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Chemistry of Natural Products

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properties of garlic have been widely reported. Sulphur containing compounds Alliin and Allicin have been shown to lower cholesterol levels in cholesterol fed as well as normal rats and other species.

Malaria continues to be one of the most widespread health hazards. Over two hundred million people worldwide are currently estimated to be suffering from this infectious disease.

The bark of cinchona *Cinchona officinalis* has been used widely in India against malaria, the active principle being the alkaloid quinine. As a consequence, effective therapeutic agents against malaria are continuously being sought, particularly against those strains, which are resistant to conventional quinine based drug. The medicinal plant *Artemisia annua* L. (Qinghao, Fam. Compositae) was used in China for many centuries against fever and, malaria. In 1972, a sesquiterpene lactone artemisinin was isolated and was found to be superior to the conventional antimatariat drugs such as chloroquine and quinine, against several strains of malaria without obvious adverse reactions or side effects in patients.

Cancers and related diseases represent the second major cause of death in man. Presently surgery, radiotherapy and chemotherapy remain the three most important methods of treating cancers. Pacific Yew tree *Taxus brevifotia,* which grows in the forests of the Western USA and Canada, has unique ability to produce the diterpene taxol, a significantly efficient anticancer drug. This compound has become one of the most promising medicines for the treatment of ovarian and breast cancers, especially those cases incurable by other forms of treatment.

One of the most famous of the Chinese folk herbs is the ginseng root *Panax ginseng,* used for health maintenance and treatment of various diseases. The biological effects are due to synergistic activity between saponins called ginsenosides and flavonoids. Another popular folk drug, the extract of the *Ginkgo biloba* L., is mentioned in the Chinese materia medica to have an effect in improving memory and sharpening mental alertness. The main constituents responsible for biological activity are ginkgolides and flavonoids. Plants have been used for centuries for the treatment of heart problems, the most important being the foxgloves *Digitalis purpurea* and related species and *Strophantus gratus* containing active principles digitalin and ovabain respectively. The latex of the upas tree *Antiaris toxicaria* (cardiac glycosides) was used in Java, for cardioionic properties.

When rye is infected by the fungus *Claviceps purpurea** the toxins ergotamine and a number of ergot alkaloids are produced. These toxic alkaloids cause ergotism or the "devils curse", which leads to convulsions, miscarriages, loss of arms and legs and finally death. In lower doses, however, some of these alkaloids are used in medicine for their beneficial effects. These alkaloids are derived from lysergic acid. Lysergic acid diethyl amide (LSD) was first prepared by A. Hofmann, at Sandoz Laboratories, Basel, in 1943 during his efforts to improve the psychotropic effects of ergot alkaloids.

The stimulant cocoa, used by Incas around the tenth century, was introduced into Europe by the conquistadors.

Numerous psychedelic plants have been used since ancient times, producing visions, mystical fantasies, sensations of flying and glorious feelings etc. The seeds of morning glory *Rivea corymbosa* one of the psychedelic plant 'Ololiqui' an important Aztec concoction, contains lysergic acid amides. The Indian hemp plant *Cannabis sativa* has been used in central Asia, China, and India & Near East for its pleasure giving effects since ancient times. Marijuana, hashish, charas, ganja, bhang, kef and dagga are the names given to various preparations of

Paul Karrer (NP 1932) established the foundation of carotenoid chemistry through the structural determination of lycopene, carotene, vitamin A and the synthesis of squalene and carotenoids. George Wald (NP 1967) showed that vitamin A was the key compound in vision. Henrik Dam (NP 1943) discovered vitamins K and E. Doisy studied its structure.

Melvin Calvin (NP 1961) at the University of California, Berkeley, elucidated the complex photosynthetic pathway in which plants produce carbohydrates from carbon dioxide. The entire structure of the photosynthetic reaction center (>10,000 atoms) from the purple bacterium *Rhodopseudomonas viridis* was established by X-ray crystallography by J. Deisenhofer, R. Huber and H. Michael (combined NP 1988). Emil Fischer was the father of carbohydrate chemistry. W. Haworth noticed the cyclic structures of monosaccharides and the presence of a-, p-anomers. He also established structures of important di- and polysaccharides.

1.6 BIOTECHNOLOGY

Biotechnology is the use of microbiology, biochemistry and engineering in an integrated fashion with the goal of using microorganisms and cell and tissue cultures or their components to manufacture useful products. Industrial microbiology, the major foundation of biotechnology, arose out of empirical developments in the production of wine, vinegar, beer etc. and with the traditional microbial fermentations used in Asia and Africa for the production of food. An experimental approach to the production of microbial metabolites began only at the beginning of 20th century. Antibiotics are anti-bacterial compounds derived from microorganisms.

A major breakthrough in biotechnology occurred after World War II as a result of the large-scale production of the first antibiotic, penicillin. In order to produce this antibiotic economically, important engineering developments had to be made, including the improvements in the techniques for large-scale sterilization, aeration, and growth of microorganisms and downstream processing. In addition, antibiotic productivity was improved through genetic modifications of microorganisms.

From World War II up until about 1960, the major biotechnology products were antibiotics. During this period around 20 new antibiotics were put into commercial production. In addition, in this early post World War II period, processes were developed for the chemical transformations of steroids and the culture of animal cells for the production of virus vaccines were perfected.

Antibiotics are also used advantageously in agriculture as preparations for the treatment of diseases in animals, birds, bees and plants. Few antibiotics are used as stimulants of growth of animals. Some antibiotics are used successfully to preserve perishable foods such as meat, fish, cheeses, fruits and vegetables.

Antibiotics are also widely used in scientific research as substances for the study of metabolic processes in living organisms to decipher fine molecular mechanisms such as protein syntheses, membrane function and many other biochemical conversions. For example an antibiotic can inhibit specifically separate stages of protein synthesis on ribosomes (e.g. chloramphenicol, puromycin, tetracycline) while others inhibit synthesis of nucleic acids at various stages (e.g. azaserine, sarcomycin, actinomycin, bleomycin), and alteration of cell membrane function (valinomycin, nystatin, gramicidin S).

Most attention has been devoted to the accidental poisoning of domestic animals especially cows, which graze on alkaloid containing plants of the leguminosae (e.g. *Lupinus* species) and in the compositae (e.g. *Senecio* species) families The toxicity of the pyrrolizidine alkaloids of rag wood *Senecio jacobeae* and other senecio plants is notorious and - 50% of all domestic cattle deaths worldwide are due to poisoning by these alkaloids. Another group of toxic alkaloids are the quinolizidine alkaloids of lupin plants. They are toxic to animals and have also been implicated as teratogenic agents.

The rotenoids, which occur in the roots of legumes such as *Derris elliptica* are well known to be insecticide and toxic to fish. Again, the prenylated flavones present in *Lonchocarpus* seed are toxic to the predating mouse.

One of the most remarkable features of plant animal interactions in the natural world is the ability of certain insects to sequester plant toxins from their food plants in the larval stage. They then move these toxins into the adult and both larva and adult thus gain protection from predation.

The floral volatiles as well as colors play an important role in attracting pollinators to the plant. Fruity or aminoid odour are attractive to beetles, sweet smells to bees, moths, and butterflies, fruity to bats, fecal odours to dung flies etc. Nector is also important source of food for most animal pollinators. The major components of nectars are simple sugars such as glucose, fructose, and sucrose in solution, the sugar content varying from 15 to 75%.

Pollens, like nectar, are largely nutritional to insects. Carotenoids and flavonoids are present in many pollens, providing yellow colour, and function in improving pollen detection by the pollinator.

The ripe fruit is usually exposed to herbivores by its attractive and distinctive odour and colour, since it is provided for animals, birds in return for the widespread dispersal of the seeds that lay within the fruit. In contrast, the seed and the seed coat usually possess some bitter chemicals, although they are often well protected by physical structures. This is to ensure that the seed is not consumed along with the fruit.

Because of the co evolution of plants with their microbial parasites, plants have elaborated a complex series of defensive barriers, capable of providing them resistance to disease.

Phytoalexins are formed, when the plant is infected by or inoculated with a microorganism. Such a defense system involves the *de novo* synthesis of new secondary metabolites, which are not present normally in the healthy plant. Phytoalexins are significantly antimicrobial in their properties.

L12 STRUCTURE ELUCIDATION

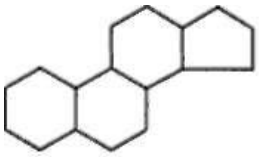
One classic method for establishing structure of natural products is based upon a stepwise degradation of an unknown molecule into smaller and known compounds. This degradation is followed with a logical restructuring of the original natural product based upon the identity of the fragments. This route is time consuming and requires a substantial amount of the compound to be degraded. Moreover in the course of the degradation some structural information, especially related to the stereochemistry of the broken bonds is sometimes lost.

For many cases in which the amount of natural product available is very small (less than a milligram or even a microgram) degradation methods are not feasible.

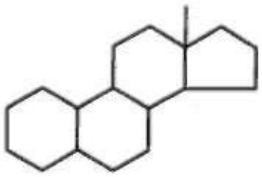
The modern powerful spectral methods for structural analyses, such as nuclear magnetic resonance spectrometry (NMR), Infrared spectroscopy (IR), Ultraviolet spectroscopy (UV), Electron spectroscopy (EI) and Mass spectroscopy (MS) are capable of establishing the

Table *l.l* **Parent steroidal Skeletons**

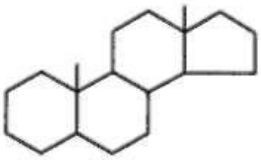
Gonane



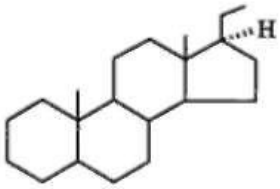
Estrane



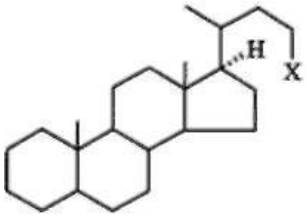
Androstane



Pregnane

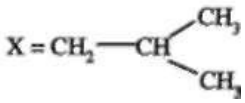


Cholane



$X = CH_3$

Cholestane



(contd. ...)

I.2.D. Configuration In the Side Chain

As per earlier traditions, to specify configuration at C-20, the terms 'a' and 'p' are applied as shown in 1.6 when there is a two- carbon chain attached at C-17. However, the current practice is to use the sequence rules or *'RS'* nomenclature to specify the stereochemistry at C-20 and other positions in the steroid side chains longer than ethyl. In case of cardenolides and bufanolides, the configuration at C-20 is *20f* when the lactone ring is saturated. In case of spirostan, the *A*S' nomenclature is used to specify the stereochemistry at C-20 and C-22 if it is differing from that shown in Table 1.1. The *'RS'* nomenclature is also used to specify other substituents at C-23, C-24, C-25 and C-26 of spirostan.

I.2.E. Conformation

The term 'conformation' denotes the different possible arrangements of atoms in space. These arrangements can be transformed into one another by mere rotation of bonds without breaking bonds. In a completely saturated steroid, rings A, B, C are cyclohexane rings and ring D is a cyclopentane ring. A cyclohexane ring prefers to assume the least energy geometry of a chair 1.7 and 1.9 rather than a boat conformation 1.8.

1.7 I * 1.9

When two rings are fused together, the geometry at the point of fusion has to be specified. The two rings could be fused in two different ways. In one form, the two substituents at the ring junction are oriented in opposite direction and are said to be *'trans'* to each other. These rings are *'trans* fused' 1.10. In the other form, the two substituents at the ring junction are oriented in the same direction and are said to be 'c/V to each other. The rings are **cis* fused' 1.11.

1.10 1.11

A six membered ring can invert from one chair form to another if the other rings fused to it are connected by **cis* junction' but not if they are attached by *'trans* junction*.

A/B rings

Fusion of rings A and B may be either *cis* or *trans.* Hence variation in stereochemistry at C-5 is more common than those at other junctions to give two isomeric 5a and 5fJ hydrocarbons. The typical saturated sterols like cholesterol are *AJB-trans* or 5a compounds. The bile acids are *AfB-cis* or 5|J compounds. Formerly, the term 'alio' was used to indicate 5a configuration.

Enzymes involved in cholesterol biosynthesis are as follows

1. Hydroxymethylglutaryl-CoA synthetase
2. Hydroxymethylglutaryl-CoA reductase
3. Mevalonic kinase
4. Phosphomevalonic kinase
5. Pyrophosphomevalonate decarboxylase
6. Isopentenyl pyrophosphate isomerase
7. Geranyl pyrophosphate synthetase
8. Farnesyl pyrophosphate synthetase
9. Presqualene synthetase
10. Squalene synthetase
11. Squalene epoxidase
12. Squalene epoxide cyclase

1.4 PHYSICAL METHODS OF CHARACTERISATION

Amongst the physical properties of steroids, the spectroscopic parameters have been important tools for providing valuable information about structure.

1.4.A. Melting Points

Most steroids are solids and their melting points fall usually in the range 100-250°C with a few exceptions (e.g. coprostane, m.p. 63°C; 5a-pregnane-3p,16ct, 200-triol, m.p. 304°C). The melting points are sensitive to the presence of small amounts of impurities causing steroids to have high freezing-point depression constant. This fact makes melting point an important aid in identification.

Some of the other characteristics of steroid melting points are crystallization of a steroid from different solvents may lead to different crystalline forms with different melting points. Sometimes, steroids crystallize with water of hydration such that the melting point differs significantly from that of anhydrous state (e.g. anhydrous aldosterone, m.p. 164-169°C; aldosterone hydrate 108-112°C). Another characteristic of some steroids is their ability to form liquid crystals. This is melting of the solid at a well-defined temperature to a turbid liquid (the liquid crystal state) followed by transition to a clear liquid at another well-defined temperature.

I.4.B. Solubility

Steroids are generally insoluble in water but soluble in many organic solvents like petroleum ether, benzene, acetone, ethanol, etc. A very unusual soluble property of steroids first discovered by Windaus in 1909 is the selective and quantitative precipitation of 3p-hydroxy steroids from solution by digitonin. The 3a-hydroxy steroids are not precipitated. The 3p-hydroxysteroid is precipitated as a complex of the glycoside. This property is used clinically as an easy method for the separation of free cholesterol in human blood plasma.

I.4.C. Ultraviolet Spectra

The position and intensity of an ultraviolet absorption band are usually given citing the wavelength of maximal absorption, the solvent and molecular extinction coefficient € as log e. Since

1.4.G. Optical Rotation and Rotatory Dispersion

The 'optical rotation* of steroids, specially the sterols are very useful in identification of steroids and structure determination. A system of classifying sterols according to their optical rotation has been proposed by Bergmann. The classification is as follows:

i. Sterols containing a conjugated system of double bond system in ring B exhibit a 'strong negative rotation' together with the ultraviolet absorption around 280 nm.
ii. Sterols with isolated double bonds show an 'intermediate negative absorption*.
iii. Sterols with only one double bond show a 'low negative absorption'.
iv. Sterol with no double bonds exhibit a 'slightly positive rotation*.
v. Sterols with a double bond between C-8 and C-9 show 'positive rotation*.

The method of 'molecular rotation differences* developed by Barton is based on the rotation contribution of the functional groups in a steroid. This rotation contribution gives rise to the difference observed between the molecular rotation of a steroid and that of the parent hydrocarbon. This difference is characteristic of the position and orientation of the substituents and is an additive physical property.

The determination of optical rotation at a series of wavelengths is 'rotatory dispersion*. This has been introduced to the steroid field by Djerassi and is an extension to the method of molecular rotation differences. This technique is particularly useful for studies of absolute and relative stereochemistry and also for determining the position of functional groups. The phenomenon is based on the difference between the refractive indices of asymmetric compounds for the right-handed and left-handed circular polarized light.

Some of the other physical methods for characterization are 'circular dichroism* and X-ray crystallographic analysis for study of structure and stereochemistry.

1.5 STEROLS

The sterols are 3-monohydroxy steroids having C-27, C-28 or C-29 skeleton. They are crystalline, widely distributed in nature and occur both in free form as well as esters of higher aliphatic acids. All naturally occurring sterols have a 3P-hydroxyI group and also one or more double bonds. The most common position of the double bond is C-5 followed by C-22 and C-7.

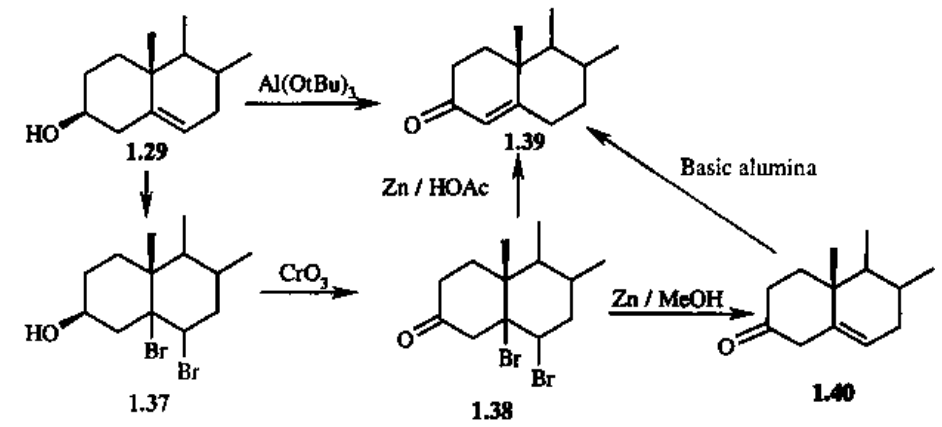
1.5.A. Classification of Sterols

Based on occurrence, the sterols are broadly classified as follows:

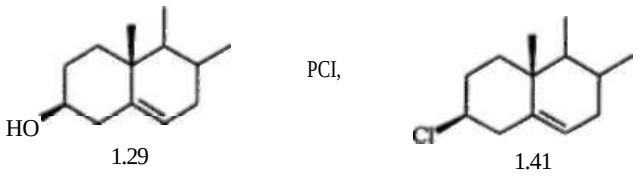
i. Zoosterols—These are obtained from animal sources, e.g. 5a-Cholestan-3p-oI; 5p-choIestan-3P-ol
ii. Phytosterols—These are found in plants, e.g. Stigmasterol.
iii. Mycosterols—These occur in yeast and fungi, e.g. Mycosterol.
iv. Marine sterols—These occur in marine organisms

However, it is becoming increasingly clear that some sterols like ergosterol occur in plants and animals. It is observed that lower animal species contain a great variety of sterols. As the evolutionary scale is ascended, variations are eliminated and cholesterol becomes more and more prominent.

