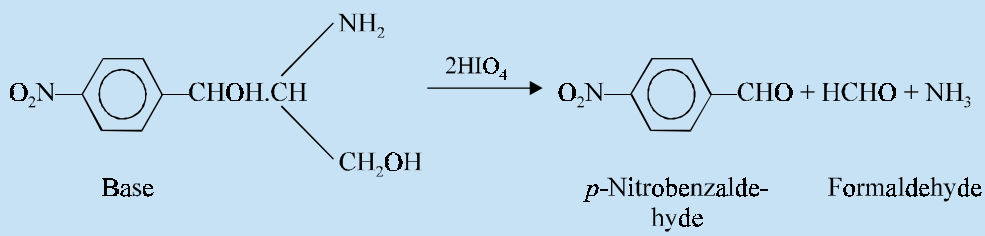
$$\underset{\text{Chloramphenicol}}{C_{11}H_{12}O_5N_2Cl_2} \xrightarrow{H_2O} \underset{\text{Dichloro-acetic acid}}{Cl_2CH.COOH} + \underset{\text{(Base)}}{C_9H_{12}O_4N_2}$$

(*vi*) The resulting base was shown to contain a primary amino group, and on being treated with methyl dichloroacetate, the base regenerated **chloramphenicol** (Rebstock *et al.* 1949).

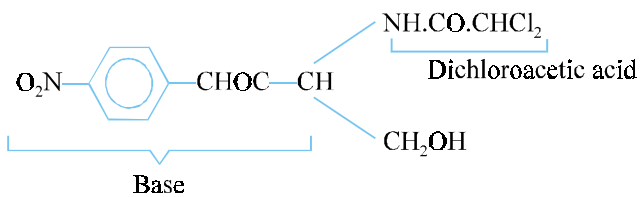
(*vii*) **Chloramphenicol** is converted into a diacetyl derivative on treatment with acetic anhydride in pyridine ; whereas the base obtained from **chloramphenicol** yields a triacetyl derivative on similar treatment thereby suggesting that **chloramphenicol** probably contains two-OH groups.

(*viii*) When the **chloramphenicol** base is treated with periodic acid (HIO_4) two molecules of the latter are consumed with the formation of one molecule each of ammonia, formaldehyde and *para*-nitrobenzaldehyde respectively.

However, these products may be accounted for provided the base is assumed to be 2-amino-1-nitrophenyl propane-1, 3-diol (Rebstock *et al.* 1949). Thus :

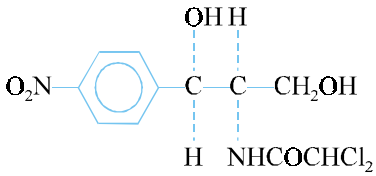


Hence, **chloramphenicol** may be written as :



D-(–)-*Threo*-2-dichloroacetamide-1-*p*-nitrophenylpropane-1, 3-diol.

