CHAPTER II

HISTORICAL

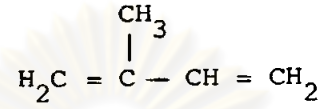
1. STEROIDS

1.1 Chemistry of Steroids

Steroids belong to a large group of compounds known as terpenoids or isoprenoids (39,40). Terpenes are formed by the polymerization of isoprene units 2 and steroids are triterpenes or triterpenoids. The term triterpene refers to a group of natural products containing 30 carbon atoms which are derived from six isoprene units. However, this definition is by no means rigid, and compounds with fewer than 30 carbon atoms, if derived from six isoprenes are included in the steroids. Discussion on isoprenoids will deal only with those triterpenes that have the steroidal ring structure of cyclopentanoperhydrophenanthrene (39-41) and the number system used for steroids is shown in 3.

Most steroids have an oxygen atom attached to C-3 and the ring junction of C and D ring is *trans* except cardiac aglycones and toad poison which have *cis* configuration. The ring-junction of A and B ring is *trans* in some steroid and *cis* in others but B and C ring is *trans* in all naturally occuring steroids. In all natural products the substituent at C-10 to C-9 positions are on opposite sides of the molecule (*trans*) and at C-8 to C-14 positions (except cardiac aglycones and toad poisons) are also *trans*. So the stereo--

chemistry of the steroids is a series of *trans* relationships between substituents at adjacent ring junctions along the 'backbone' of the molecule C-5-10-9-8-14-13 which gives an almost flat structure 4 (41).



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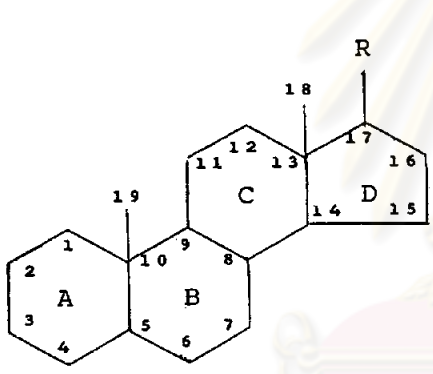

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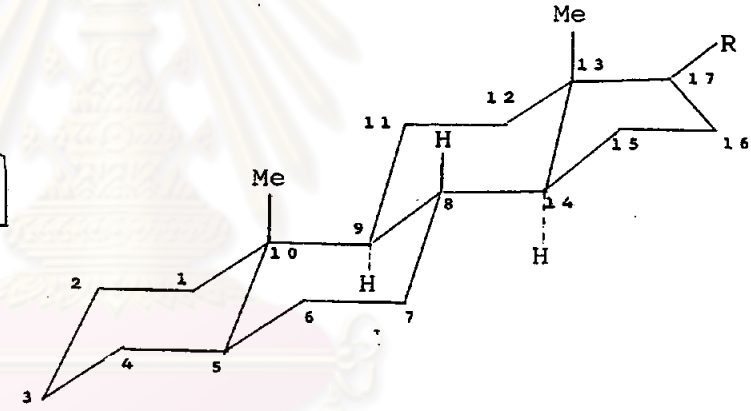

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1.2 Classification of Steroids

Steroids which are based on the cyclopentanoperhydrophenanthrene ring system, can be divided into at least five groups of compounds : sterols, steroid hormones, steroid saponins, steroid alkaloids and cardiac glycosides.

1.2.1 Sterols

The sterols are 3-monohydroxysteroids of the C_{27}, C_{28} and C_{29} series : all the naturally occuring compounds have a

3β-hydroxy group, and nearly all have one or more double bonds. The commonest position is at 5; next come 22 and 7 (41). Ring A/B, B/C and C/D are *trans* fused. The hydrocarbon skeleton has a few angular methyl group at C-10 and C-13 generally an 8 to 10 carbon chain at C-17. The hydroxy, the two methyl groups and the C-17 side chain all have the β-orientation (39).