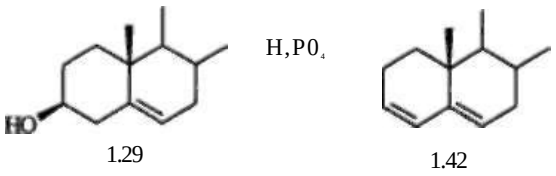
Oxidation



Nucleophilic displacement



Elimination



Eplmerisation

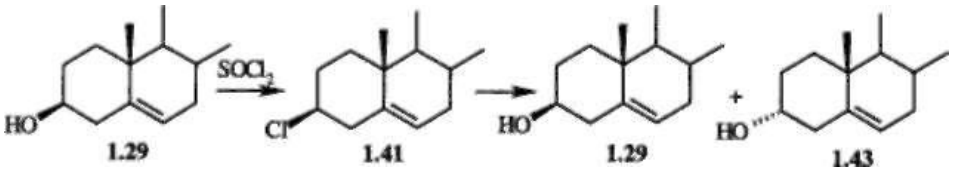
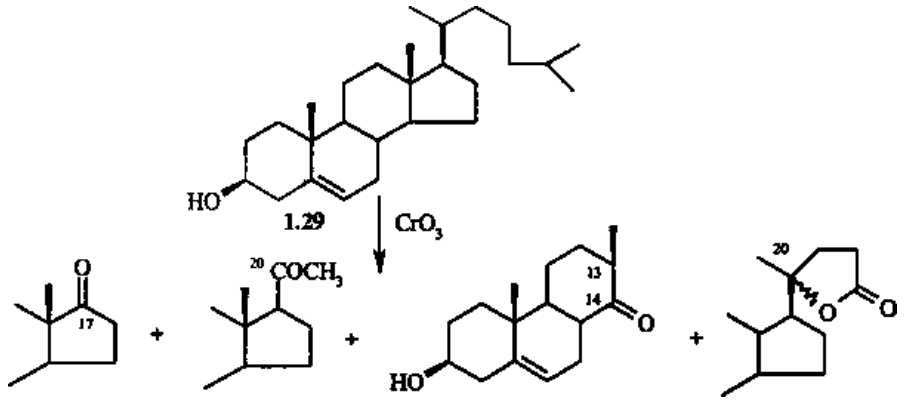


Figure 1.3 Reactions of the hydroxy! group in cholesterol (cont.)

Oxidative degradation



1.63 **1.64** **1.65** **1.66**

1.67 **1 68** **1.69** **1.70**

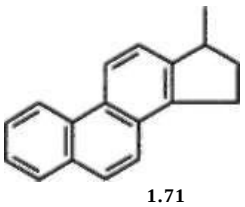
Figurel.**6** Oxidative degradation of the side chain of cholesterol

1.6.G. Structure Elucidation

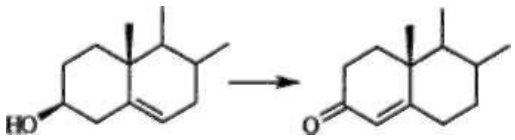
The empirical formula of cholesterol was established as $C_{27}H_{46}O$ in 1888. In 1927, Otto Diels discovered that dehydrogenation of cholesterol with selenium at high temperatures yielded a hydrocarbon (Diels' hydrocarbon, **1.71)** amongst other products. This paved the way for the structural elucidation. The correct structure was established in 1932 by Windaus and Dane and confirmed with the X-ray work of Bernai in 1950. The structure was elucidated in four parts:

i Structure of the nucleus—sizes of the rings A,B.C. and D,
ii. The position of the hydroxyl group and the double bond,
iii. Position and structure of the side chain,
iv. Position of the angular methyl group.

Structure of the nucleus



1.71

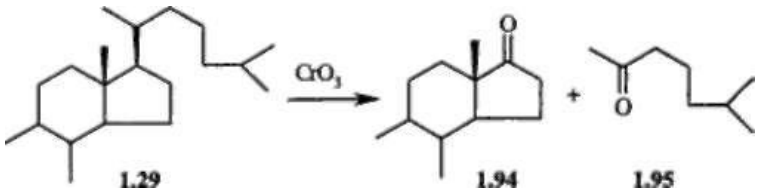


1.29 L39

Position and structure of the side chain

Cholesteryl acetate 1.35 on chromium trioxide oxidation yields a volatile ketone, which was shown to be isohexyl methyl ketone 1.95 and a non-volatile ketone 1.94. Thus isohexyl methyl ketone is the side chain and the point of attachment is C-17. This is because selenium dehydrogenation is known to degrade a side chain to a methyl group and cholesterol yields Diel's hydrocarbon 1.71 on dehydrogenation with selenium.

X-ray Crystallographic studies and surface film measurements also confirm the point of attachment as C-17 and as having *p-configuration\



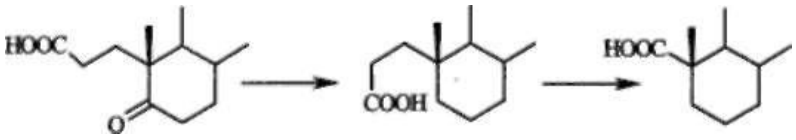
1.29 1.94 1.95

Position of the angular methyl groups

The cyclopentenophenanthrene nucleus accounts for 17 carbon atoms and the side chain accounts for 8 carbon atoms. Therefore the remaining two carbons are shown to be angular methyl groups as follows:

C-10 methyl group

The keto-acid 1.96 from deoxycholic acid was reduced to 1.97 followed by degradation to the monoacid 1.98 which is difficult to esterify. This shows that the carboxylic acid group was attached to a tertiary carbon. Thus one of the angular methyl groups is at C-10.



1.96 1.97 1.98

Formation of ring A

The compound **1.113** was condensed with elhyl formate to yield **1.114** followed by treatment with N-methylaniline to give methylanilinomethylene compound **1.115.** This was condensed with acrylonitrile and hydrolyzed to give two isomeric keto-acids **1.116.** One of the isomer was converted to the lactone **1.117.** This on treatment with methylmagnesium bromide gave **1.118,** which was converted to enone **1.119** by treatment with alkali forming the ring-A.

Conversion of ring D equivalent to five membered ring

Hydrolysis of the acetonide and oxidation with periodic acid gave dialdehyde **1.120,** which was cyclized to the aldehyde **1.121.** Oxidation and esterifcation with diazomethane gave the methyl ester **1.122.**

Modifications of ring A and attachment of side chain

The compound **1.122** was reduced with sodium borohydride to a mixture of (±>3a-hydroxy and (±)-3p-hydroxy forms from which the (+) form of the 3p-aIcohol was precipitated by digitonin and oxidized to the desired stereoisomer **1.123.**

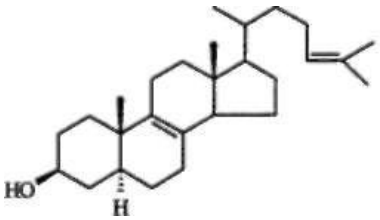
1.123 was hydrogenated to the keto-ester **1.124.** This on reduction with sodium borohydride followed by hydrolysis gave the acid **1.125. 1.125** was acetylated, treated with thionyl chloride followed by dimethyl cadmium to give ketone **1.126.** Grignard reaction with isohexyl-magnesium bromide, dehydration, hydrogenalion and hydrolysis gave 5a-cholestanol **1.44.**

The compound **1.44** on oxidation followed by treatment with bromine gave the 2ot-bromo compound **1.127,** which at high temperature in pyridine was pyrolyzed to **4**-en-**3**-one **1.39.** This was converted to cholesterol **(1.29)** via its enol acetate **1.128.**

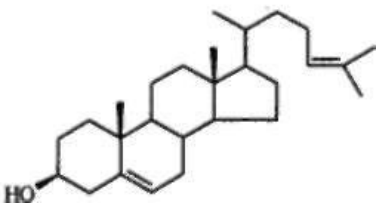
The keto- ester **1.124** can be converted by known reactions to estrone **1.177.**

1.7 OTHER C-27 STEROLS

Two other C-27 sterols, which are considered important precursors in the biosynthesis of cholesterol include zymosterol **1.129** and desmosterol **1.130.** Zymosterol (Gr. Zyme = yeast) is a mycosterol isolated from yeast while desmosterol (Gr.desmo = link) has been isolated from chick embryos. Both are efficiently converted by the rat liver to cholesterol.



1129



M M

1.8 C-28 STEROLS—ERGOSTEROL

The most important C-28 sterol studied extensively includes ergosterol **1.131.** It is the commonest of the mycosterols. Its importance stems from the finding that ergosterol is the precursor of vitamin **D2** (ergocalciferol **1.147).**

the hydroxy! group at C-3 and also the presence of the geminal dimethyl group at C-4. This reaction does not occur in sterols without the geminal dimethyl group.

Reactions of the C-8, C-9 double bond (Figure 1.9)

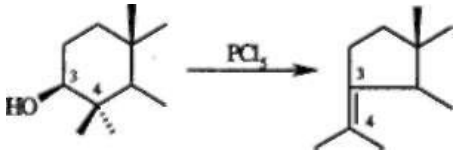
Reduction

The double bond at C-8, C-9 cannot be directly reduced. Instead it is hydrogenated to 1.142 with hygrogen-platinum or zinc-acetic acid after oxidation to diketone 1.141.

Shifting the double bond to C-7, C-8

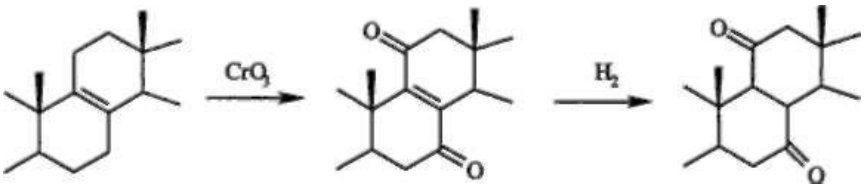
The double bond is readily shifted to C-7, C-8 1.143 under acidic conditions.

Rearrangement of ring A



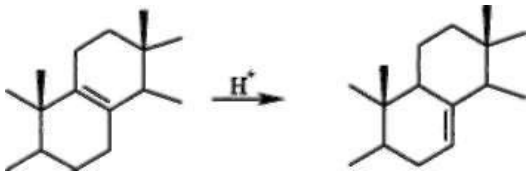
1.138 1.140

Reactions of the C-7(8) double bond Reduction



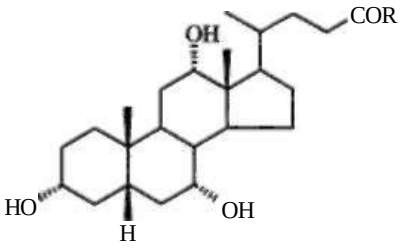
1.138 1.141 1.142

Shifting of the C-7, C-8 double bond

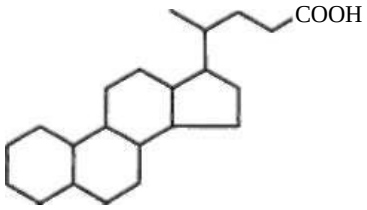


1.138 1143

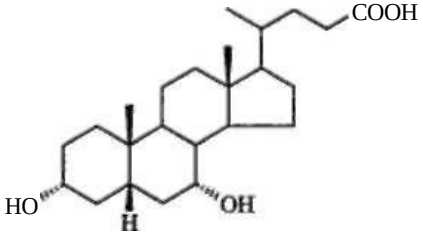
Figure 1.9 Reaction of lanosterol



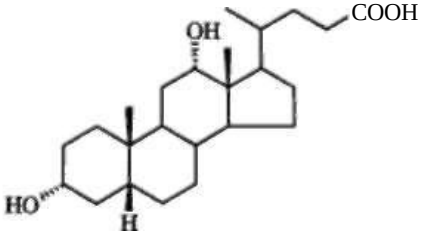
1.151 R OH
1.152 R $NHCH_2COOH$
1.153 R $NHCH_2CH_2SO_3H$



1.154



1.155



1.156

cholic acid. Details of the reaction sequence are still missing. However, much has been learned by administering radioactive precursers. These results indicate that the ring skeleton of cholesterol remains intact, while the isopropyl group of the side chain is removed. Also studies show that C-12-hydroxylation of the ring precedes degradation of the side chain. Once the side chain is oxidized, no hydroxyl group can be introduced into the 12a position.

1.12.B. Clinical Significance

The bile salts play a major role in fat emulsification whereby the fat particles are converted to fine droplets that can be hydrolyzed by the action of lipases. In addition, studies show that bile salt may participate in activation of lipases needed for fat hydrolyses and / or activation of the enzymes needed for fat resynthesis.

1.12.C. Colour Reactions

Some specific colour reactions for bile acids have been listed below.

Mylius reaction for cholic acid

Cholic acid dissolved in etbanol and treated with a few drops of iodine solution gives on warming a blue colour in transmitted light and needles, which appear yellow in reflected light.

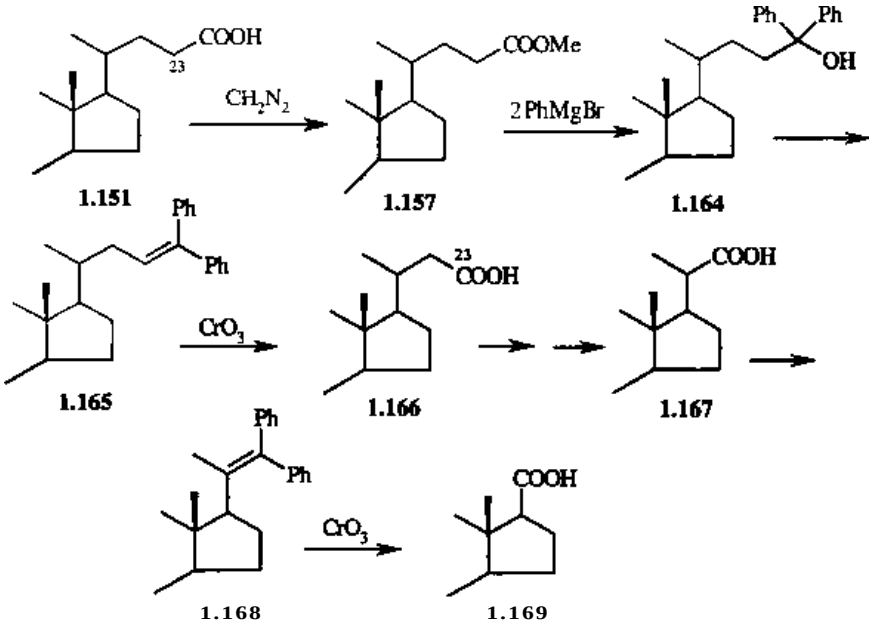
that sequence gives etianic acid **1.169.** A variation is the use of ozone as oxidizing agent in the penultimate step to give 20-oxopregnanes **1.170.**

A more economical method is the Meystre Miescher procedure involving the use of N-bromosuccinimide to the dehydrated compound **1.165** to give **1.171,** which is dehydrobrominated to **1.172** and directly oxidized to **1.170.**

Conversion to cortisone series (Figure 1.14)

An important reaction of the diene **1.172** is the treatment with N-bromosuccinimide to give a 21-bromo-diene **1.173.** This is oxidized with chromium trioxide to give 27-bromo-ketone **1.174,** which is converted to cortisone side chain **1.175.**

Conversion to etianic acid (Barber **Wieland** procedure)



Conversion to pregnane

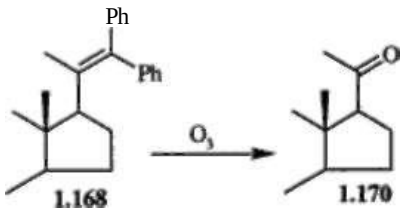


Figure **1.13** Reactions of the bile acids side chain

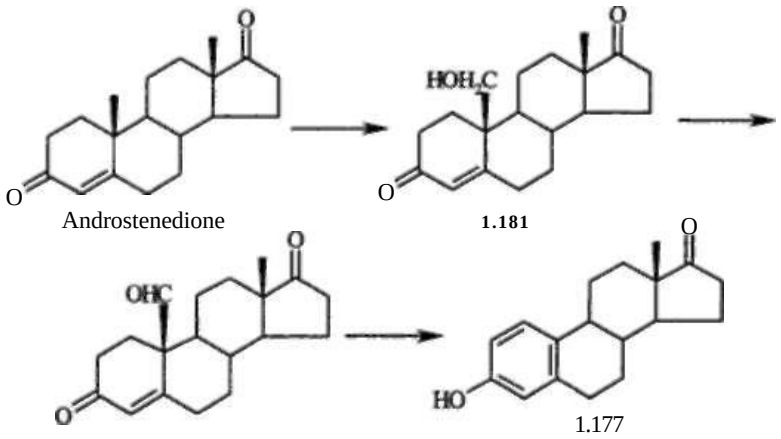


Figure 1.15 Biosynthesis of estrone

1.15.E. Colour Reactions

Kober Test

This reaction is used to determine urinary estrogens. Estrogens are heated with 60% sulphuric acid containing phenol, hydroquinone or p-naphthol to give a yellow colour with green fluorescence. On dilution with water and reheating, a pink colour is produced.

Diazo coupling test

Diazotised p-nitroaniline or sulphanilic acid develops an orange or red colour on treatment with estrogens. This is due to the coupling of diazo compound with estrogen, which reacts as a phenol.

David test for estriol

A blue colour is produced by the treatment of estriol with sulphuric acid and arsenic acids.

Bachmann test for estriol

A violet colour is produced by the treatment of estriol with sodium p-toluene sulphonate and phosphoric acid.

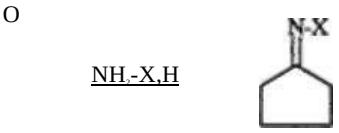
Benzoyl chloride test

Estrone and estradiol by treatment in chloroform with benzoyl chloride and a solution of zinc chloride in acetic acid form complexes, which absorb at 502 mu,. Estriol gives a negative test.