A. *Chloramphenicol* INN, BAN, USAN,

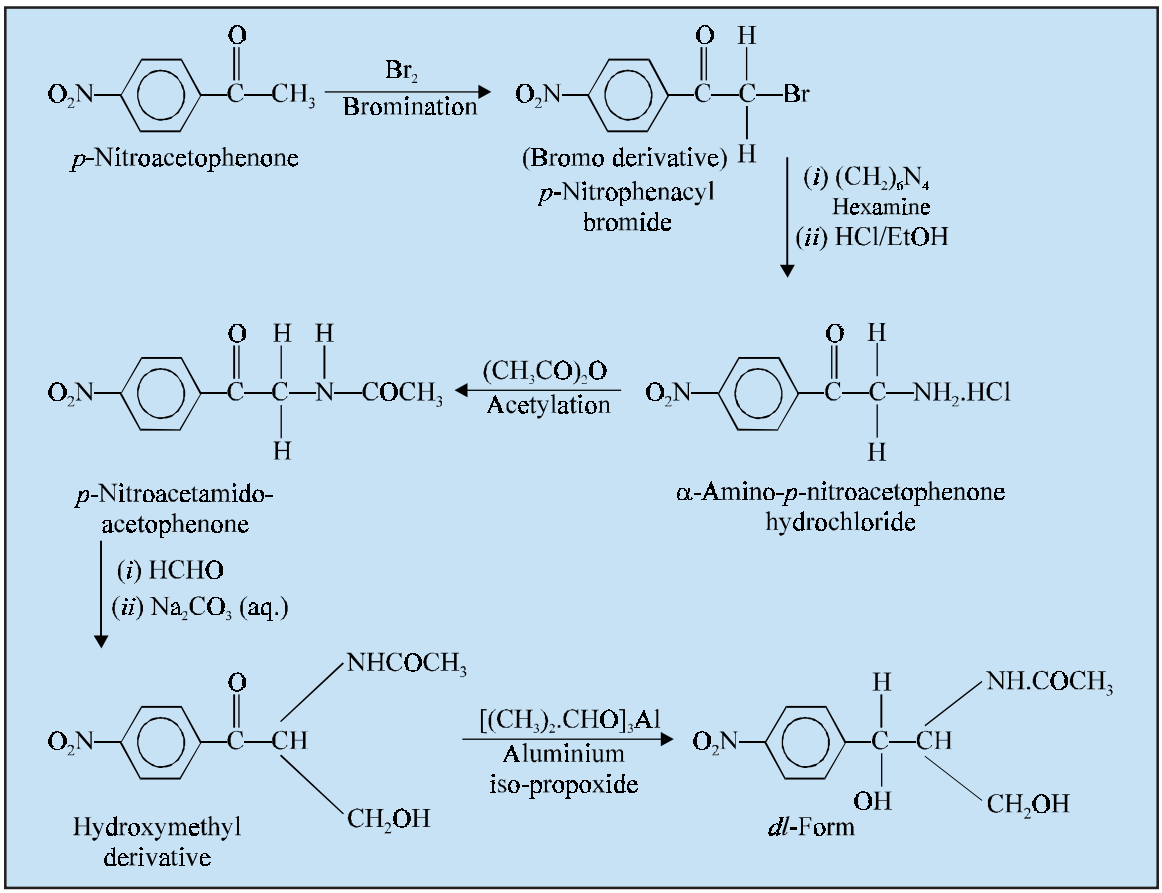


D-*threo*-(–)-2, 2-Dichloro-N-[β-hydroxy-α-(hydroxy methyl)-*p*-nitrophenyl] acetamide ; Acetamide, 2,z-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) etheryl]-, [R-(R*, R*)]-; BP ; USP ; Eur. P ; Int. P ; Ind. P ;

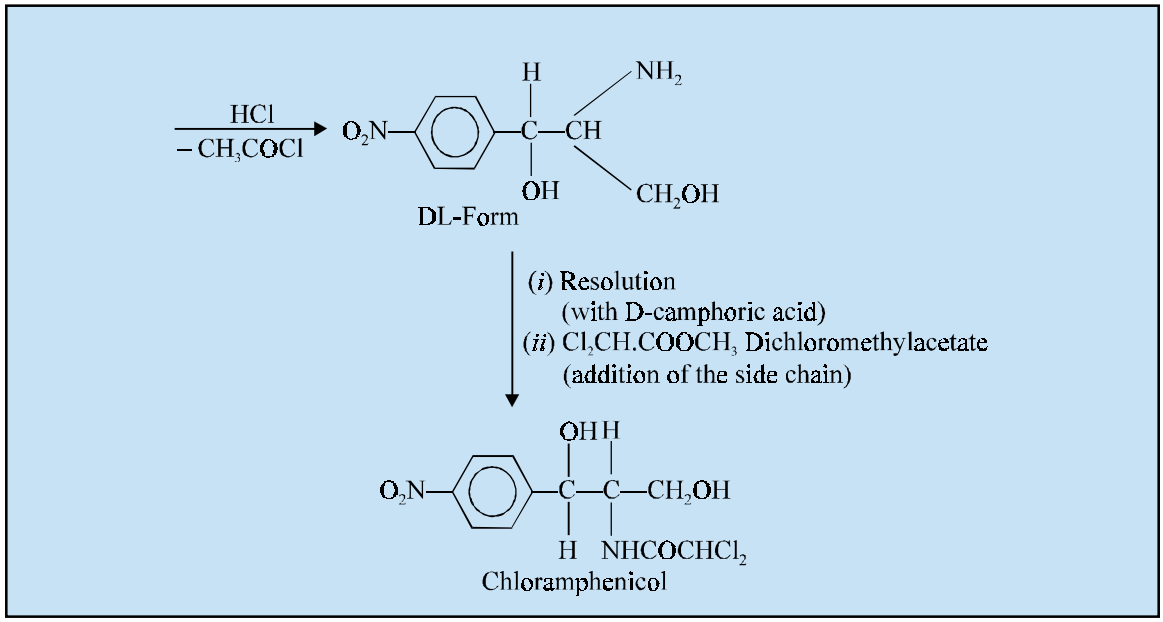
Chloromycetin$^{(R)}$ (Parke-Davis).