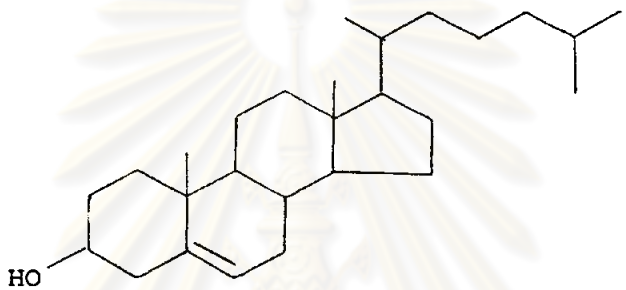
The sterols are secondary alcohols which are crystalline solid at room temperature so the name sterol is derived from the Greek *stereos* (solid) plus -ol (39).

The number of sterols encountered in nature is quite large so the classification has three groups and takes according to the number of carbon atoms in molecule :

C_{27} sterols

Cholesterol 5, once believed to be the typical animal sterol, has recently been found to be rather widely distributed among plants. So far, cholesterol has been found in the red algae, in blue-green algae, in bacteria, in fungi and in the pollens of many plants; the date palm, cotton wood, sunflower and mustard, in the spores of the fern *Polystichum filix-mas*, in the seeds of many plants; soybean, peanut, oat, apple, avogado and oil palm, in the bark of pine tree and *Erythrina suberosa* Roxb. and in the roots of the cactus *Wilcoxia viperina* Britton & Rose (40). Cholesterol is the key intermediate from which animals make all the other steroids; such as molting hormones, vitamin D_3, bile acids, progesterone, adrenocortical hormones, androgenic and estrogenic hormones. It has recently shown that cholesterol may occupy a similar key position in the biogenesis

of most of the different types of steroids found in plants : other sterols, such as molting hormones and related products; C_{27} sapogenins and alkaloids; various pregnene derivatives, including progesterone and the C_{21} alkaloids; cardiac aglycones; and probably also the sex hormones (40).



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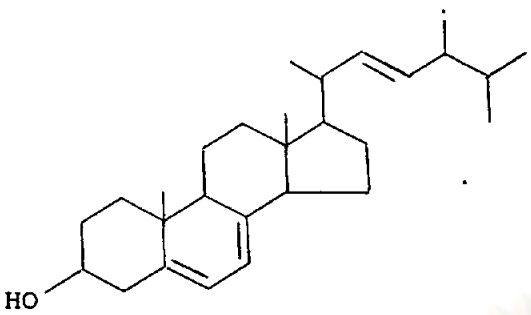
C_{28} sterols

The C_{28} sterols derived from the C_{27} sterols and from either the carboxyl or methyl carbon of acetate for C-28 (42). The most important C_{28} sterol is ergosterol 6 which was first isolated from ergot (42,43) but also occurs in yeast and in most fungi, but more recently it was also been discovered in higher plants. Irradiation with ultraviolet light converts ergosterol to vitamin D_2, so it is quite possible to be found vitamin D_2 in plant. Fecosterol 7, fungisterol 8 and 5-dihydroergosterol 9 are biogenetically related to ergosterol and also occur in yeasts and other fungi (42).



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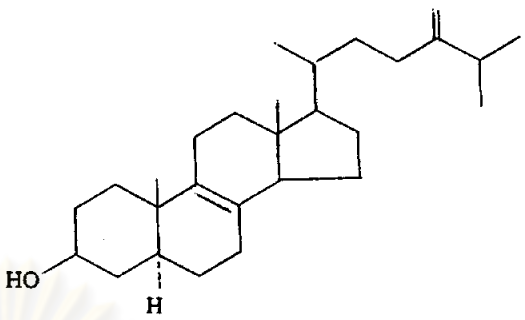