Zimmermann reaction

This is used for the quantitative estimation of 17-ketosteriods. Treatment of an alcoholic solution of the keto steroid with m-dinitrobenzene and potassium hydroxide gives a coloured

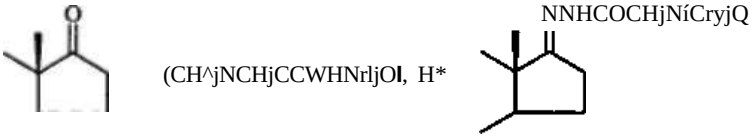
Formation of semicarbazone, oxime and 2,4-dinitrophcnvl hydrazone



1.177

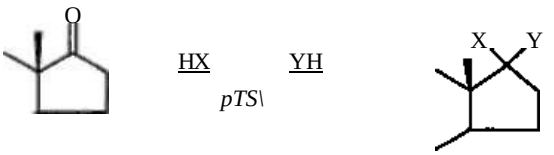
1.187, X = NH.CO.NH_2
1.188, X = OH
1.189, X = NH.C_6**H3**(NO_2)$_2$

Formation of Girard derivatives



1.177 **1.180**

Formation of ketals and thioketals



1.177

1.190X = Y = O
1.191 X s S, Y = 0
1.192X = Y = S

Reduction to alcohol

1.177 1.178

Reduction to methylene group

1.177 1.192 1.193

Figure 1.17 Reactions of the carbonyl group of estrone

3,4-tetrahydronaphthalene **1.208** with succinic anhydride **1.209** to yield the keto acid **1.210.** This on reduction followed by cyclisation yielded the cyclohexanone **1.212.**

Formylation and subsequent reaction with hydroxylamine gave the oxime **1.214,** which on heating gave the cyano compound **1.215.** a-alkylation with methyl iodide followed by hydrolysis of the cyano group to carboxylic acid and subsequent esterification afforded the methyl ester **1.217.** Three of the four possible racemic modifications were obtained crystalline and one of these (m.p. 133°C) was subjected to further reactions.

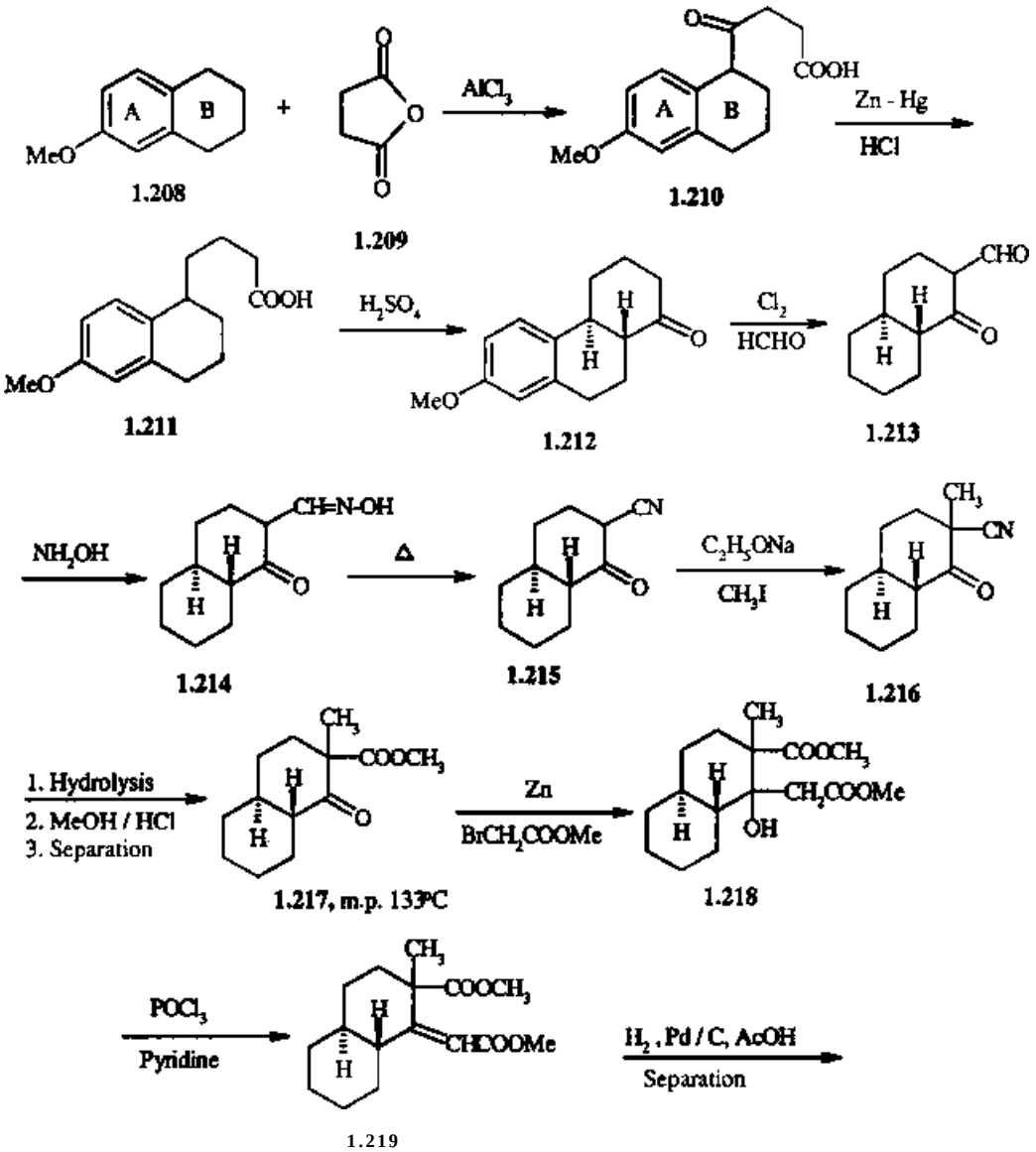


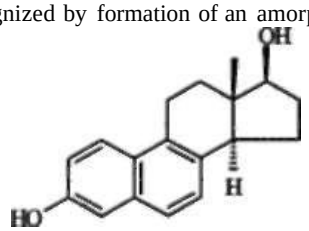
Figure **1.20** Anner-Miescher synthesis of estrone

1.19 EQUINE ESTROGENS

Marcs' pregnancy urine processed by Girard, using the reagent Girard T, resulted in the isolation of equine estrogens, equilenin 1.231 and equilin 1.232. These estrogens have additional double bonds in the ring B. Equilenin and equilin are less potent compared to estrone 1.177.

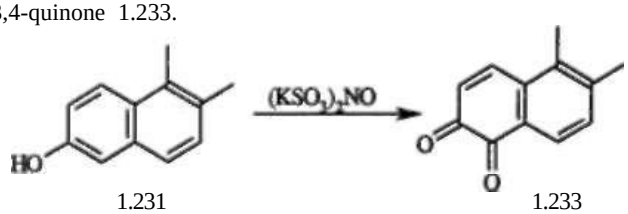
1.19.A. EQUILENIN, $C_{18}H_{M}0_{2}$, [a]$_{D}^{B}$ +53° (dioxane)

Equilenin 1.231 is readily recognized by formation of an amorphous red coloured compound on heating in air.



1.231

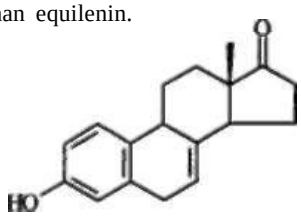
A characteristic reaction of equilenin is its oxidation with potassium nitrosodisulfonate (Fremy' salt) to a red 3,4-quinone 1.233.



1.231 1.233

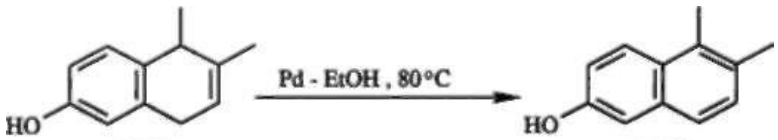
1.19.B. EQUILIN, C^H^Oj, [ctJ$_{b}^{M}$ + 308°

Equilin 1.232 is more potent than equilenin.



1.232

Dehydrogenation of equilin 1.232 with palladium-ethanol at 80°C gives equilenin 1.231.



1.232 **1.231**

In the animal body, progesterone is synthesized by degradation of cholesterol. Bloch and coworkers fed deuterated cholesterol to a pregnant woman and isolated labeled pregnanediol 1.250 (the chief metabolite of progesterone) from her urine. Incubation of radioactive cholesterol with placental, testes, corpus luteal and adrenal tissues yield progesterone. The probable route is the 20-hydroxylation to give 1.248 followed by cleaving of the isocaproic acid unit.

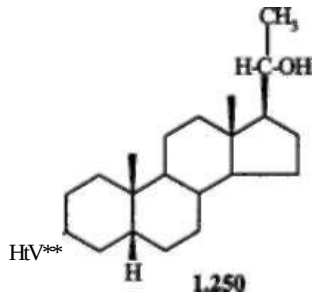
1.21.D Clinical Significance

Progesterone is required for the maintenance of pregnancy. It prepares the uterus for implantation of the fertilized ovum by inducing specific changes in the uterine mucosa. Together with other hormones, progesterone stimulates mammary development.

1.21.E. Commercial Significance

Progesterone occupies the key position in the industrial preparation of steroidal hormones.

1.21.F. Colour Reactions for Pregnanediol (Metabolite of Progesterone)



1.250

The most abundant metabolite of progesterone in human pregnancy urine is pregnane-3a,20a-diol 1.250, also called pregnanediol. Hence most methods of determination depend on the colour reactions of pregnanediol. Pregnanediol in urine occurs as glucuoronide, which has to be hydrolyzed by the enzyme ^-glucuronidase before determination.

i. Pregnanediol with concentrated sulphuric acid gives an orange-red colour with absorption maxima at 430 m|i.
ii. Reverse Zimmermann reaction—This is based on the purple colour produced on the addition of ethanolic solution of potassium hydroxide to the 3,5-dinitrobenzoate of pregnanediol.

1*J21JG* HYDROXYPROGESTERONE CAPROATE $C_{y}H_{O}$, , $[a]_{D}$ + 61° (Chloroform)

IUPAC name: 17-ï(l-OxohexyI)oxy]-4-pregnene-3,20-dione, m.p. 117-122°C

UV: $L^{W}{}^{H}$ 241nm £ = 17000

IR (nujol mull): 1670 (<x,|i unsaturated ketone) 1720 (side chain C-20 C = O), 1740 (ester C = O) cm^{-1}.

From stigmasterol 1.263 (Figure 1.26)

An alternative route to progesterone 1.247 started with stigmasterol 1.263 obtained from soyabean oil. From this, in four simple and high yielding steps, progesterone was obtained. Oppenauer oxidation of stigmasterol 1.263 gave stigmastadienone 1.264, which was ozonised directly to ketodehyde 1.265. Azeotropic distillation of the aldehyde 1.265 with piperidine in benzene gave the enamine 1.266. This on oxidation with sodium dichromate afforded progesterone 1.247.

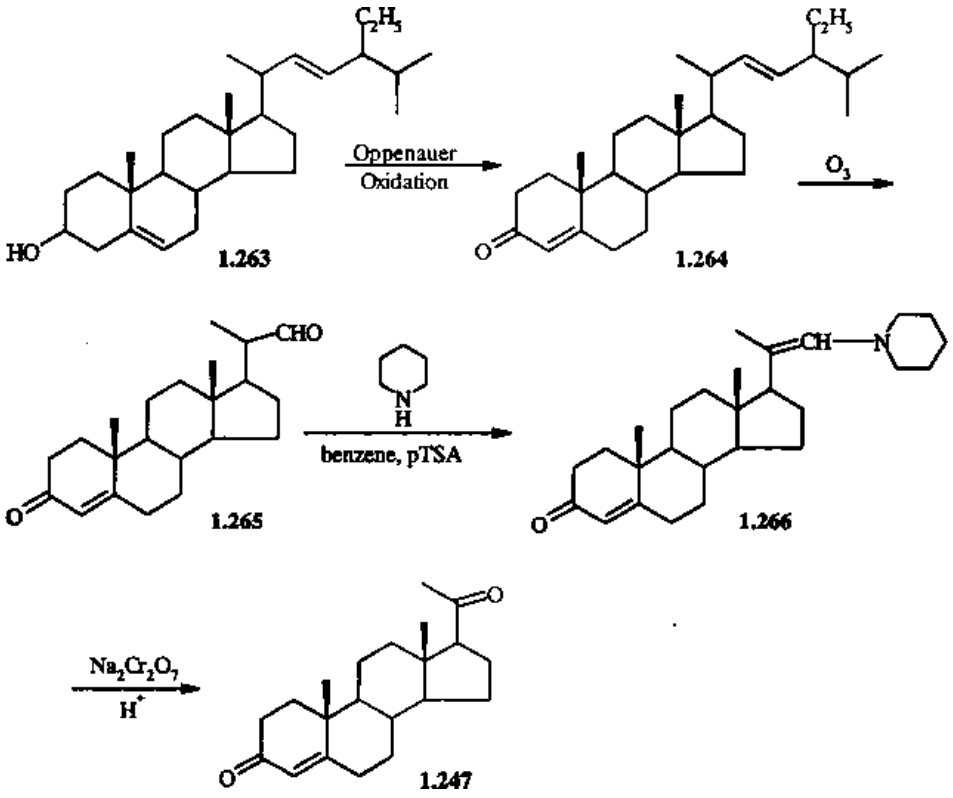


Figure 1.26 Partial synthesis of progesterone 1.247 from stigmasterol 1.263

1.21.J. Synthetic Analogues of Progesterone

Clinically, progesterone is used in many ovarian and menstrual disorders. Progesterone with or without an estrogen component are used as contraceptives and in the prophylactic management of habitual and threatened abortions. However, a major drawback is that progesterone is ineffective when given orally and parenteral administration frequently causes painful local reactions.

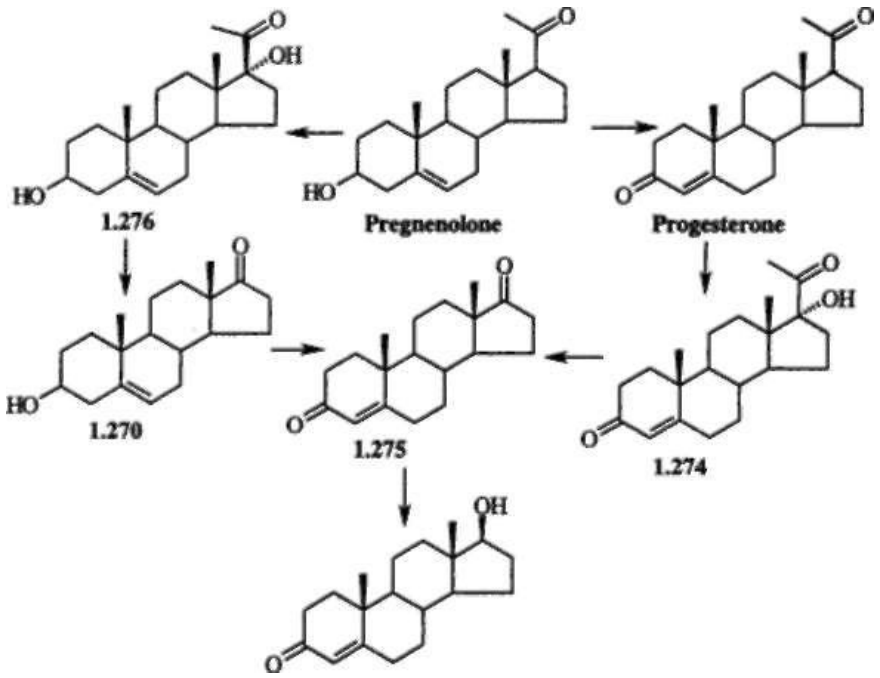


Figure 1.28 **Biogenesis of androgens**

1.24.D. Clinical Significance

Testosterone is necessary in the male for spermatogenesis and proper functioning of the reproductive tract glands and maintenance of secondary sexual characteristics. Androgens, in general, stimulate the development of male reproductive organs and secondary sexual characteristics. In addition the androgens cause an increased vascularity of the tissues, flushing of the skin, increase both the red blood cells count and the haemoglobin content of the blood and also have a profound effect on enzyme activity and metabolism. The androgens promote bone growth. The anabolic effect of androgens on the muscular development is called myotropic action.

1.24.E. Colour Reactions

Androgens occur as conjugates in the urine and hence are first hydrolyzed by heating with acid and the free steroid extracted into organic solvents. This treatment does not affect the 17-keto group on which the final estimation is usually based. The specificity of the tests can be improved by measuring the absorption of the coloured product at more than one wavelength and correcting for the absorption due to interfering chromogens.

androst-4-en-3,17-dione 1.275. This is reduced with *Saccharomyces cervisiae* to afford testosterone 1.271.

From androstenedione 1.275 (Figure 1.31)

Improved partial synthesis of testosterone 1.271 from androstenedione 1.275 by Rosenkranz includes reduction to the diol 1.276 followed by oxidation with manganese dioxide in chloroform at 20°C.

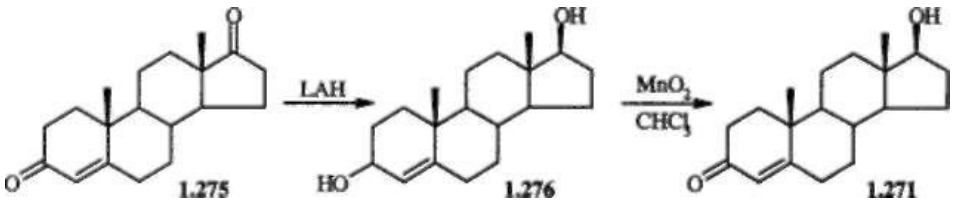


Figure 1.31 Partial synthesis of testosterone from androstenedione

From dehydroepiandrosterone 1.270 (Figure 1.32)

The Butenandt-Ruzicka method for the partial synthesis is as follows. Dehydroepiandrosterone as its acetate 1.284 on hydrogénation with a nickel catalyst was selectively reduced at the carbonyl group. Benzolylation of the product to the 3-acetate-17-benzoate 1.285 and partial saponification in methanol gave the 3-ol-17-benzoate 1.286. Conversion to the 5,6-dibromide, oxidation, debromination with sodium iodide and hydrolysis afforded testosterone 1.271.

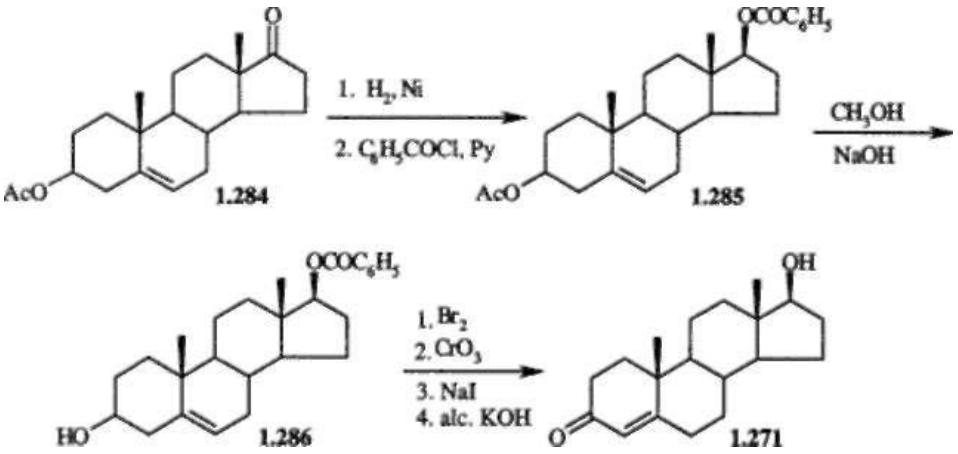


Figure 1.32 Partial synthesis of testosterone from dehydroepiandrosterone

The cortex of the adrenal gland produces a number of steroid hormones (corticoids) essential for the maintenance of life. Deficiency of these hormones produces a number of disturbances in the metabolism of water, electrolytes, carbohydrates and proteins. The corticoids involved in salt and water metabolism are called 'mineralocorticoids' while those involved in carbohydrate metabolism are called 'glucocorticoids*. The research groups led by Reichstein, Kendall, Wintersteiner and Wettstein have isolated more than forty six corticoids. The active compounds amongst these are corticosterone 1.297, cortisone 1.298, Cortisol 1.299, aldosterone 1.300, 11-desoxycorticosterone 1.301 and 11-dehydrocorticosterone 1.302.