5.2. Synthesis of Chloramphenicol

Chloramphenicol has been successfully synthesized by different methods and the present global demand of this drug is adequately met exclusively by chemical synthesis. The synthesis put forward by Long *et al.* (1949) is discussed below :



(*Contd...*)



para-Nitroacetophenone on bromination gives the corresponding bromo derivative which on treatment with hexamine followed by acidic ethanol yields α-amino-*p*-nitroacetophenone hydrochloride. This on acetylation gives the acetamido derivative which on treatment with formaldehyde followed by aqueous sodium carbonate affords the corresponding hydroxy methyl analogue. Reduction of the keto moiety is effected by treatment with aluminium iso-propoxide to give the product in DL-form which on reaction with HCl removes the acetyl function to yield the **chloramphenicol base** in its DL-form. The resulting product is first subjected to resolution with α-camphoric acid and secondly with dichloromethyl acetate to afford the addition of the side chain to yield **chloramphenicol**.

Typhoid fever and similar salmonellal infections are usually considered the prime indications for the use of **chloramphenicol**. It is also employed *in acute infections due to Heamophilus influenzae, including meningitis attributed to ampicillin-resistant strains.* It also find its enormous applications in *topical infections of eye and skin.* It has also been used *to eradicate vibrios from patients with cholera.* It is employed *for rickettsial infections like typhus and Rocky Mountain spotted fever.*

Chloramphenicol is particularly recommended for the management and treatment of serious infections produced by the strains of both Gram-positive and Gram-negative organism that have developed eventually resistance to either **ampicillin** or **penicillin G,** for instance : *H. influenzae, Salmonella typhi, S. pneumoniae, B. fragilis,* and *N. meningitidis.*

It is used topically extensively for the superficial *conjunctival infections* and *blepharitis* essentially caused by *E. coli, H. influenzae, Moraxella lacunata, Streptococcus hemolyticus,* and *S. aureus.* However, it is still the drug of choice for the **typhoid fever.**

Dose. *Usual, adult, 500 mg every 6 hours.*

Mechanism of Action. The '**drug**' has specifically the ability to penetrate right into the **central nervous system (CNS)** ; therefore, it is still an important alternative therapy for meningitis. The major course for the metabolism of **chloramphenicol** essentially involves the formation of the 3-*o*-glucuronide.

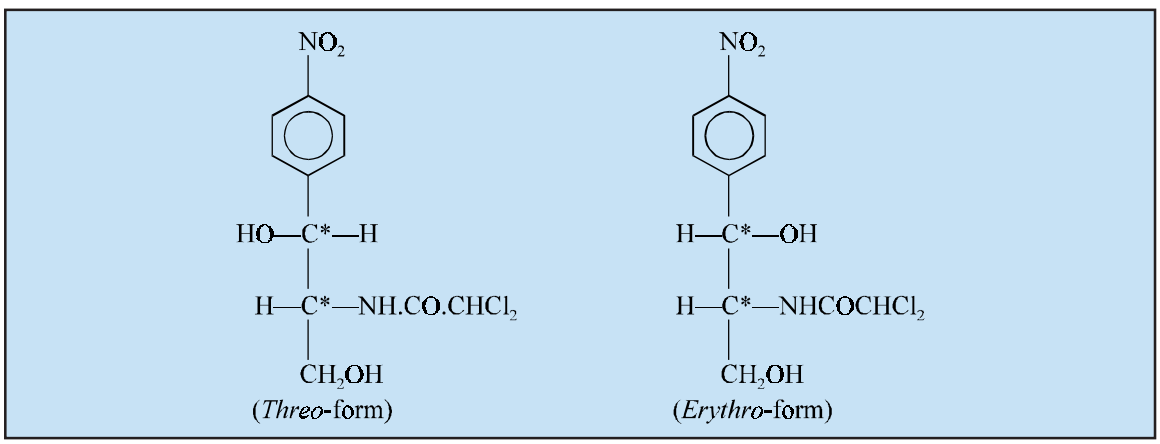
Howerver, the minor reactions necessarily include : (*i*) reduction of the inherent *para*-nitro moiety to the corresponding '*amine*' function ; (*ii*) hydrolysis of the amide moiety ; (*iii*) hydrolysis of α-chloroacetamido group ; and (*iv*) reduction to yield α-hydroxyacetyl analogue.*

Chloramphenicol gets absorbed very fast from the GI tract, having a bioavailability of almost 90%. It has been observed that about 60% of the drug in blood is bound to serum albumin. It is biotransformed in the liver within a range of 85-95%. The volume of distribution v_d^{ss} stands at 0.7 mL. g^{-1}. The plasma half-life varies between 1.5–5 hours, except over 24 hours in neonates 1-2 days old, and 10 hours in infants 10-16 days old. The '*drug*' may cross the placental barrier and in turn intoxicate the fetus ; therefore, it must be avoided as far as possible in pregnant women.