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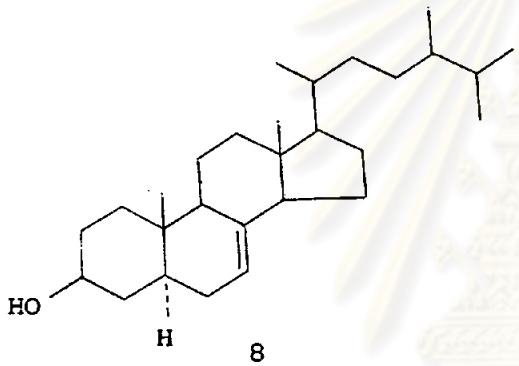

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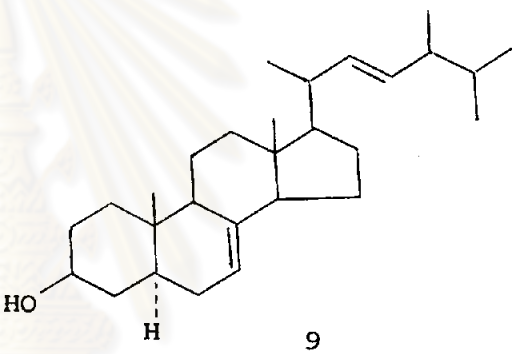

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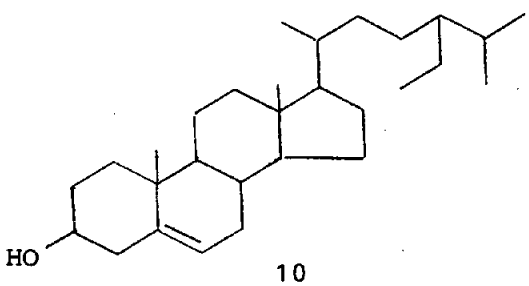
C_{29} sterols

The most widely distributed sterols in higher plants are sitosterol 10, stigmasterol 11 and campesterol 12 which called 'phytosterols'. These common sterols occur both free and as simple glycosides (42,44). The sterol most often isolated from plant is sitosterol but stigmasterol and campesterol are also quite common (39).



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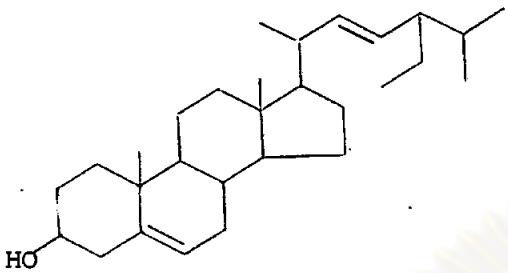

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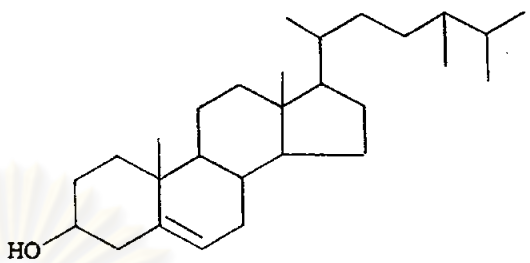

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The trivial name of the sitosterols 9 (Greek *sitos*, grain) antedates precise knowledge of their structure and the Greek letters are, of course, not related to the stereochemistry. α-Sitosterol was shown to be a mixture. β-Sitosterol happens to be 24β-ethyl Δ^5 cholesten-3-β-ol, or named with reference to the parent hydrocarbon of stigmasterol, Δ^5-stigmasten-3β-ol. It is probably the most common sterol in plants. The main commercial sources of β-sitosterol are sugar cane wax, teel oil and cottonseed oil. C_{28} and C_{29} sterols are only absorbed to a minor extent by most animals and are usually excreted unchanged. β-Sitosterol is hydrogenated to 24β-ethyl-coprostan-3β-ol in their intestines. γ-Sitosterol is the principal sterol of soybean oil, but it also occurs in many other vegetable oils. It is one of the most widely distributed sterols in marine vertebrates, and was called clionasterol before its identity with γ-sitosterol was recognized. Toads also secrete γ-sitosterol through their skin glands (42).