The corticoids are C-21 steroids with highly oxidized side chains, an oxygen atom at C-1l, a 4-en-3-one group and a 20,21-ketol or a 17,20,21-dihydroxyacetone group. Some stereochemical regularities observed are as follows:

C-3 - All 50 compounds of human urine are 3a,
C-5 - Most compounds from human urine are 5p,
C-1l - All hydroxy groups are 1ip
C-17 - All side chains are 17p and hydroxy! groups when present are 17a
C-20 - Most compounds from human urine are 20a.

1.29.A. Isolation

The adrenal cortex is extracted with ethanol to precipitate proteins and the extract is partitioned between pentane (fats) and 30% methanol (adrenocorticoids). The mixture of adrenocorticoids is separated by the use of Girard's reagent into ketonic and nonketonic fractions. The water soluble ketonic fractions are fractionally hydrolyzed at varying pH. Chromatography of acetates on neutral alumina gives the different corticosteroids.

1.29.B. Biogenesis (Figure 1.36)

Adrenal tissue contains hydroxylating enzymes capable of introducing hydroxyl groups into progesterone in specific positions and orientations. Thus, progesterone plays the central role in

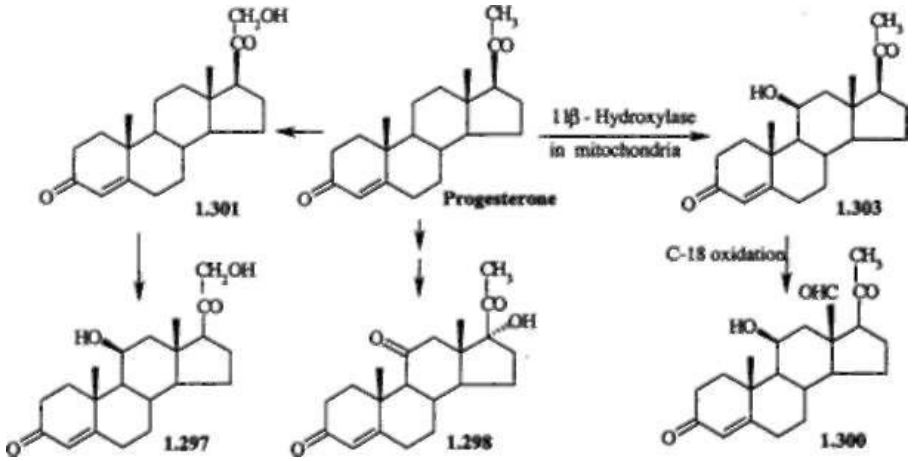
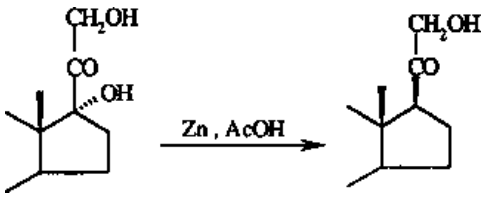


Figure 1.36 Biogenesis of adrenocortical hormones

Reactions of the C-17 hydroxy I group

Removal of the C-17 hydroxyl group

The C-17 hydroxyl group can be removed by zinc in boiling aqueous acetic acid to yield 20,21-ketol 1.310.



1.298 1310

1.30.B. Total **Synthesis** (Figure 1.39)

A stereospecific total synthesis of cortisone has been achieved by Sarett around 1953. This is of the A <— B «- C <- D type.

Formation of rings B/C equivalent

The Diels-Alder reaction of 3-ethoxypenta-l,3-diene 1311 and benzoquinone 1.312, which eventually formed ring C, gave the adduct 1.313. This was hydrogenated with nickel to the *cis* B/C-diketone 1.314, reduced with lithium aluminum hydride to the diol 1315 and hydrolyzed to the bicyclic dihydroxy ketone 1.316.

Formation of ring A equivalent

Addition of methyl vinyl ketone to 1.316 and cyclization yielded the tricyclic dihydroxy ketone 1.317. This was converted to the 3-ethylene ketal 1.318 and selectively oxidized to the hydroxy diketone monoketal 1.319.

Formation of ring D equivalent

Alkylation with methyl iodide followed by a second alkylation with methylallyl iodide afforded 1.320. Oxidation with chromium trioxide in pyridine gave the diketone 1321, which was condensed with ethoxyethynylmagnesium bromide to give the acetylenic ether 1.322. This rearranged to the unsaturated ester 1.323 with sulphuric acid.

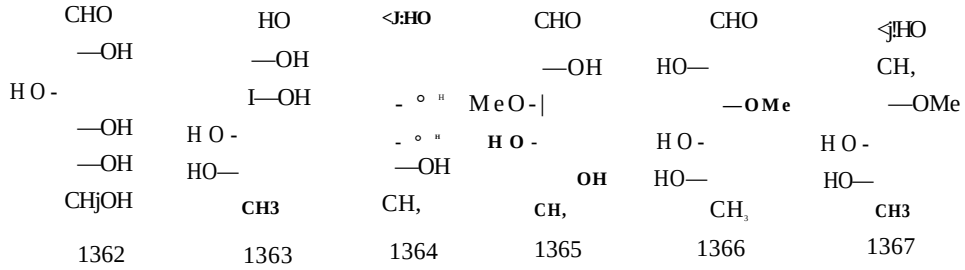
1.323 was hydrolyzed to the corresponding acid, reduced with sodium borohydride (carbonyl), potassium-isopropanol in liquid ammonia (conjugated double bond) and lithium aluminum hydride (carboxyl) in that order to the diol 1.324. This was converted to the tosylate 1.325.

Oxidation with chromium trioxide in pyridine gave the C-ll keto compound 1326. Osmium tetroxide followed by periodic oxidation gave the dioxo ester 1327. Cyclization with sodium methoxide afforded the 17-isoprogesterone derivative 1328. This was isomerised by alkali to the (±)-ll-ketoprogesterone derivative 1.329 which was converted by condensation with methyl oxalate to the 21-oxalylacid 1330. Resolution with strychnine afforded the (+)-acid 1330. .

may also be present at C-5, C-l, C-ll, C-12, C-16 and C-19. Some genins carry an aldehyde function at C-19, e.g. adonitoxigenin 1358 found in *Adonis vernalis*, a Ranunculacea. The C-16 oxygenated cardenolides appear in various species and may perhaps, constitute a link between this group and sapogenins. These C-16 hydroxy aglycones are found in *Neriun oleander*, an Apocyanaceae.

5a-Cardenolides occur in *Coronilla glauca* (Papilionacea), *Gomphocarpus fruticosus* (Asclepidaceae) and *Strophantus bovinii* (Apocyanaceae). *Strophantus* seeds have been long used by African aborigines for the preparation of arrow poison. Reichsten has investigated the glycosides in these seeds. The three major cardenolides, which occur in various *Strophantus* seeds include periplogenin 1.359, strophanthidol 1.360 and strophanthidin 1361.

The sugars, which are linked as glucosides to the 3p-hydroxyl group of the steroid, include glucose 1.362, rhamnose 1.363 and a number of unusual sugars which have not so far been found elsewhere in nature. These include 2,6-deoxy-sugars (digitoxose, 1.364), 6-desoxy-3-methoxysugars (digitalose 1.365 and thevetose 1.366) and 2,6-desoxy-3-methoxy-sugars (oleandrose 1367). The glycosidic linkage is always p in the case of D-sugars and a in the case of L-sugars.



1362 1363 1364 1365 1366 1367

135.A. Isolation

The isolation of cardiac glycosides from plant material is complicated due to the presence of enzymes, which hydrolyze the glycosides. To prevent this, a cold ammonium sulphate solution is used to extract the leaves. Solvent extraction of the aqueous solution permits the recovery of the glycosides.

In case of seeds, the material is defatted with petroleum ether followed by extraction of the hydrolytic enzymes with cold water and extraction of the glycosides with alcohol. The alcohol treatment inactivates the allomerizing enzymes. After precipitation of tannins, the glycosides are hydrolyzed and further purified by chromatographic methods. The glycosides can be hydrolyzed by enzymes obtained from the aqueous plant extracts or snail intestinal juice or acid hydrolysis (hydrochloric acid in acetone at room temperature).

1.35.B. Colour Reactions

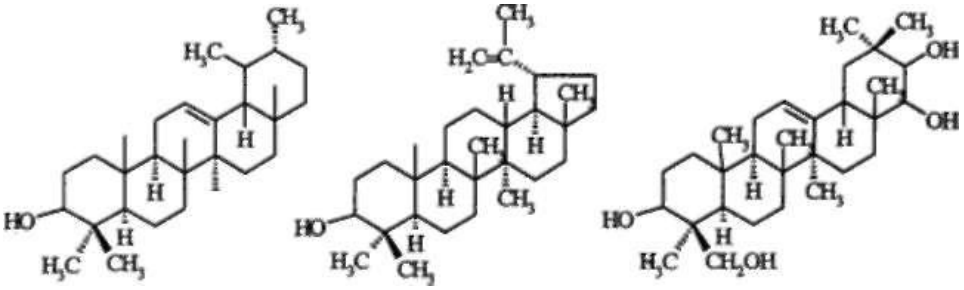
Keller-Kiliani test

This is specific for glycosides with 2-desoxysugars. The glycoside is dissolved in glacial acetic acid containing a trace of ferric chloride and concentrated sulphuric acid is added beneath the solution. The acetic acid layer turns blue.

1.36.B. Differentiation of Bufadienolides from Cardienolides

Bufadienolides may be differentiated from cardenolides by their ultraviolet absorption. The coumalin ring absorps around 300 mji.

1.37 SAPOGENINS



1383 1384 1385

The saponins (latin Sapo = soap) are glycosides which lower the surface tension of water. The genin portion may be a pentacyclic triterpene C-30 sapogenin or a C-27 steroidal sapogenin with a spiroketal side chain (hence also called spirostans). Pentacyclic triterpene sapogenins are widely distributed in plant kingdom. a-Amyrin 1383 obtained from elemi resin, lupeol 1384 from the lupine plant and soyasapogenot A 1385 obatined from soya beans are examples.

Steroid sapogenins form molecular compounds with cholesterol and other 3p-hydroxy steroids. They are haemolytic when injected directly into the blood stream due to the reaction with cholesterol in the red cell membrane. Saponins are used as fish poisons owing to their detergent properties. However, fish killed by saponins are edible as ingested saponins are not absorbed. The function of saponins in plants is largely unknown.

137.A. Spirostans

Spirostans are internal ketals of 16,27-dihydroxy, 22-ketosteroid and contain two heterocyclic rings. Ring E is a five membered furan ring and ring F is a six membered pyran ring. The rings are joined at C-22 in spiroketal fashion. The majority of known spirostans are a compounds (A/B *trans* fused); but some 5p- and A^{5}—compounds are also known. The C-3 hydroxyl group is invariably P oriented. Hydroxyl groups may be also present at C-2, C-6, C-12 and C-15. Digitoxigenin appears to be unique among the natural steroids in having the hydroxyl at C-15.

The carbon-oxygen bond at C-16 and 17, 20 positions are p oriented. The methyl group at C-20 is also p oriented. The pyrane oxygen is located behind the plane of ring E.

Formerly, spirostans were classed into two groups depending on the position of the pyrane ring oxygen with respect to the plane of the furan ring. Those with oxygen behind the plane of the E ring were grouped in the 'a' series and those with oxygen in the front in the *b* series.

It has been shown that all natural sapogenins have the same configuration at C-22 and the isomerism is at C-25 with methyl group being either axial ('a* and p) or equatorial ('e* and a) to the pyrane ring.

Compounds in which F ring is open are called furostans. Workers principally concerned in establishing the structure of spirostans were Windaus, Jacobs, Tschesche, Kon, Fieser and Marker.

cyclised forms. As examples, various forms of sesquiterpene and diterpene skeletons are given in Figures 2.2 and 2.3 respectively

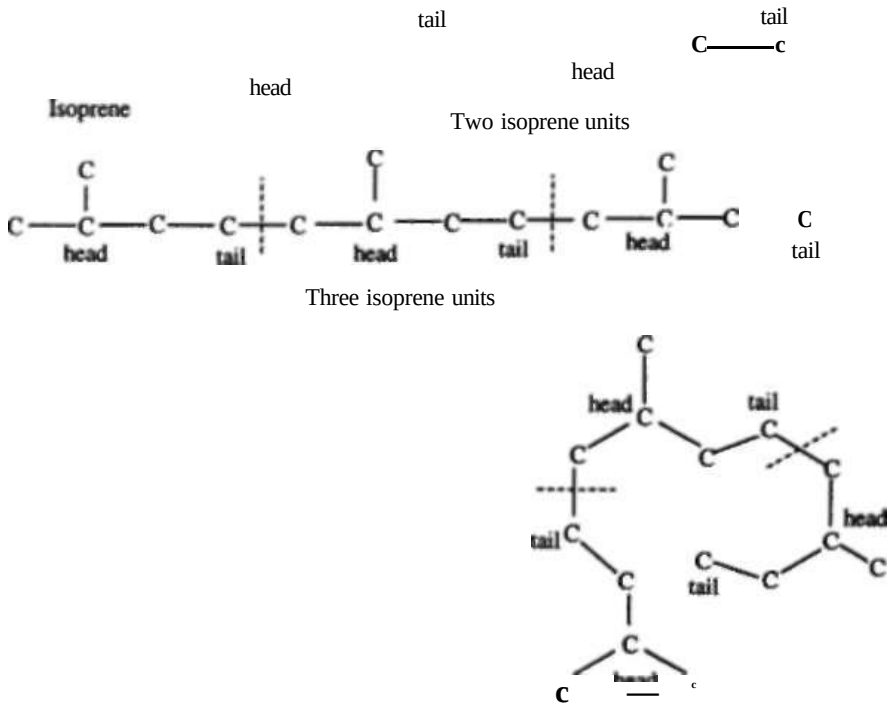


Figure 2.1 Arrangement of isoprene units in mono- and sesquiterpenoids

***23* BIOSYNTHESIS** (Figure 2.4)

Biosynthesis of terpenoids consists of the following steps

1. Photosynthesis converts 6 molecules of carbon dioxide and 6 molecules of oxygen to the glucose molecule, which breaks down through glycolysis to pyruvate.
2. Pyruvate is further converted to acetyl coenzyme-A 2.2 and two molecules of acetyl coenzymes couple with each other to form acetoacetyl coenzyme-A 2J.
3. Acetoacetyl-coenzyme-A reacts with another molecule of acetyl coenzyme-A to produce hydroxymethylgluuric acid 2.4, which in turn forms the key-intermediate, 3fl-mevalonic acid (MVA) 2.5.
4. MVA is phosphorylated by ATP to give mevalonic acid MVA-5-diphosphate 2.6.
5. MVA-5-diphosphate undergoes decarboxylation with the simultaneous elimination of pyro-phosphate to furnish the CS- isoprene unit, isopenetenyl diphosphate (IDP) 2.7.
6. IDP is isomerised by the catalytic action of sulphhydryl enzyme IDP- isomerase to afford dimethylallyl diphosphate (DM A DP) 2.8.

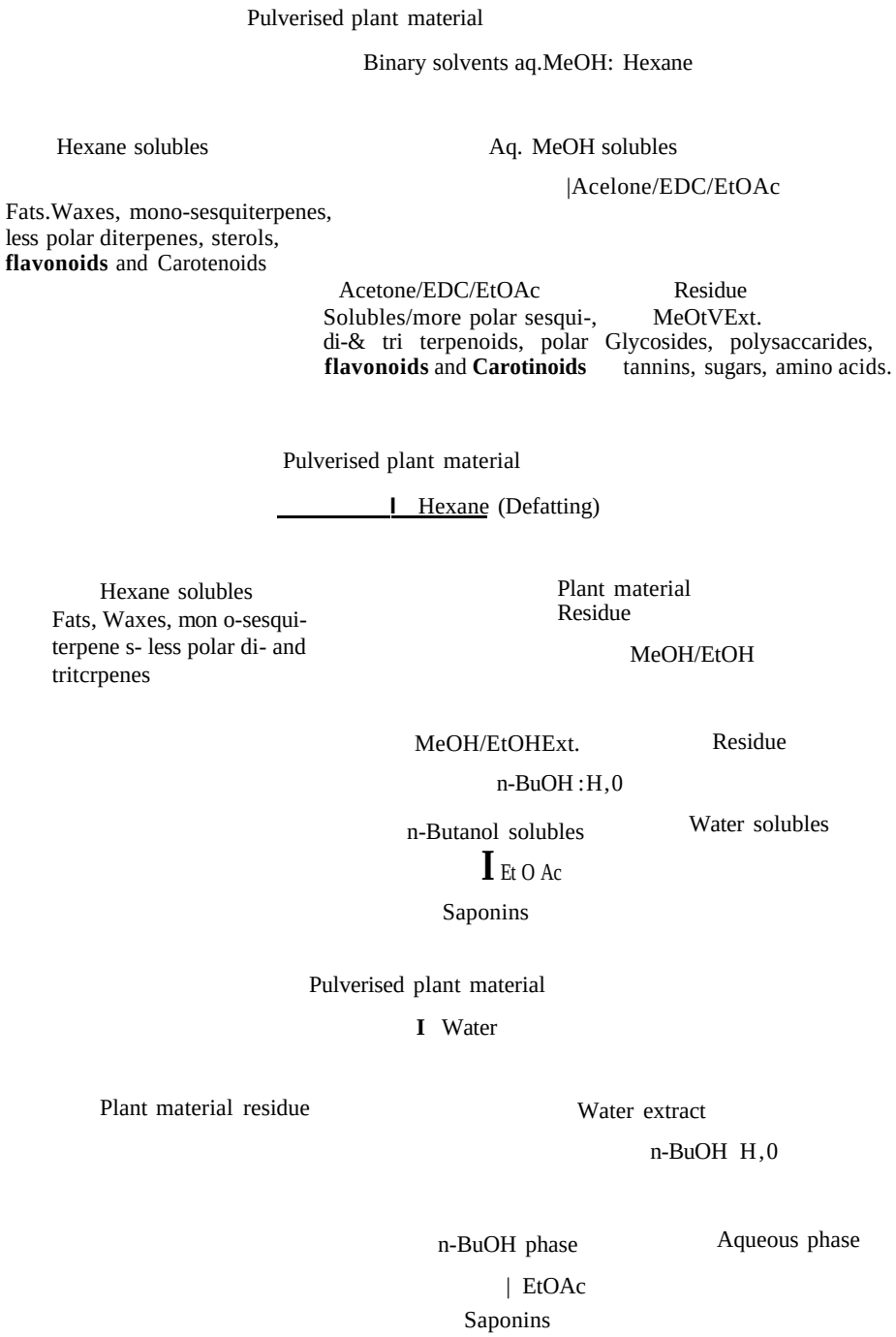


Figure 2.9 Procedures used for extraction and separation of terpenoids

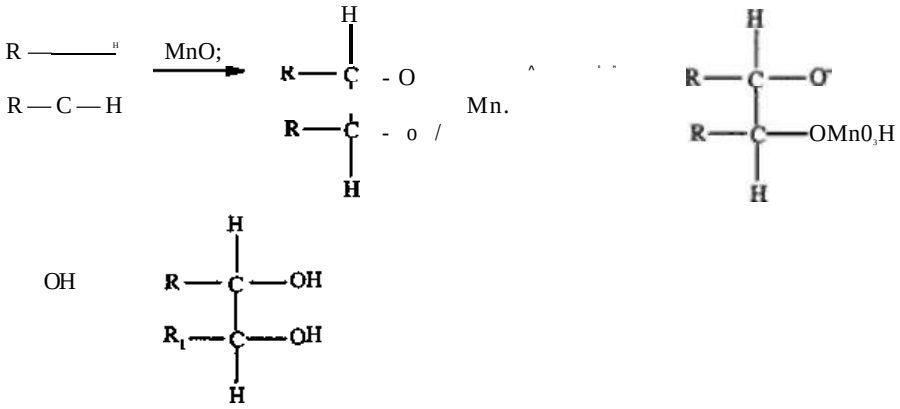
Carbonyl compounds formed after ozonolysis could be aldehydes or ketones depending on the substitution on the double bond.

iv) Oxidation

Oxidation by different oxidizing agents is one of the important methods to determine the presence and position of double bonds.