Note. The '*prodrug*' of chloramphenicol viz., chloramphenicol palmitate (USP), which is a tasteless product, is solely intended for pediatric usage profusely, because the parent drug has a distinct bitter taste.

5.3. Structure Activity Relationship

Chloramphenicol possesses *two chiral (asymmetric) carbon atoms* in the '**acylamino-propanediol chain'** as shown below :



Thus there are *two* possible pairs of enantiomorphs.

It has been observed that the biological activity resides almost exclusively in the **'D-Threo-isomer'** whereas the **L-*Threo***, and **D- and L-*Erythro* isomers** are virtually inactive.

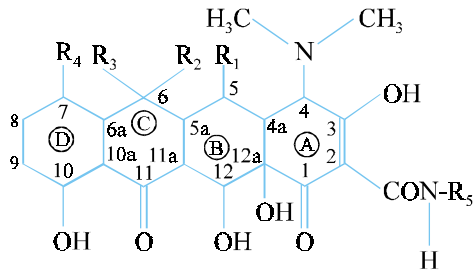
A large number of structural analogues of **chloramphenicol** have been prepared on the basis of the following themes : removal of the chlorine atom, transference of chlorine atom to the aromatic nucleus, transference of the nitro moiety to the *ortho*- or *meta*-position, esterification of the hydroxyl function(s), replacement of the phenyl ring with furyl, naphthyl and xenyl rings respectively, addition of alkyl or alkoxy substituents to the aryl ring and lastly replacement of the inherent nitro group by a halogen atom. It is, however, pertinent to mention here that none of these structurally modified analogues showed an activity approaching to that of **chloramphenicol** towards *Shigella paradysenteriae*

*Glazko A : *Antimicrob Agents Chemother.*, 655, 1966.

6. THE TETRACYCLINES

The epoch-making discovery of **chlortetracycline** (**aureomycin**) in 1947 by Duggar paved the way for a number of structural analogues used as broad-spectrum antibiotics that belong to the tetracycline family. **The tetracyclines** which are found to be effective therapeutically are listed in the following table.

6.1. Salient Features of the Tetracyclines



Name of Compound	Official Status	Brand Name(s)	R_1	R_2	R_3	R_4	R_5
Tetracycline	BPC ; (1973); USP ;	Tetracyn(R) (Pfizer) ; SK-Tetracycline(R) (SK & F)	H	OH	CH_3	H	H
Oxytetracycline	USP ;	Terramycin(R) (Pfizer)	OH	OH	CH_3	H	H
Chlortetracycline HCl	BP, USP ; Eur. P.; Int. P.; Ind. P.;	Aureomycin(R) (Lederle)	H	OH	CH_3	Cl	H
Demeclocycline HCl	BP, USP ; Eur. P.;	Ledermycin(R) (Lederle, UK)	H	OH	H	Cl	H
Methacycline HCl	BP (1973); USP ;	Rondomycin(R) (Wallace)	OH	=	CH_2	H	H
Doxycycline	USP ;	Vibramycin(R) (Pfizer)	OH	H	CH_3	H	H
Rolitetracycline	USP ;	Syntetrin(R) (Bristol)	H	OH	CH_3	H—CH_2—N 	

6.2. Nomenclature

Based on the above conventional numbering of various carbon atoms and subsequent labelling of the **four** aromatic rings present in the **tetracycline** nucleus, oxytetracycline is chemically designated as :

"4-Dimethylamino-1, 4, 4*a*, 5, 5*a*, 6, 11, 12*a*-octahydro-3, 6, 10, 12, 12*a*-penta-hydroxy-6-methyl-1, 11-dioxo-2-naphthacenecarboxamide".

Some other members of the **tetracycline** family may conveniently be named as follows :

Methacycline : 6-Methylene-5-oxytetracycline ;

Doxycycline : α-6-Deoxy-5-oxytetracycline ;

Rolitetracycline : N-(Pyrrolidinomethyl)-tetracycline.

6.3. General Chracteristics of the Tetracyclines

Following are the **general characteristic features** of all the members of the **tetracycline** family :

(*a*) The **tetracyclines** are obtained by fermentation procedures from **streptomyces species** or by the chemical transformations of the **natural products**.

(*b*) The important members of this family are essentially derivatives of an **octahydron-aphthacene**, *i.e.*, a hydrocarbon made up of a system of four-fused rings.

(*c*) The **antibiotic spectra** and the chemical properties of these compounds are quite similar but not identical.

(*d*) The **tetracyclines** are amphoteric compounds, *i.e.*, forming salts with either acids or bases. In neutural solutions these substances exist mainly as **Zwitter ions**.