Stigmasterol 11 was first isolated from the calabar bean (*Physostigma venenosum* Balf.). The commercial source is the soybean,

but sugarcane wax also contains substantial amounts of this sterol. Its abundance and the double bonds at C-22 and C-5 make stigmasterol to be an important starting material for the synthesis of progesterone and other steroid hormones. The 24-epimer of stigmasterol occurs in various marine invertebrates (42). Recently, 5-dihydrostigmasterol has been isolated from a slime mold, *Dictyostelium discoideum*. This substance has acrasin activity because it causes the amoeboid cell of the mold to aggregate in a multicellular unit, which undergoes further differentiation (42).

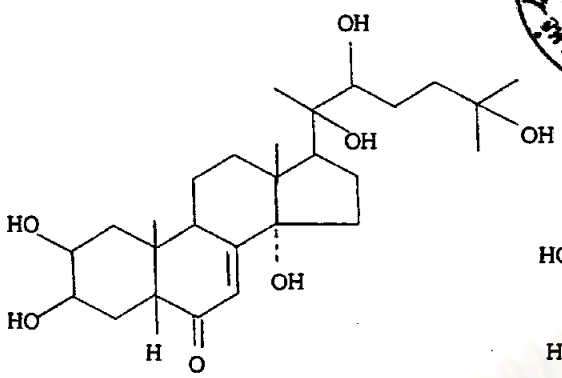
1.2.2 Steroid Hormones

The number of steroid hormones found in nature are quite large so. They are classified into two groups, according to the number of carbon, such as steroid hormones with 27 to 29 carbon atoms and with 18 to 21 carbon atoms.

C_{27} to C_{29} Steroid Hormones

This group is the most insect-molting hormone. Compounds of this type were first isolated from insects, but the variety and amounts of insect-molting hormones in plant are far greater than insects. The molting hormones interact directly with the chromosomes of insects to release genetic information. This result is in metamorphosis.

Ecdysterone 13, Makisterone A 14, Makisterone C 15 and Cyasterone 16 are representatives of this group. They are characterized by a Δ^7-6-keto group and a 14α-hydroxyl group. Other hydroxyl group may be attached to various positions (40).



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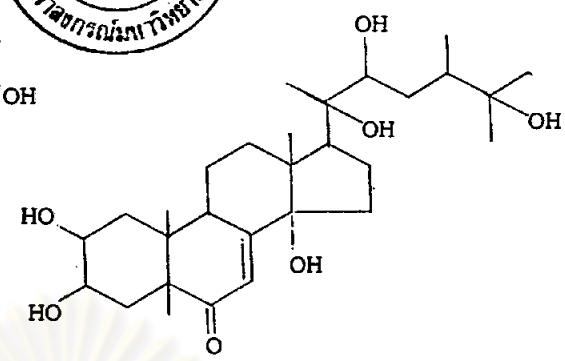

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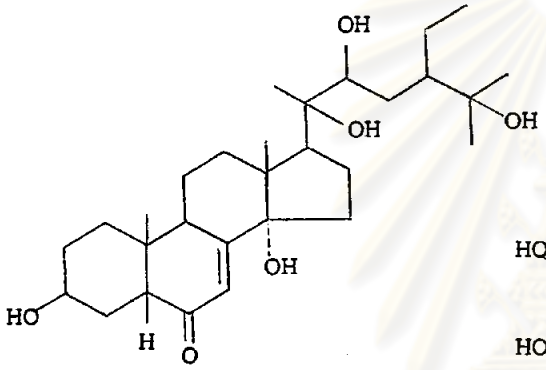