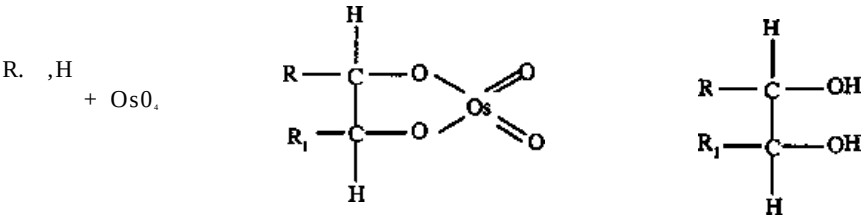
a. Alkaline permanganate hydroxylation

Cold and dilute alkaline potassium permanganate adds to the double bonds to give *cis* diols.



b. Osmium tetroxide hydroxylation

Another method of introducing a cw-dihydroxy group in the place of the double bond is the oxidation with Osmium tetroxide.



v) Epoxidation

Since peracids such as perbenzoic acid, monoterphthalic acid and other peracids react with double bonds to form epoxides by oxidation process, their consumption is calculated by titration methods to determine the number of double bonds.

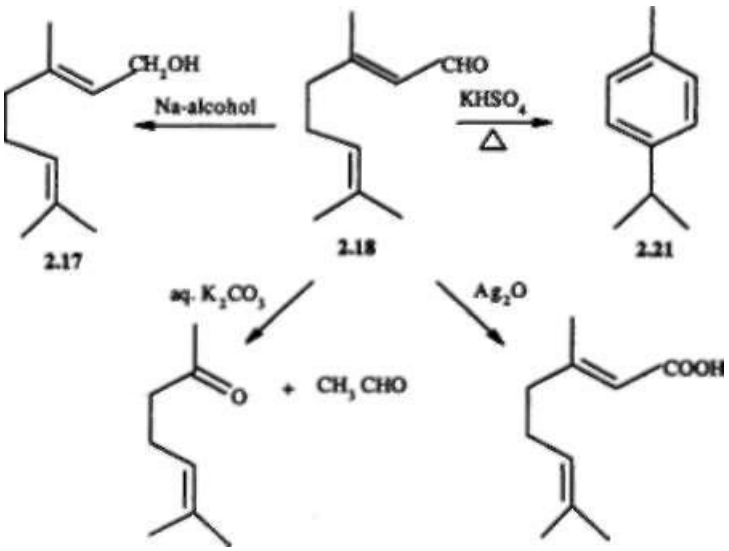
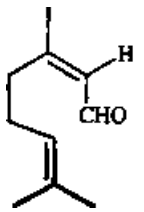


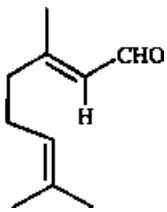
Figure 2.15 Reactions of citral

Isomerism of citral

Two geometrical isomers occur in nature. When the olefinic proton and the methyl group on the double bond are *cis* to each other, the isomer is known as ci'j-citral or neral and when they are *trans* to each other, the compound is identified as /rans-ciirai or geranial



cis Citral



irons Citral

2.8.D. ct-Pinene 2.24, $C_{10}H_{18}$, b.p. 155-I56°C. ***r***\\ 1.4. $[a]_D$+52.4°

a-Pinene is one of the major constituents of turpentine oil obtainable from various ***Pin us*** species. It is present as both (+)-a-pinene and (-)-a- pinene. The racemic (±) a-pinene is also present in terpentine oil.

4

2.24

This was further supported by the fact that both eudesmol and p-selinene 2.105 gave selinene dihydrochloride.

Position of the hydroxyl group

Three alternate. Structures A1, Bl and 2.104 were possible for eudesmol.

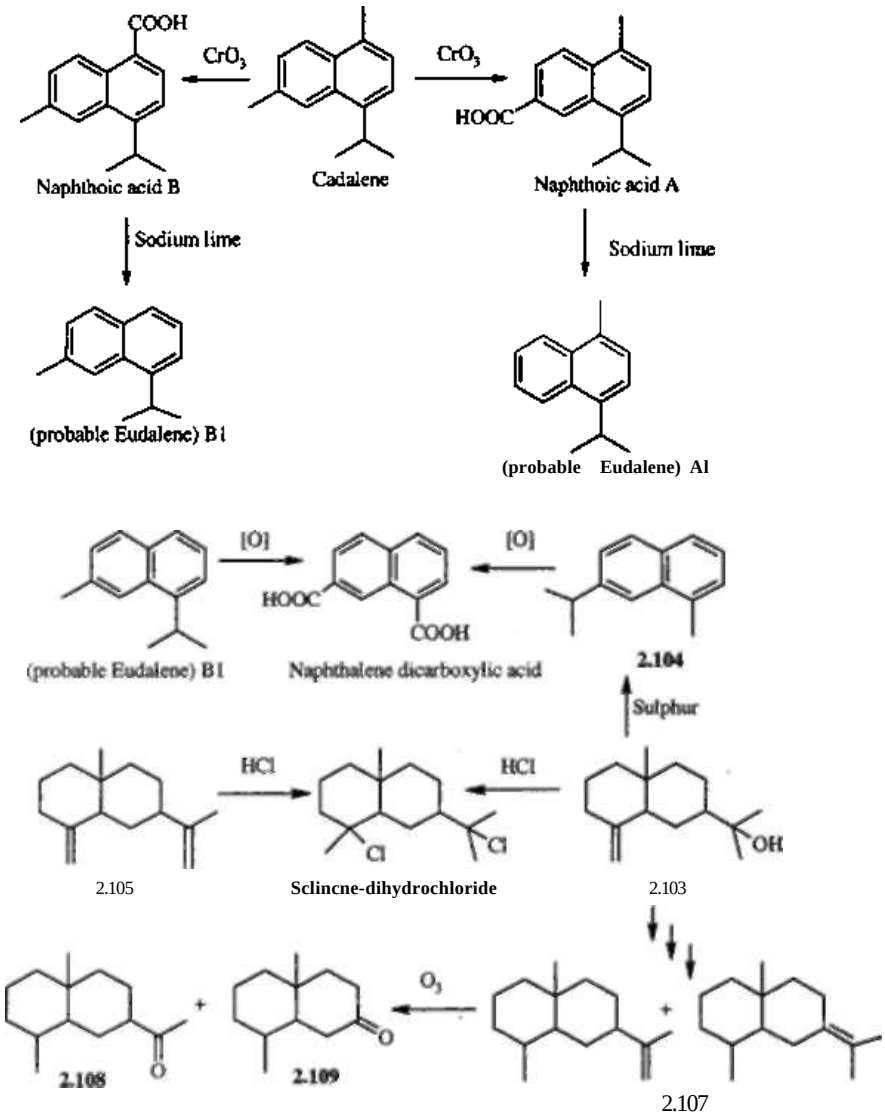


Figure 2.32 **Reactions of eudesmol**

presence of an a, p-unsaturated ketone was confirmed by UV (Xmax 227nm, e 6200), IR (1695 cm" '), and 'H NMR spectra. The appearance of an AB quartet for the olefmic protons of the newly introduced double bond clearly showed that C-9 position is substituted. Further, one of the quaternary methyls other than the assigned at C-5 had moved downfield to 8 0.92 from its original position of 8 0.82 due to its ally lie nature of the newly introduced double bond. Therefore the quaternary methyl group must be at C-9.

The position of the secondary methyl group at C-8 and its configuration as a-oriented followed from the evidence presented below. The enone diester on oxidation with RuO_4 followed by alkaline hydrolysis yielded the monoester of the dicarboxylic acid 2.185 which on refluxing with acetic anhydride and distillation yielded the cyclised cyclopentanone methylcarboxylate 2.186. The CD curve of this ketone showed positive cotton effect suggesting a a-configuration for the C-9 methyl group. In addition, signals due to the secondary methyl group centered at 5 0.83 in all the derivatives had now moved downfield to 8 1.2 due to periposition of the

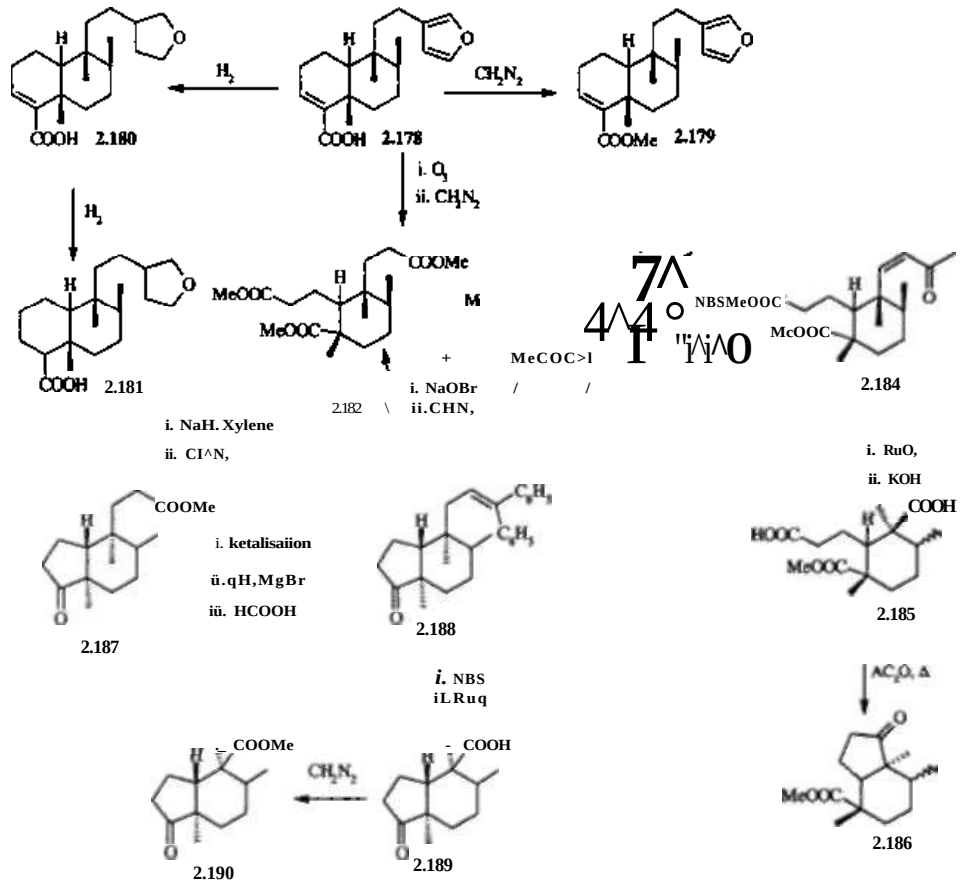


Figure 2.42 Reactions of hardwickiic acid

On treatment with lithium aluminum hydride, the carboxylic group can be reduced to alcohol. Fatty acids undergo specific a-halogenation (Hell-Volhard-Zelinski reaction) in the presence of bromine or chlorine and red phosphorus.

3.7 COMMERCIAL USES OF OILS, FATS AND WAXES

The fixed oils and fats are important products used industrially, pharmaceutical!)' and as food. Fixed oils and fats are employed in pharmaceuticals for their emollient properties. They also serve as vehicles for other medicaments. For example calcium or magnesium stearate is used as tablet lubricants and ethyl oleate is employed as pharmaceutical vehicle. In industry, fats and oils are used in the manufacture of soaps (sodium and potassium salts of fatty acids), as drying oils in the manufacture of paints and varnishes, as lubricants and emulsifying or stiffening agent etc. A few oils such as castor oil, chaulmoogra oil have cathartic and antileprosy activities respectively. Certain fatty acids find use as topical antifungal agents.

Waxes are employed in pharmaceuticals for 'hardening' ointments and in cosmetic creams. In industry and in the arts they are used for providing protective coatings. Waxes are also used in the manufacture of candles, wax varnishes, leather and furniture varnishes.

3.8 SOAPS AND MICELLE

Detergents or surfactants are washing agents and have wetting, dispersing and emulsifying properties.

Alkaline hydrolysis (saponification) of fats and oils produces glycerol and a mixture of salts of long chain fatty acids (sodium or potassium salts), which are called soaps (Figure 3.2). After complete hydrolysis sodium chloride is added to the reaction mixture to precipitate soap. The crude soap is filtered and purified by subjecting *in situ* to several reprecipitations.

The dilute solutions of the sodium salts of long-chain fatty acids are almost completely soluble in water. However, as we increase the concentration of salt, at critical concentration, molecules aggregate and there is formation of micelles. The soap micelles (Figure 3.6) are usually spherical clusters of carboxylate ions (hydrophilic) facing towards aqueous phase at the surface and hydrocarbon chains in the interior. These hydrocarbon chains are associated together through inter-chain hydrophobic interactions. The sodium ions are scattered throughout the

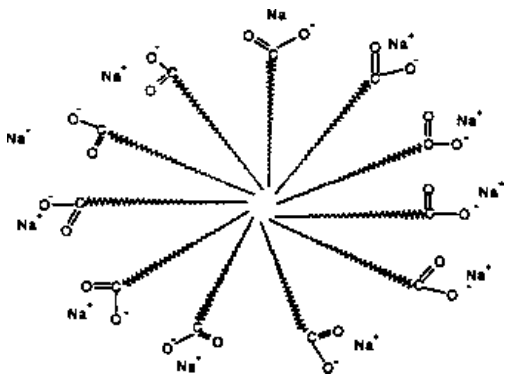


Figure **3.6 A** soap micelle

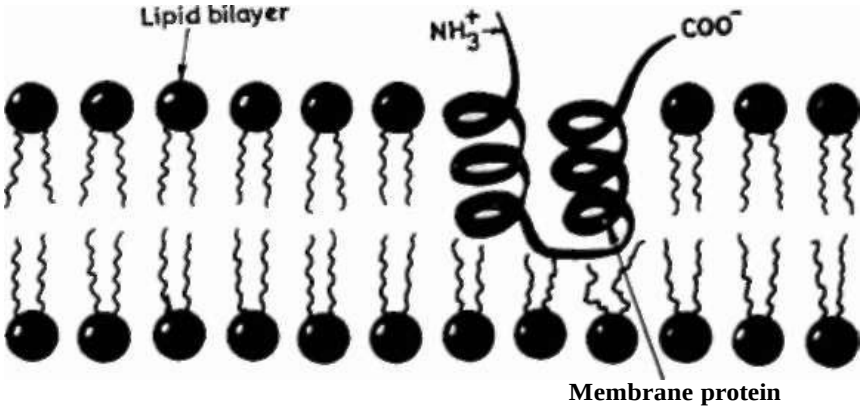


Figure 3.14 The structure of a lipid bilayer composed of phosphatidyl-choline and phosphatidyl-ethanolaminc and membrane protein

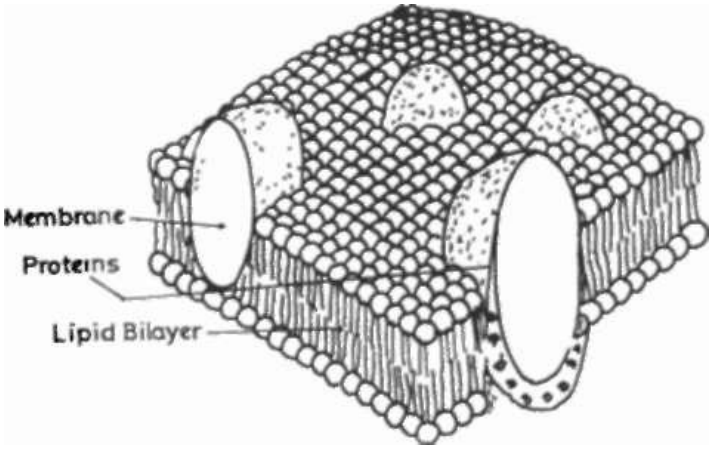
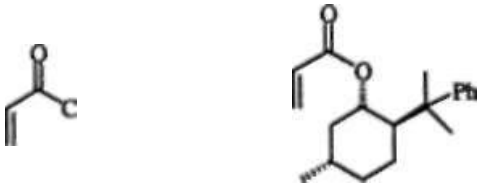


Figure 3.15 Cell membrane with integral proteins

particular nutrients or ions in the cell. Receptors on the plasma membrane sense extracellular signals, converting them into molecular changes within the cell. The energy transducers in mitochondria and chloroplasts are membrane bound. Proteins and phospholipids account for almost all of the mass of biological membranes (Table 3.4); the small amount of carbohydrate is also present in the form of glycoproteins or glycolipids.