(*e*) The acid salts of the **tetracyclines** that are formed through protonation of the dimethylamino group of C-4, usually exist as crystalline compounds which are found to be very much soluble in water. However, these **amphoteric antibiotics** will crystallize out of aqueous solutions of their salts unless they are duly stabilized by an excess of acid.

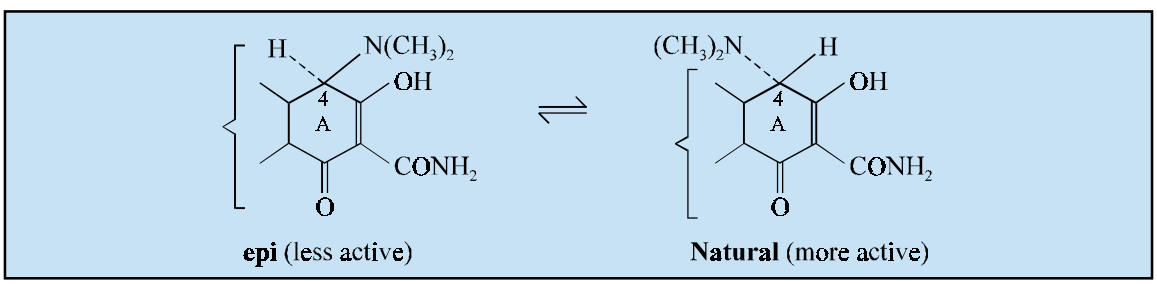
(*f*) The corresponding hydrochloride salts are used most commonly for oral administration and are usually encapsulated owing to their bitter taste.

(*g*) The water soluble salts are obtained either from bases such as sodium/potassium hydroxides or formed with divalent/polyvalent metals, *e.g.*, Ca^{++}. The former ones are not stable in aqueous solutions, while the latter ones, *e.g.*, calcium salt give tasteless products that may be employed to prepare suspensions for liquid oral dosage forms.

(*h*) The unusual structural features present in the **tetracyclines** afford three acidity constants (pKa values) in aqueous solutions of the acid salts. The thermodynamic pKa values has been extensively studied by Lesson *et al.* and discussed in the chapter on 'Physical-chemical factors and biological activities'.

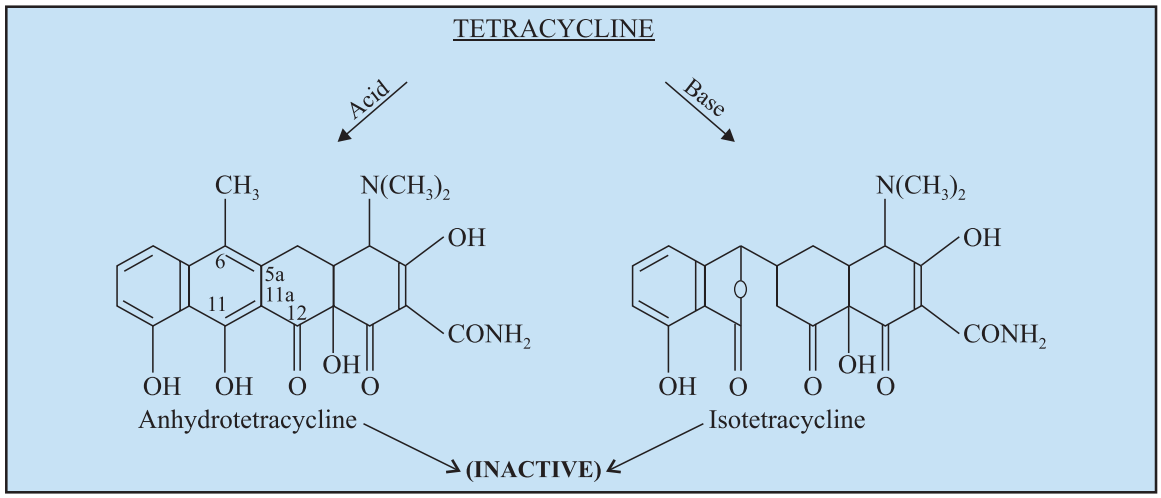
(*i*) An interesting property of the **tetracyclines** is their ability to undergo epimerizaton at C-4 in solutions having intermediate pH range. These isomers are called **epitetracyclines**.