3.12 LARGE RING LACTONES

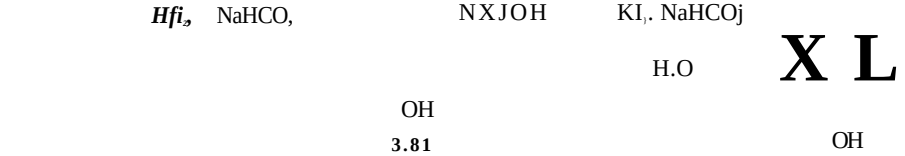
Lactones containing 15-17 ring carbon atoms are used in perfumery. These include ambrettolide, 3.24, a perfume of oils from musk seeds and a lactone exaltolide 3.25 from *Angelica.* They are synthesized through oxidation of cyclic ketones with peroxomono-sulphutic acid (H_2SO_5).

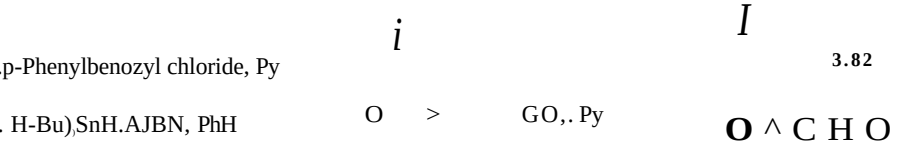


3.75

3.77

(89 % endoadduct)





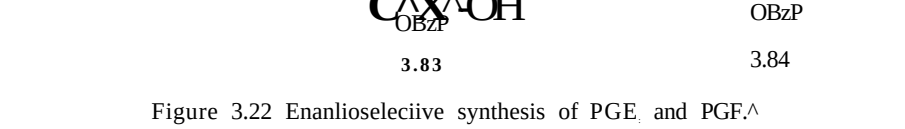
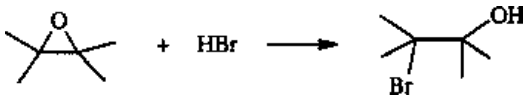


Figure 3.22 Enanlioseleciive synthesis of PGE, and PGF.^

vii) Epoxide and ether linkage

Epoxide or ether linkages are cleaved by the addition of hydrogen bromide or hydroiodic acid.

R-O-R, + HBr———^ ROH + IfBr



viii) Nature of nitrogen

In the majority of alkaloids nitrogen atom(s) is present in heterocyclic ring therefore it can be secondary or tertiary.

The acetylation or benzoylation can distinguish tertiary amine from secondary amine, the former being inert whereas the latter gives acetate or benzoate derivative. This distinction can also be done by treatment with **HNO2.** In this test secondary amine gives N-nitroso derivative (Figure 4.2).

Secondary amine

>N-H + **HNO2** -* >N-NO + HjO
>NH + CH,I -> >N-Me + HI

Tertiary amine

>N + CH,I -* >N* -Me I
>N + H_2Oj -» >N'-0 + $H_2$0

N-Me thy I group

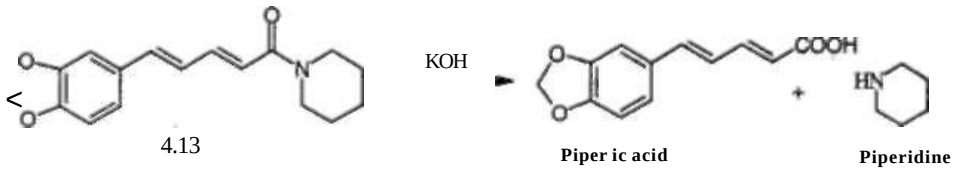
$$>\text{N-Me} + \text{CaO} \xrightarrow{A} \text{CH}_3\text{NH}_2$$

$$>\text{N-Me} + \text{HI} \xrightarrow{A} \text{N-H} + \text{Mel} \xrightarrow{\text{AgNOj}} \text{Agl (Estimated gravimetrically)}$$

Figure 4.2 Detection of nature of amino group

On reaction with one mole of methyl iodide, secondary amine gives N-methyl derivative whereas the tertiary amine yields crystalline quaternary salt. Oxidation with 30% hydrogen peroxide tertiary amine yields amine oxide whereas secondary amine gives amine hydroxide.

The presence of N-methyl group is often detected by distillation of amine with sodalime when methylamine is obtained. Alternatively, N-methyl group can be detected and estimated by the treatment of alkaloid with hydroiodic acid at !50-300°C and conversion of methyl iodide produced to silver iodide (Herzig-Meyer method) as mentioned for estimation of methoxy groups, (see Zeisel method, section 4.9A. iv).



4.13 **Piper ic acid** **Piperidine**

v) *Alkali fusion*

This is a very drastic method, which is often employed to break down the complex alkaloid molecule into simpler fragments, which gives information on the type of skeleton present in the alkaloid molecule. For example quinine on fusion with alkali gives methoxy-quinoline. Similarly papaverine on fusion with alkali yields an isoquinoline derivative.

vi) *Dehydrogenation*

In this method alkaloid is heated with a catalyst such as sulfur, selenium or palladium to yield dehydrogenated product, which provides clue to the skeleton of the alkaloid. Pyrolysis or zinc dust distillation produces aromatic ring systems. Thus pyrolysis of leucenol gave 3,4-dihydroxy-pyridine.

4.9.**C.** Physical Methods

Recently physical methods are used, in conjunction with chemical reactions to elucidate structure of alkaloids.

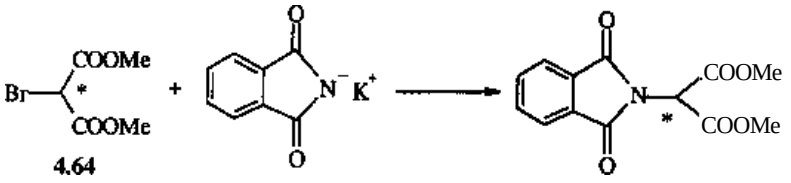
Infrared spectrum gives information about many functional groups. Ultraviolet spectra are used to indicate the nature of unsaturation or aromatic rings. NMR spectroscopy is more versatile for detecting many functional groups, the nature of protons, carbons, heterocyclic rings etc. Mass spectral fragmentation gives the information about molecular weight and degradation of the skeleton (See appendix).

Single crystal X-ray analysis has offered means for determining or confirming stereochemistry as well as distinguishing between alternate structures that appear to fit well for a particular alkaloid. Further support for the stereochemistry can be obtained by using optical rotatory dispersion or circular dichroism studies.

4.10 BENZYLISOQUINOLINE AND RELATED ALKALOIDS

These alkaloids occur in a considerable number of widely separated plant families. The structure of papaverine 4.44 is typical of the benzylisoquinoline alkaloids. This class of alkaloids also includes laudanosine 4.45, corydine 4.46, protopine 4.47, emetine 4.26, hydrastine 4.48, tubocurarine 4.11, berberine 4.49 (Figure 4.5). Opium alkaloids e.g. morphine 4.34, codeine, thebaine contain a hydrophenanthrene nucleus. It has been shown that these phenanthrene alkaloids are derived bio-synthetically from benzylisoquinoline intermediates. For this reason opium alkaloids are included in this group. Thus alkaloids in this group are representative of seven structural types of which six of them (4.45-4.49, Figure 4.5) occur in opium including phenanthrene alkaloids.

Phenylalanine with ^{u}C (radioactive isotope of carbon) label on the carbon attached to nitrogen was prepared as indicated in Figure 4.8 starting from malonic ester with $^{\cdot 4}C$ label in the a-position. Malonic ester was brominated in a-position to give a-bromomalonate 4.64, which was treated with potassium salt of phthalimide to give N-substituted phthalimide. The base catalyzed alkylation with benzyl bromide followed by the hydrolysis and decarboxylation yielded labeled phenylalanine. Labeled tyrosine was also prepared using a similar route, except that p-acetoxybenzyl bromide was employed as the alkylating agent in place of benzyl bromide



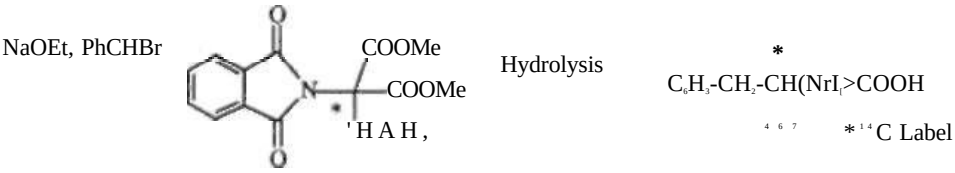


Figure 4.8 Synthesis of labeled phenylalanine

The biosynthetic pathway starting with $^{14}C2$ tyrosine leading to papaverine, morphine and corydine is shown in Figure 4.9. A key feature of this pathway is the enzymatically controlled methylation pattern and oxidative cyclization; Norlaudanosine is probably the key intermediate. Laudanosine 4.45 an optically active base isolated from opium is N-methyl-tetrahydropapaverine. Laudanosine is also related to the series of alkaloids, such as corydine 4.46, protopine 4.47, hydrastine 4.48 and berberine 4.49 etc. (Figure 4.9). Tubocurarine chloride 4.11 is an example of bisbenzylisquinoline alkaloid.

A key feature of biosynthesis of morphine is the formation of the dienone, salutaridine 4.68 that is the first intermediate with a phenanthrene nucleus. Another interesting aspect of this pathway is the biosynthetic relationship of thebaine. codeine and morphine. Stepwise demethylation of thebaine leads first to the relatively mild analgesic codeine and subsequently to the potent narcotic morphine.

Papaver bracteatum Lindley, a thebaine- producing poppy appears to lack any significant demethylation capability. Since thebaine can be converted to codeine semisynthetically, *P. bracteatum* becomes an important source of thebaine without concomitant production of morphine, which is subject to more abuse by drug addicts.

Presumably, morphine and other opium alkaloids are formed primarily in various cells of the poppy plant and excreted into the laticiferous ducts. It is known, however, that isolated latex is also capable of alkaloid biosynthesis in the presence of suitable precursors and cofactors indicating the enzymes required for biosynthesis are also present in the latex.

4.11.A. Structure Elucidation of Morphine

The presence of a hydrophenanthrcne ring system was suggested in 1881 by the isolation of phenanthrene as a product of zinc dust distillation of morphine. The location of the three oxygen functions was established by degradations leading to phenanthrene derivative 4.69 oxygenated at 3,4,5 positions (Figure 4.11).

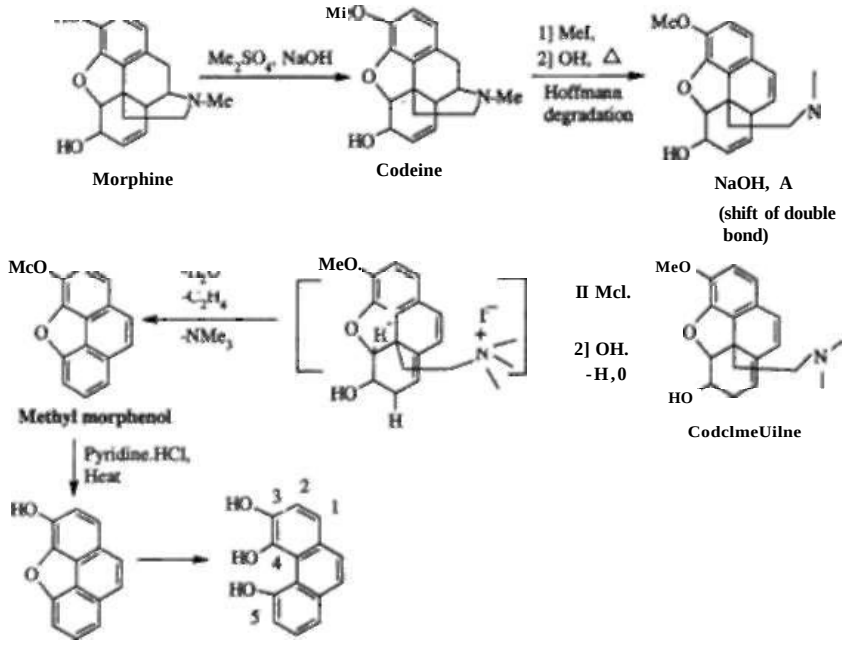


Figure 4.11 Degradations of morphine

Morphine on heating with hydrochloric acid gave apomorphine. The morphine-apomorphine rearrangement was clarified by Small (Figure 4.12).

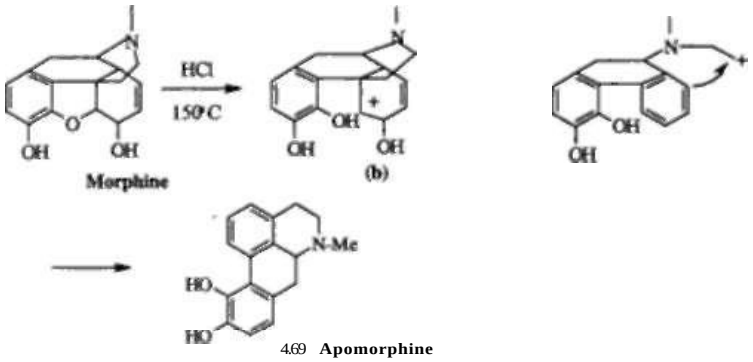


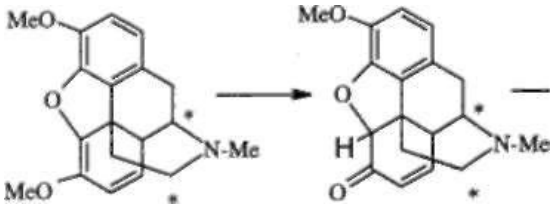
Figure 4.12 Morphine apomorphine rearrangement

anthranilic acid that contained only half of the label present in the phthalic anhydride. This experiment proved the position of label to the carbonyl group of the anhydride.

These experiments demonstrated that phenylalanine is a precursor of tyrosine and that two molecules are indeed used by the plant in the construction of the morphine skeleton. In fact, two tyrosine molecules account for all the carbon atoms of the morphine molecule except the N-methyl group. To study the source of N-methyl group, methionine labeled with ^{14}C in the S-methyl group was fed to the poppy plant. The N-methyl group of the morphine produced contained the label.

The idea that these amino acids are precursors of alkaloids has been very profitably applied to elucidate of the structures of the subsequently isolated related molecules.

These experiments led to the following biosynthetic pathway for morphine.



Thebaine Codenone

Codeine———** Morphine

4.12 QUINOLINE ALKALOIDS

Cinchona contains around 25 closely related quinoline alkaloids, of which the most important are quinine 4.29, quinidine 4.30, cinchonine and cinchonidine, the average yield being 6 to 7%, of which half is quinine in the yellow bark, whereas in the red bark cinchonidine exists in greater proportion.

Cinchona bark is dried bark of the stem or of the root of *Cinchona succirubra* Pavon et Klotzsch or of *C. ledgeriana* Howard or of C. *calisaya* Weddell, their hybrid *Remjia pedunculata* (Fam. Rubiaceae).

Cinchona and its alkaloids have been used in the treatment of malaria for many years. The structure of quinine has provided lead to important synthetic antimalarial drugs such as chloroquine, mafloquine etc.

Quinidine sulfate 4.30 is a cardiac depressant and is used particularly to inhibit auricular fibrillation.

4.12.A. Quinine, $C_{20}H_{24}N_2O_2$ m.p. 177°C, $[a]_D$-169° (c = 2 in 97% alcohol)

UV: **An.,** 277, 321, 332 nm (€ 2381, 2858 and 3334 respectively).

IR (KBr): ***v^MOO*** (OH bonded), 2580 (NH+), 1615 (C = N, C = C), 1600. 1512, 1480 (C = C, Ar). 1248, 1230, 1100, 1030 (ether linkage). 860, 838, 808, 725 (trisubstituted benzene) CUT'.

'H-NMR (CDCb): 5 8.6 (lH.d, H-2), 7.67 (1H, d, H-3), 7.53 (1H, s, 5H), 6.93 (2H, d,H-7. H-8), 6.33 (2H, bs, NH, OH), 5.53, 5.07.4.50 (IH each, m, -CH = CH*), 3.63 (3H, s, OCH,), 3.20, 2.03, 1.27 (quinuclidine).

4.13 INDOLE ALKALOIDS

These alkaloids contain either indole nucleus or an oxidized, reduced or substituted equivalent of it.

Indole alkaloids predominantly occur in the plants of families Apocynaceae, Rubiaceae and Loganaceae. There are >250O different indole alkaloids with respect to their structural features. These alkaloids can be divided into two main classes. The first group comprises of simple compounds derived from simple or substituted tryptamine 4.104, examples are Harman 4.105, koenigine 4.106. (Figure 4.18)

Indole alkaloids of the second class, which are more in number, contain two structural units: tryptamine 4.104 or tryptophan and C* or Cio monoterpene moiety derived from secologanin 4.107. These alkaloids have been classified into eight different skeletal types:- carynanthin 4.108, vincosan 4.109, strychnan 4.110, aspidospermatan 4.111, vallesiachataman 4.112, eburnan 4.113, plumacran 4.114, ibogan 4.115 (Figure 4.19).

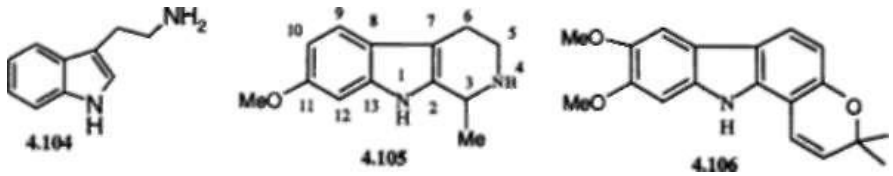


Figure 4.18 Simple indole alkaloids derived from trypatamine

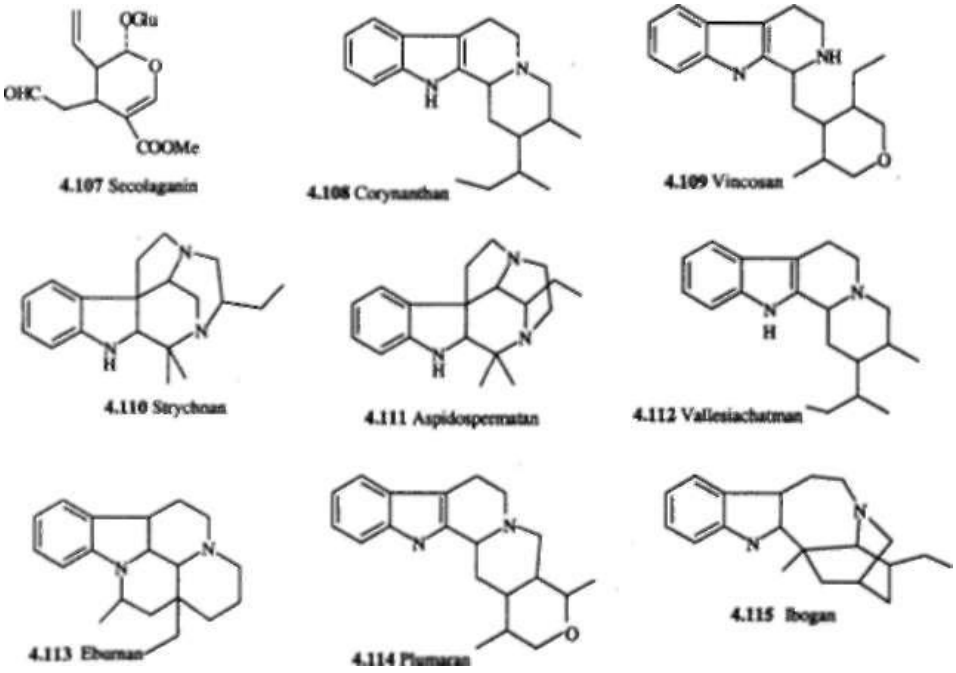


Figure 4.19 Indole alkaloids containing monoterpene fragment

Reserpic acid has one carboxyl, one hydroxyl and two methoxy groups. On oxidation with potassium permanganate reserpic acid yielded 4-methoxy-N-oxaloyl anthranilic acid 4.130 thus confirming the presence of indole nucleus and the presence of methoxy group in m-position to -NH group in reserpic acid.

Reserpic acid on fusion with KOH yields 5-hydroxyisophthalic acid 4.131. This compound indicated the presence of hydroxyl and carboxyl group in m-position in reserpic acid, which was confirmed by the fact that reserpic acid when heated with acetic anhydride yields a y-lactone 4.132.

Methylation of reserpic acid gave methyl ester 4.133. Methyl reserpate on dehydrogenation with selenium yielded a compound with molecular formula C^Hi^ as one of the principal product, which is called yobyrine 4.134 as it is also obtained through dehydrogenation of yohimbine with selenium.

•Yobyrine 4.134 on distillation with zinc dust yielded 3-ethylindole 4.135 and isoquinoline 4.136. Yobyrine on oxidation with permanganate yielded phthalic acid. Alternatively, yobyrine on oxidation with chromic acid yielded o-toluic acid.

The structure of yobyrine was confirmed through its synthesis (Figure 4.22).

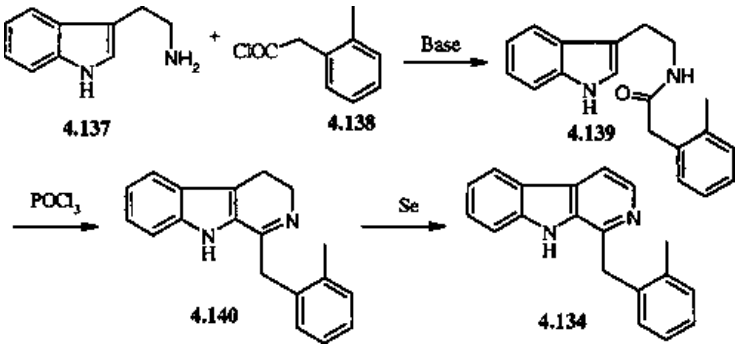


Figure 4.22 Synthesis of Yobyrine

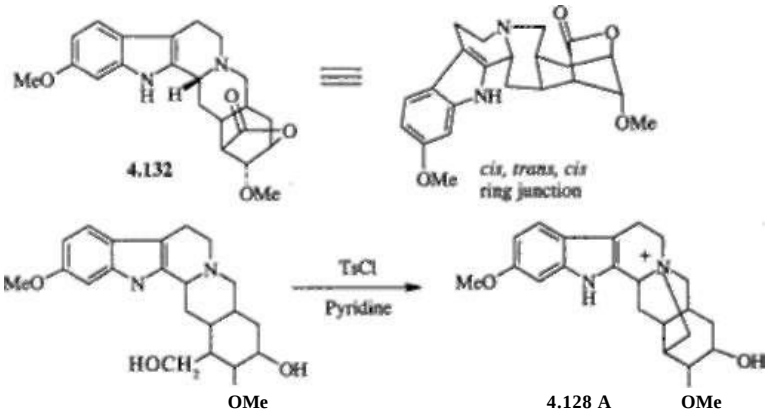


Figure 4.23 Stereochemistry of reserpine

lactam 4.157. Subsequent Bischler-Napieralski reaction of the compound by the treatment with phosphorus oxychloride gave immonium chloride 4.158 through ring closure. Reduction of the imine linkage with sodium borohydride led to compound 4.159, formed by the approach of the hydride from the least hindered side of the ring system. The compound was resolved through diastereomeric salt of optically active di-p-toluyl 1-tartaric acid, and the (-) rotating antipode was isolated in optically pure form.

Subsequently the two ester functions of 4.159 were hydrolyzed to give hydroxy acid, which on dehydration with DCC yielded y-lactone 4.160. Treatment of lactone 4.160 with pivalic acid in boiling xylene gave the thermodynamically more stable isomer 4.132 through epimerisation at the asymmetric carbon of the CD ring junction. The epimer 4.132 was subjected to methanolysis to give methyl ester, which was further esterified with 3,4,5-trimethoxybenzoyl chloride 4.161 in pyridine to give reserpine 4.9. This substance was identical in all respects with the naturally occurring alkaloid.

4.15 ERGOT ALKALOIDS

Ergot is a dried sclerotium of parasitic fungus *Claviceps purpurea* Fries (Fam. Hypocreaceae) that grows on cereal grasses, particularly rye. Ergot produces a large number of alkaloids, the most important of which are ergonovine 4.22, a mixture of ergocristine 4.162, ergocryptine 4.163 and ergocornine 4.164 (which has been marketed for many years under the name ergotoxine) and ergotamine 4.165 (Figure 4.26). All known ergot alkaloids are amides of lysergic acid. Lysergic acid contains asymmetric centers at Ca & C» and it is readily isomerised by acids to the Cg epimer, isolysergic acid 4.166, amides of which have no physiological activity. Lysergic

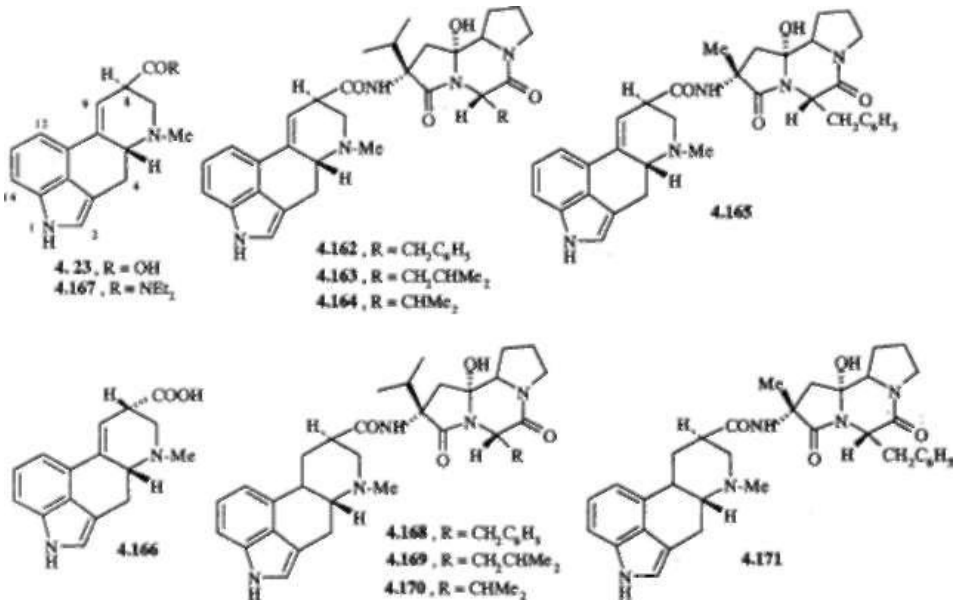


Figure 4.26 **Some examples of ergot alkaloids**

4.15.D. Synthesis of Peptide Portion of Ergotamine (Hofmann, Frey and Otto 1961)

This synthesis is given in Figure 4.29. Methylmalonic acid diethyl ester, 4.181, on bromination followed by the treatment with sodium benzyl oxide gave corresponding ether 4.182. This on partial hydrolysis followed by treatment with thionyl chloride yielded acid chloride 4.183. Treatment of dipeptide 4.184 with acid chloride 4.183 resulted in the formation of tripeptide 4.185, which on hydrogenolysis of protecting benzyl group gave cyclised tripeptide 4.186. Controlled hydrolysis of 4.186, followed by conversion to acid chloride and treatment with sodium azide gave acid azide 4.187. Curtius rearrangement of acid azide 4.187 by heating in benzyl alcohol followed by hydrogenolysis yielded the required tripeptidic molecule 4.188 which was condensed with lysergic acid chloride to yield ergotamine 4.165.

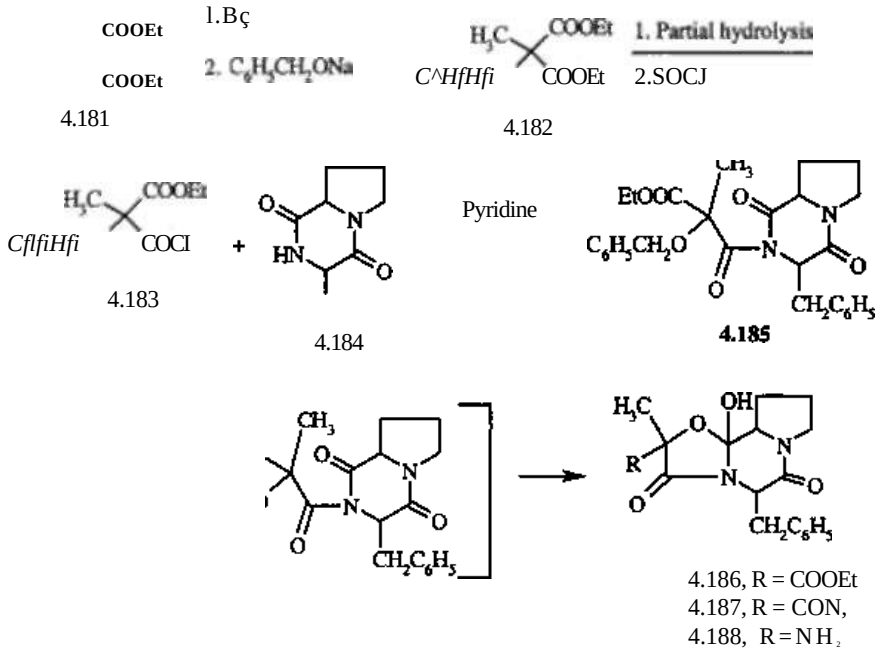
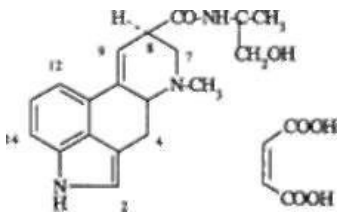
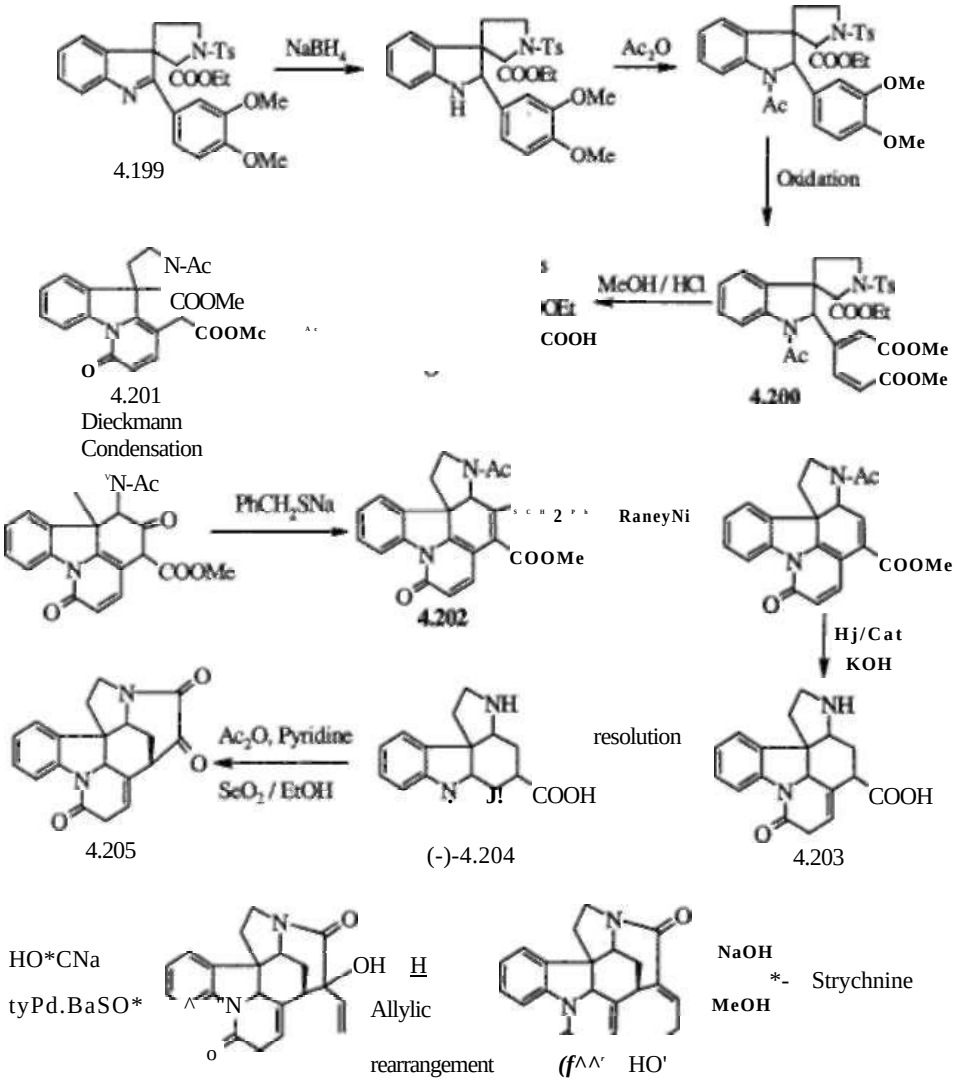


Figure 4.29 Synthesis of peptide portion of ergotamine

4.15.E. Ergonovine Maleate—$C_{19}H_{23}N_3O_2C_4H_4O_4$





Scheme 4.31 Synthesis of Strychnine

4.17 THE DITERPENE ALKALOIDS

Alkaloids based on diterpene skeleton have been isolated from several genera of the families Ranunculaceae (e.g. Aconitum, Delphinium) and Garryaceae (e.g. Garrya) and from a single species each of asteraceae *{Inula royleana)* and Rosaceae *{Spiraea japónica).* Many of these alkaloids are highly poisonous. Extracts of the roots and leaves of *Aconitum* species were

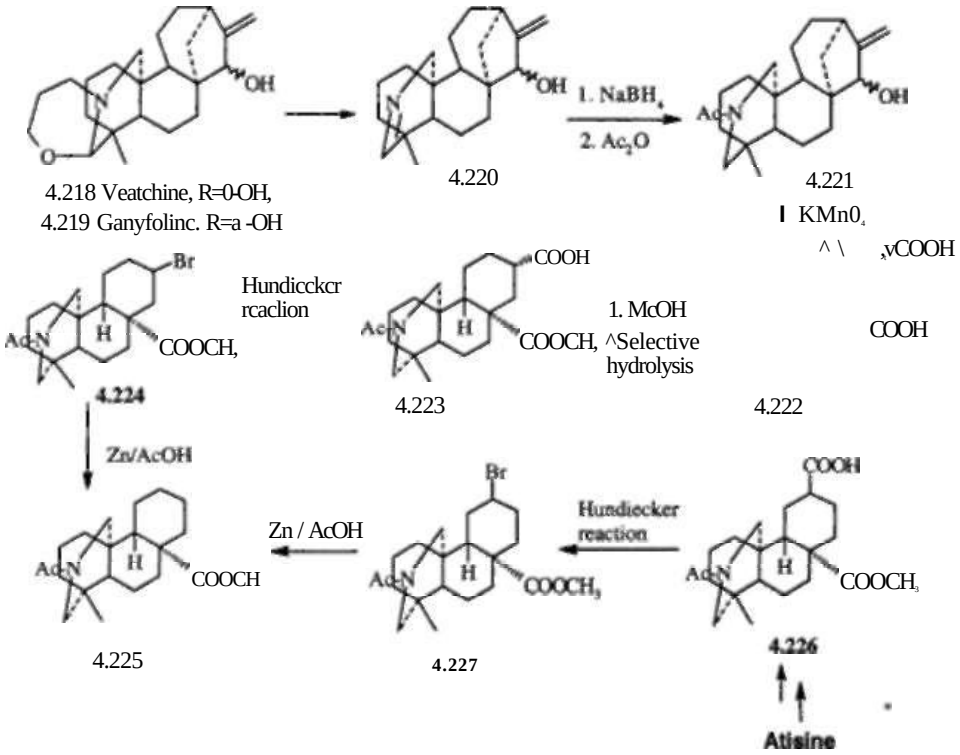


Figure 4.34 Conversion of vealchine to ester 4.225

aldehyde 4.238, which was reduced to hydrocarbon 4.239 by Wolff-Kishner reduction. This hydrocarbon was identical with (-) dihydrokaurene, which was the minor hydrogenation product of (-) kaurene 4.240. Thus absolute configuration of Cg, C* and C 1 3 positions in garryfoline and therefore C\. C and C.. positions in atisine was established.