The **four *epi*-tetracyclines** have been isolated and characterized. They exhibit much less, activity than the corresponding **'natural' isomers ;** thus accounting for an apparent decrease in the therapeutic value of aged solution.



epi (less active) **Natural** (more active)

(*j*) It has been observed that the strong acids and bases attack the tetracyclines having a hydroxy moiety at C-6, thereby causing a considerable loss in activity through modification of the C-ring as shown below :



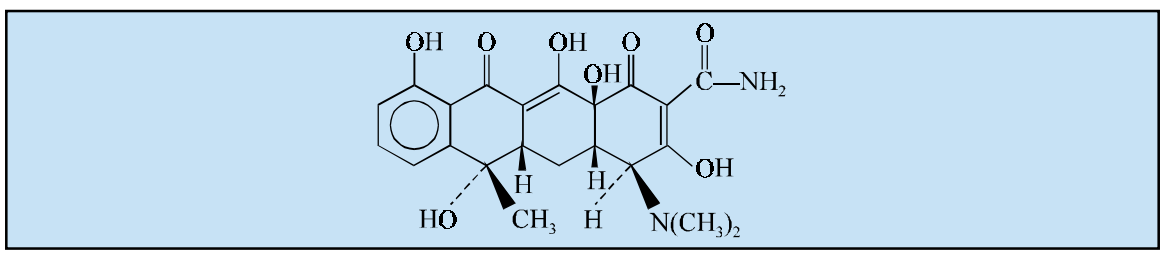
Strong acids produce a dehydration through a reduction involving the OH group at C-6 and the H atom at C-5*a*. The double bond thus generated between positions C-5*a* and C-6 induces a shift in the position of the double bond between the carbon atoms C-11 and C-11*a* thereby forming the relatively more energetically favoured resonant system of the naphthalene group found in the **inactive anhydrotetracyclines**.

The strong bases on the other hand promote a reaction between the hydroxyl group at C-6 and the carbonyl moiety at C-11, thereby causing the bond between C-11 and C-11*a* atoms to cleave and eventually form the lactone ring found in the **inactive isotetracyclines**.

(*k*) The **tetracyclines** form stable chelate complexes with many metals, *e.g.*, Ca^{++}, Mg^{++}, Fe^{++}, etc.

A few typical examples of the tetracyclines shall be dealt with in the sections that follows :

6.3.1. Tetracycline USAN, BAN, INN



2-Naphthacenecarboxamide [4S-(4α, 4*a*α, 5*a*α, 6β, 12*a*α)]-4-(dimethylamino)-1, 4, 4*a*, 5, 5*a*, 6, 11, 12*a*-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-; USP;

Achromycin(R); Cyclopar(R); Panmycin(R) ; Tetracyn(R) ;

The '**drug**' is the durg of choice in the treatment of chloera, relapsing fever, granuloma inguinale and infections produced by rickettsia, *Borrelia*, *Mycobacterium fortuitum* and *marinum*, and *Chlamydia psittaci* and *trachomatis* (except pneumonia and inclusion conjunctivitis).

It may be employed as an '**alternative drug**' in the following *two* situations, namely :

(*a*) For silver nitrate in the prevention of neonatal ocular prophylaxis of chlamydial and gonococcal cojunctivitis, and

(*b*) For treatment of actinomycosis, anthrax, chancroid, mellioidosis, plague, rat-bite fevers, syphilis and yaws.

It has also been reported to be beneficial in the treatment of *toxoplasmosis.*

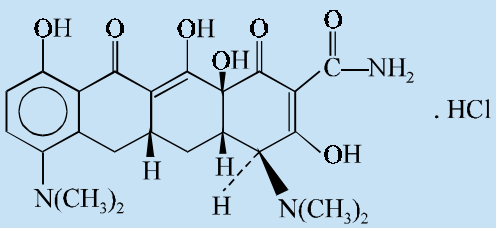
Mechanism of Action. The mechanisms of action of its combination with other agents have been established adequately, such as :

Tetracycline + $MgCl_2 . 6H_2O$—**Panmycin (R)—**Enhances the rate and peak of plasma concentration.

Tetracyclines + Aluminium/Calcium gluconates—Observed enhanced plasma levels in experimental animals.

From the above two cited examples one may evidently conclude that the **tetracyclines** may form stable complexes with bivalent metal ions (*e.g.,* Mg^{2+}, Ca^{2+};) that would appreciably minimize the absorption from the GI-tract. In reality, these '**adjuvants'** seen to compete with the tetracyclines for substances present in the GI-tract which might otherwise be free to complex with these antibiotics, and thus ultimately retard their absorption significantly. Of course, there is no concrete evidence which may suggest that the metal ions (Mg^{2+}, Ca^{2+}) *per se* serve as '**buffers**', a theoretical explanation quite often put forward in the literature.