4.17.D. Synthesis of dl-atisine

The first total synthesis of dl-atisine (overall yield 1.7%) was reported by Nagata et al. (1963). This synthesis was achieved starting from ketone 4.241 using twenty-three steps (Figure 4.37). The conjugate addition of cyanide to the ketone 4.241 gave the ?ra/i£-cyano ketone, which by a Wittig reaction with p-tolyloxymethylene-triphenyl phosphorane followed by acid hydrolysis and methylation afforded aldehyde 4.242. The latter compound was converted to the tetracyclic derivative 4.243 by a three- step sequence namely partial hydrolysis of cyanide, etherification followed by reduction of lactamol ether with $LiAlH_4$. The compound 4.243 was converted to enone through Birch reduction, N- mesylation followed by hydrolysis of methyl ether. The enone was converted to pentacyclic derivative 4.244 through the conjugate addition of cyanide, protection of carbonyl followed by the treatment with methyl lithium and cyclization of the

4.18.**B.** Isolation of Nicotine

Nicotine being liquid, the following procedure is used for its isolation. Dried and powdered leaves and stems of tobacco plant are heated with lime when nicotine distils over. The distillate is extracted with organic solvent and when the solvent is evaporated, nicotine is left as an oily liquid, which is further purified by repeated crystallization of its oxalate salt.

4.18.C. Structure of Nicotine

Both nitrogen atoms are present as tertiary amines and one bears a N-methyl group. Chromic acid oxidation of nicotine gave nicotinic acid, while distillation from lime afforded pyrrole and methylamine. Nicotine forms two different monomethiodides on treatment with one equivalent of methyl iodide, when the methiodide with pyridine ring quaternized was oxidized with ferricyanide and then with dichromate, (-) N-methylproIine was formed. These reactions led to the structure of nicotine.

4.18.D. Synthesis of Nicotine

Several total syntheses of nicotine has been accomplished. One of the syntheses mentioned in this section began with Claisen condensation of ethyl nicotinate with N-methyl-2-pyrrolidone 4.253 to form condensation product 4.254, which on acid hydrolysis underwent decarboxylation to yield the ketone 4.255. The reduction of the ketone and heating with hydroiodic acid, the amine iodide spontaneously cyclized to (±)-nicotine, which was resolved to give natural nicotine (Figure 4.38).

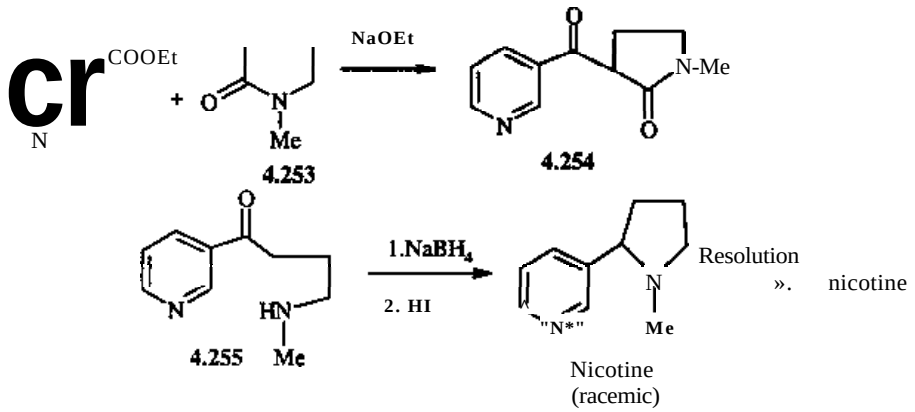


Figure 4.38 Synthesis of nicotine

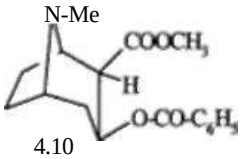
4.19 TROPANE ALKALOIDS

Tropane alkaloids occur in *Atropa belladonna. Datura stramonium* and many other solanaceous species. Atropine 4.5, Cocaine 4.10 and scopolamine 4.256 are the prominent members of this group (Figure 4.39). Tropane bases also occur in Erythroxylaceae, Convolvulaceae, Dioscoreaceae, Rhizophoraceae, Cruciferae and Euphorbiaceae families. Atropine has anticholin-

Mackenzie and Woodward synthesized tropic acid (Figure 4.42) from acetophenone. The reaction of hydrogen cyanide followed by hydrolysis yielded atrolactic acid, which on dehydration gave a-methylene-phenyl-acetic acid. The addition of hydrogen chloride followed by hydrolysis with aqueous potassium carbonate and acidification yielded (±) Tropic acid.

The reduction of tropinone followed by esterification of the resulting tropine with (±) atropic acid gives atropine.

4.19.C Cocaine, $C_{17}H_{21}N0_4$, m.p.98°C. $[a]^{w}_{D}$ -16° (c = 4 in $CHCI_5$), $[a]"_{D}$ -35° (50% alcohol). Hydrochloride $[al_{D}$ -72° (water)



4.10

H

Cocaine 4.10 occurs in dried leaves of *Erythroxylon cocoa* Lamarck, or of *Erythroxylon truxillense* Rusby (Fam. Erythroxylaceae). Cocoa leaves contain three basic types of alkaloids, derivatives of ecgonine, tropine and hygrine. Only the ecgonine 4.268 derivatives are of commercial importance. Coca leaves contain 0.5-2% of ester alkaloids, of which cocaine constitutes the major part. Cocaine is a local anesthetic. Usual dose, topically to mucous membrane, has 1-2% solution. When taken internally, cocaine and its hydrochloride are cerebral stimulant, however, in large doses or in continuous usage they are narcotics. Therefore the narcotic laws of the federal and slate governments control the sale of these drugs and they can be dispensed on prescription only. Cocaine, however, has served as a model for a large number of synthetic local anesthetics, which are less toxic than the natural product.

4.19.D. Structure Elucidation of Cocaine

Cocaine (Figure 4.43) when heated in water is hydrolyzed to methanol and benzoylecgonine $(C16H19NO4)$ 4.269, which on further hydrolysis on heating with barium hydroxide yields benzoic acid and ecgonine **(C9H15NO3.**4.268). Thus ecgonine is hydroxycarboxylic acid and cocaine is diester of the same. Ecgonine on oxidation with chromic acid yields tropinone (CiHuNO) through decarboxylation of unstable p-keto acid formed. Tropinone on further oxidation yields tropinic acid (CgHuNOj) and Ecgoninic acid $(C7H11NO3)$ 4.270. These reactions led to the structure of cocaine. The structure of cocaine has been confirmed by synthesis (Willstatter et al. 1901) starting from tropine.

Cocaine contains four asymmetric carbons; therefore 2* = 16 isomers are possible. Since, however, only the *cis* fusion of the nitrogen bridge is possible, Ci and **C5,** therefore, have only one *cis* configuration, thus only eight optically active isomers are possible. Three pairs of enantiomers have been prepared synthetically. Ecgoninic acid 4.270 has been synthesized from L-(+)-glutamic acid 4.271 (Figure 4.44). Thus, the absolute configuration of L-(-) cocaine was supported.

Fodder et al (1953,1954) and Findlay (1953,1954) have established the conformations of ecgonine and y-ecgonine and the corresponding cocaines.

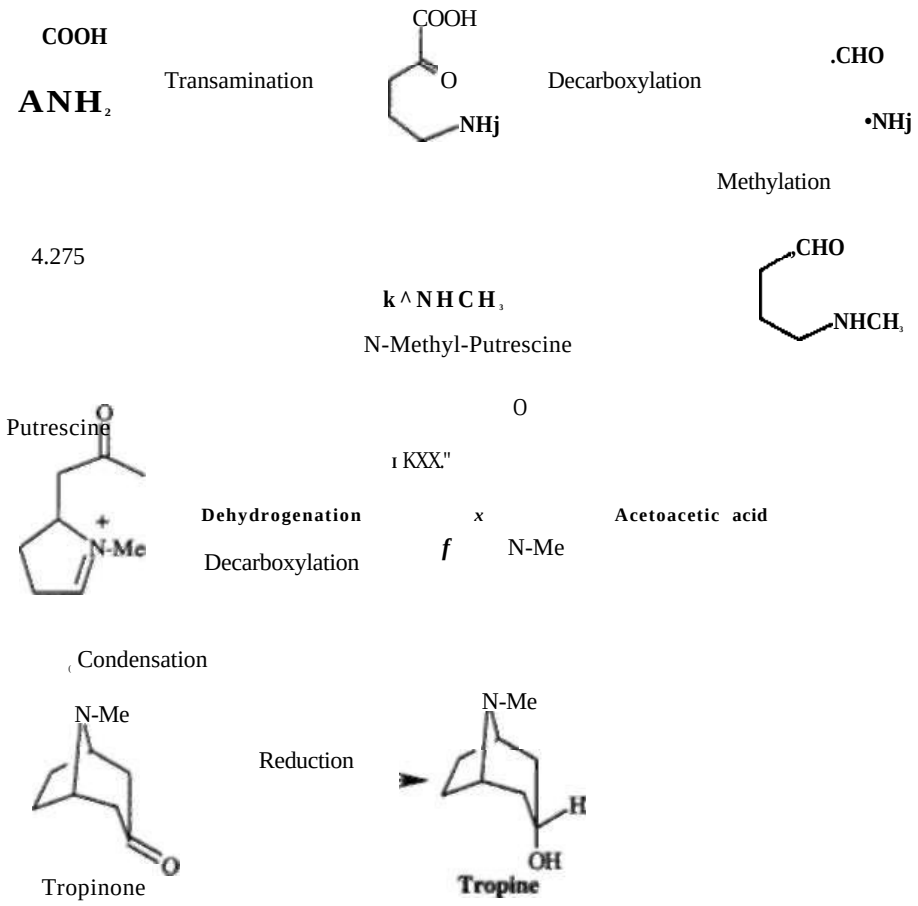


Figure 4.48 **Possible biogenetic routes for the tropane nucleus**

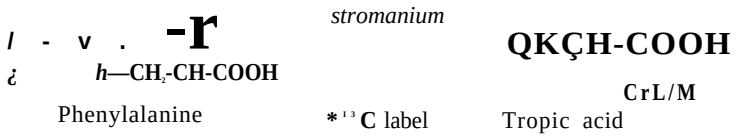
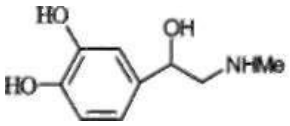


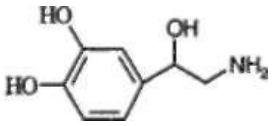
Figure 4.49 **Demonstrated incorporation of phenylalanine into the tropic acid**

4.21 ADRENALINE (EPINEPHRINE), C ^ N O , , m.p.211°C, [a]$_D$-53.5°

The adrenal medulla secretes two hormones adrenaline 4.280 and noradrenaline 4.281 (normally in a ratio of 17:3). Adrenaline is l-[3,4-dihydroxy-phenyI]-2-memylamino-ethyl alcohol. Dextro-rotatory epinephrine is almost completely inactive and racemic mixture has about half the activity of natural (-) epinephrine.



4.280



4.281

Norepinephrine is a sympathetic stimulant having predominantly a-receptor adrenergic activity. It is a strong peripheral vasoconstrictor and is especially useful in restoration of blood pressure in acute hypotensive situations. Usually it is administered by intravenous infusion of bitartrate salt. Bronchodilation by adrenaline resulting from its beta-receptor adrenergic activity is particularly useful in acute asthmatic attacks. Epinephrine is incorporated into a variety of pharmaceutical formulations for therapeutic utilization, for example aqueous solutions for topical application, inhalation, and parenteral administration, ophthalmic solution etc. Adrenaline when mixed with local anesthetics is used in dentistry and surgery to reduce bleeding.

4.21.A. Extraction of Adrenaline

The adrenal gland is minced and extracted with dilute acid and heated to coagulate proteins. After separation of the insoluble proteins by filtration, the filtrate is concentrated and treated with alcohol to precipitate the remaining biopolymers and impurities, which are separated by filtration. The resulting filtrate is concentrated in vacuum to remove alcohol and the alkaloid is precipitated from the aqueous solution by the addition of ammonia. The precipitate on filtration is further purified by repeated crystallization of oxalate salt.

4.21.B. Structure Elucidation of Adrenaline

Adrenaline is soluble in sodium hydroxide and is reprecipitated by carbon dioxide indicating the presence of phenolic hydroxy group. With ferric chloride adrenaline gives a green colouration, which indicates that adrenaline is a catechol derivative. Adrenaline on Oppenauer oxidation gave a ketone indicating the presence of secondary hydroxyl group. The presence of one aliphatic hydroxyl and two phenolic hydroxyl groups was confirmed by the fact that adrenaline, when treated with benzene sulphonyl chloride yields tri-benzene-sulphonyl derivative (Figure 4.53). The latter compound on Oppenauer oxidation yielded the corresponding keto-derivative.

Adrenaline on methylation followed by fusion with potassium hydroxide gives veratric acid and trimethyl-amine. These observations supported structure 4.280 for adrenaline.

The structure was further confirmed by total synthesis.

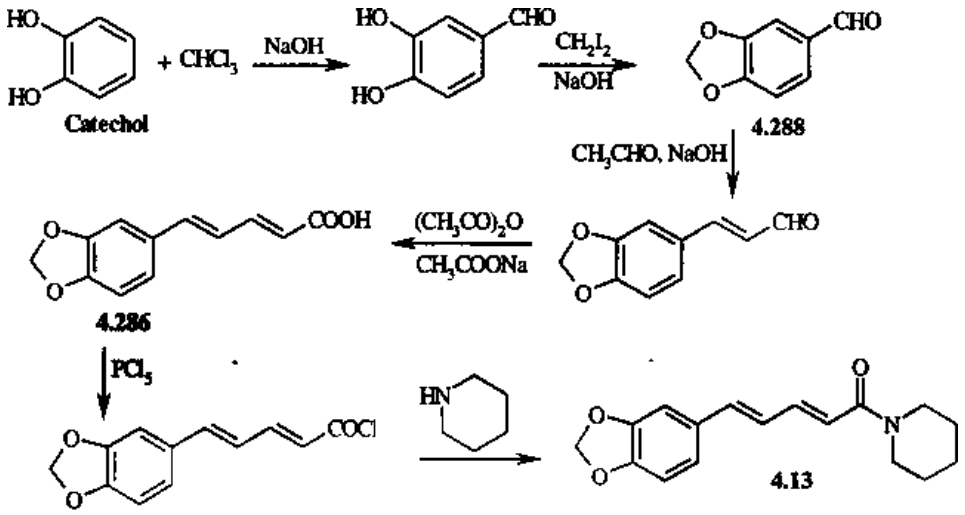
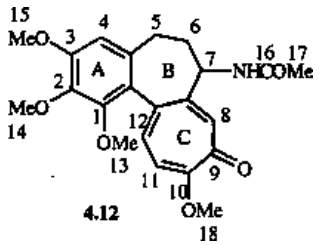


Figure 4.57 Synthesis of piperine

4.23 COLCHICINE, C^H^N, m.p. I57°C, [a]$_D^{27}$-121° (CHC1,)



UV: λ_M 243,351.nm.

IR: 1680, 1589, **1566,** 1248 cm'".

•H NMR (CDCls): 6 7.67 (s, H-8). 7.4 (d. J = 11Hz, H-12), 6.93 (d, J = 11Hz, H-l 1). 6.57 (s, H-4).

^{13}C NMR (CDCh): 5 135-6 (C-12a), 130.7 (C-12). 112.3 (C-ll), 163.8 (C-10). 178.4 (C-9), 134.7 (C-8), 151.2 (C-7a), 51.7 (C-7), 36.0 (C-6), 29.4 (C-5), 134.4 (C-4a), 108.0 (C-4), 153.2 (C-3), 141.1 (C-2), 150.7 (C-l), 125.7 (C-la), 55.9 (C-18), 22.4 (C-17), 168.9 (C-16), 56.0 (C-15), 60.7 (C-I4) and 60.9 (C-13).

MS: m/z 399(M*), 37I(M'-CO), 312 (M$^+$-CH$_3$-C = NH), 297 (M* 312-CHj), 281 (M* 312-OCH$_3$).

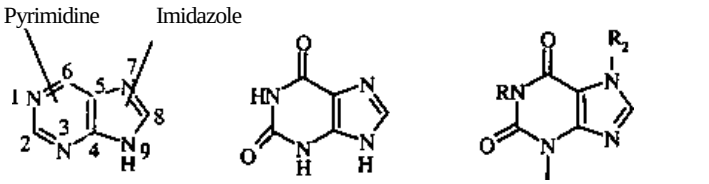


Figure 4.60 Purine bases

2,6-dioxypurine (Xanthine 4.304). Caffeine 4.305 is 1,3,7-trimethylxanthine, theophylline 4.306 is 1,3-dimethylxanthine and theobromine 4.307 is 3,7-dimethyIxanthine. (Figure 4.60).

4.24.A. Caffeine, $C_8H_{10}N_4O_2$ m.p. 235-237°C

UV: X_{max},278nm (loge 4.03).

IR(KBr): aw 3034, 2950, 1700 (C = O stretch), 1660 (C = N stretch), 1604, 1548, 1440 (aromatic stretch pyrimidine moiety), 1230, 1197,1020 (-C-N stretch) and 740 (C-H deformation) cm^{-1}.

'H NMR: 5 3.53 (N_1Me),. 3.33 (N_3Me), 3.98 (N_7Me) and 7.54 (H-8).

"C NMR: 8 151.69 (s. C-2). 148.73 (s, C-4), 107.55 (s, C-5), 155.35 (s, C-6), 141.53 (d, C-8), 29.70 (q, N, Me), 27.87 (q, N,-Me) and 33.54 (q, N_7-Me).

MS: m/z 194 (M* 100%, base peak), 165(M*-CO), 109 (**C5H7NJ**, 66%), 82 (37%), 67(54%) and 55 (80%).

The dried cotyledons of *Cola nitida* or of other species of cola contain 3.5% of caffeine. *Cola nitida* is a large tree indigenous to West Africa. It is cultivated in East Africa, Sri Lanka, Indonesia, Brazil and Jamaica. Cola possesses the central stimulating action of caffeine. It is an ingredient in several carbonated beverages.

Caffeine is present in dried ripe seed of *Cojfea arabia* Linne or *Coffea liberica* Hiern (Fam. Rubiaceae). The seeds are roasted until they acquire a dark brown colour and the characteristic aroma is developed. Coffee seeds contain from 1 to 2% of caffeine, together with tannin, glucose, fats and proteins.

4.24.B. Isolation of Caffeine

Caffeine is manufactured from tea dust or damaged tea leaves. The dried and powdered leaves are boiled with water and filtered hot. The filtrate is mixed with basic lead acetate to precipitate out tannins and albuminoids. The excess of lead in the filtrate is removed as lead sulfate by adding sulphuric acid. The precipitate is removed by filtration and the filtrate is decolourized by adding animal charcoal. Caffeine is separated from filtrate by extraction with chloroform. The chloroform layer is evaporated to dryness and the residue on recrystallization from water yields caffeine.

Caffeine occurs as a white powder or as needles and has bitter taste. Caffeine may be sublimed without decomposition on heating.