6.3.2. Minocycline Hydrochloride USAN, BAN, INN



2-Napthhacenecarboxamide, [4S-(4α,4a α, 5a α, 12a α)]-4,7-*bis* (dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-3,10,12,12*a*-tetrahydroxy-1,11-dioxo-, monohydrochloride ; USP ; Minocin$^{(R)}$; Vectrin$^{(R)}$;

The '**drug**' is found to be 2-4 folds as potent as **tetracycline** ; however, it essentially shares an equally low potency against *Enterococcus fecalis.* Besides, it is observed to be 8 times more potent against *Streptococcus viridans*, and 2-4 times against Gram-positive organisms in comparison to tetracyclines. It is the drug of choice for the treatment of infections caused by *Mycobacterium marinum.* It remarkably differs from the other structural analogues of **tetracyclines** wherein the observed bacterial resistance to the drug stands at a *low ebb and incidence* ; it is particularly true for *Staphylococci,* in that the prevailing cross-resistance is only upto 4%.

Minocycline has been indicated for the management and treatment of *chronic bronchitis* and othe **upper respiratory tract infections (URTs)**. Though it essentially possesses comparatively low renal clearance, which is partially compensated for by means of its high serum and tissue levels, it has

been duly recommended for the treatment of **urinary tract infections (UTIs)**. The '**drug**' has been equally useful in the virtual erradication of *N. meningitidis* in specific asymptomatic carriers.

Mechanism of Action. The '**drug**' is usually absorbed by the oral route upto 90-100%. However, its absorption is predominantly diminished to a small extent milk and food intake ; and appreciably by the presence of **'iron preparations'** and '**nonsystemic antacids**'. It is protein-bound in plasma between a range of 70-75%. The volume of distribution v_d^{ss} stands at 0.14 – 0.7 mL. g^{-1}. The plasma half-life ranges between 11–17 hours. It gets excreted unchanged in urine upto 10%; however, its biological half-life is usually prolonged chiefly in the incidence of renal failure.

6.4. Structure Activity Relationship (SAR)

The **structure activity relationship** amongst the various members of the **tetracycline** family has ben studied extensively.

The high level of antimicrobial activity of tetracycline established earlier reveal that the substitutions on the C-5 and C-7 were not an essential requirement.

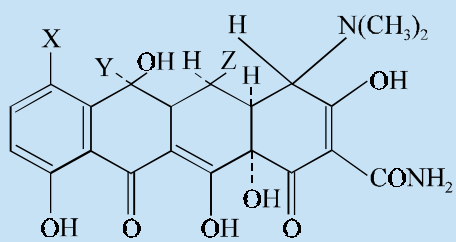
The activity of 6-dimethyltetracycline (**demecycline**) and **demeclocycline** has established that the methyl function at C-6 may be replaced by hydrogen.

The activity of **deoxycycline** and **6-deoxy-6-demethyltetracycline** (**minocycline**) shows that the presence of hydroxy moiety at C-6 is not essential either.

The **6-deoxy-6-methylenetetracyclines** and their corresponding **mercaptan adducts** possess typical characteristics tetracycline activity and illustrate further the level of modification feasible at C-6 with the possible retention of biologic activity.

It is, however, interesting to observe that the subsequent removal of the 4-dimethylamino function affords a loss of about 75% of the antibiotic effect of the **parent tetracyclines**.

The X-ray diffraction studies reveal that the following stereochemical formula represents the orientations, as observed in the **natural tetracyclines** :



Tetracycline : X, Z = H ; Y = CH_3 ;

Chlortetracycline : X = Cl ; Y = CH_3; Z = H ;

Oxytetracycline : X = H ; Y = CH_3 ; Z = OH ;

Demeclocycline : X = Cl ; Y = Z = H ;

* Tally FT *et al. J Antimicrob. Chemother*, **35,** 449, 1995.

X-ray diffraction studies further reveal that the 4-dimethylamino function is placed in a *trans*-orientation rather than the *cis*-form as inferred earlier by chemical investigations. It further establishes the presence of a conjugated system existing in the structures of **tetracycline** from C-10 through C-12.

6.5. Newer Tetracyclines

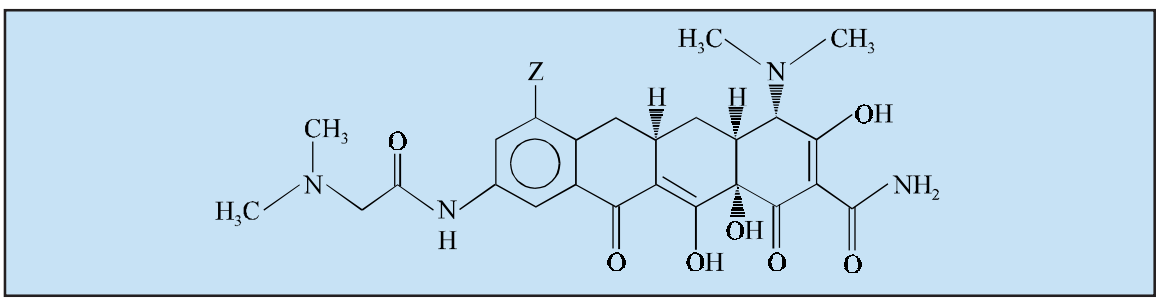
Since 1992, several newer breeds of '**tetracyclines**' have emerged that were exclusively based on the recent researches focussed on the following aspect, namely :

(*a*) superb broad spectrum antimicrobial profile of the **'tetracyclines'**, and

(*b*) recent astronomically broad emergence of bacterial **genes** and **plasmids** encoding tetracycline resistance.

Therefore, keeping in view of the stringent limitations imposed on the '**tetracyclines'** as a class has caused the researchers at the Lederle Laboratories to augment extensive and intensive studies to rediscover **SARs of tetracyclines** with strategical substitutions in the **aromatic ring 'D'** in a meaningful and sincere effort to lay hand on to certain newer breeds of tetracyclines that might give rise to such drug substances which are specifically effective against the resistant strains.*

The concerted efforts ultimately gave birth to a **few newer** tetracyclines as illustrated below :*



Examples :

(*a*) 9-(Dimethylglycylamino) minocycline : [DMG-MINO] ; Z = $N(CH_3)_2$;

(*b*) 9-(Dimethylglycylamino)-6-demethyl-6-deoxytetracycline [DMG-DMDOT] ; Z = H ;

Salient Features. The salient features of the '**glycylcyclines'** are as stated under :

(*i*) retain essentially both potency and broad spectrum profile as displayed by the '**parent tetracyclines'** against specifically the **tetracycline-sensitive microbial strains**, and

(*ii*) exhibit predominantly maximum activity against bacterial strains which show tetracycline resistance either through the ribosomal protecting determinants or afford mediation by efflux.

The future prospects of a possible '**second generation tetracyclines'** are almost written on the wall provided the meaningful and fruitful clinical trials of the ongoing **glycylcyclines** do emerge both favourable *pharmacokinetic* and *toxicological* profiles for such '**medicinal compounds'** in the near future.

* Tally FT *et al. J Antimicrob. Chemother,* **35,** 449, 1995.

Probable Questions for B. Pharm. Examinations

1. (*a*) What are the four cardinal requirements of a substance to be called an **'antibiotic'** ?
(*b*) Give the structure, chemical name and other names of the **six** naturally occurring **Penicillins.**

2. How would you establish the structure of the Penicillins as per the following steps ?
(*a*) Hydrolysis by hot dilute inorganic acid
(*b*) D-Penicillamine
(*c*) Penilloaldehyde
(*d*) Presence of CO_2 molecule
(*e*) Combination of Penicillamine and Penilloaldehyde in Penicillins
(*f*) Presence of Thiazolidine ring
(*g*) Evidence for Penicilloic acid
(*h*) Probable structure for Penicillin.

3. Discuss the synthesis of phenoxy methyl penicillin from *tert*-butyl-alpha-*phthalimidomalonalhydate and D-penicillamine.*

4. Give the structure, chemical name, official status of at least **two** clinically useful :
(*a*) Penicillins
(*b*) Penicillins related to Ampicillin
(*c*) Ester of Ampicillin.

5. (*a*) What are **'Aminoglycoside Antibiotics'** ?
(*b*) Give the structure, official status and uses of any **three** potent drugs.

6. Based on vital chemical evidences, how will one establish the structure of an **'antibiotic'** produced from *Streptomyces venezualae*.

7. (*a*) What are the **three** reasons of paramount interest of **chloramphenicol** for its cognizance as a potent antibiotic ?
(*b*) Discuss the SAR and stereochemistry of **chloramphenicol.**
(*c*) Describe the synthesis of **chloramphenicol** from *p*-nitroacetophenone.

8. (*a*) Discuss the salient features of the **'Tetracylines'.**
(*b*) Give a brief account of the SAR of **'Tetracylines'.**

9. Elaborate the characteristics of the 'Tetracylines' with specific reference to :
(*a*) pKa values
(*b*) Epimerization
(*c*) Effect of strong acids and bases.

10. Give a comprehensive account of a 'CEPHALOSPORINS' and provide appropriate examples.

OR

Write short note on the following :
(*a*) Minocycline Hydrochloride
(*b*) Newer Tetracyclines.

RECOMMENDED READINGS

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