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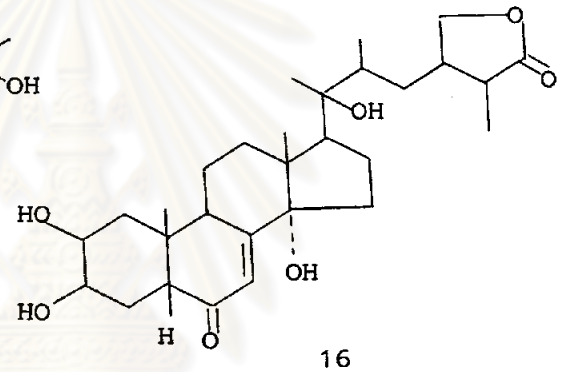

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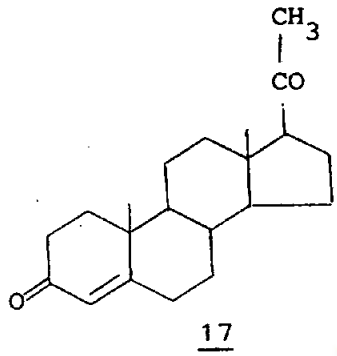
C_{18} to C_{21} Steroid Hormones

In the biosynthesis of sex hermone in higher plants, sitosterol is converted to progesterone (C_{21}) 17 which further convert to the adrenocortical hormone, deoxycorticosterone. Allo-pregnenolone (C_{21}) 18 is a degradation product of tomatidine or neotigogenin, which were alkaloid and sapogenin in the plants. Microorganisms are also known to be capable of converting sapogenin to C_{21} and C_{19} steroids. Thus, the degradation of sapogenins and alkaloids in plants apparently lead to the intermediates on which the manufacture of most semisynthetic steroid pharmaceuticals is based (45).



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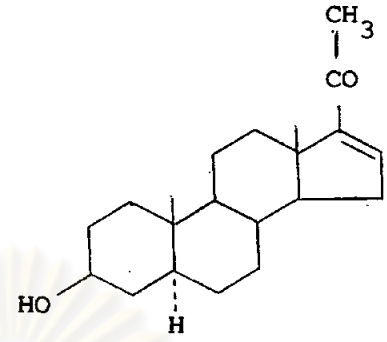

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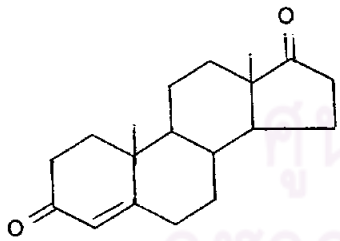
In animals, the C_{19} and C_{18} steroids are formed from C_{21} steroids, this reaction sequence has also been observed in fungi but the biogenesis of C_{19} and C_{18} steroids in higher plants is still unexplored. Androstenedione 19, testosterone 20 and rubrosterone 21 show C_{19} steroids identified in plants. Rubrosterone and testosterone stimulate protein synthesis in animals and it is not impossible that they have similar effects on plant cells (40,45).



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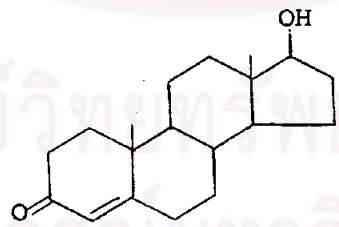

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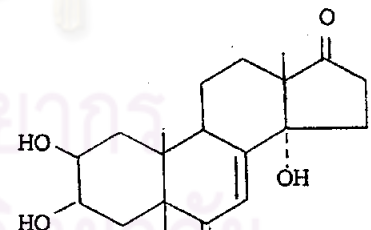

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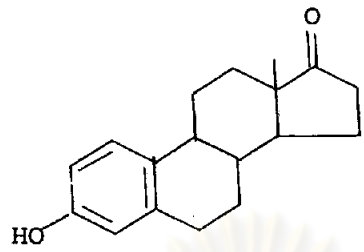
The C_{18} steroid, estrone 22, has now found in the seed of palm, pomegranate and apple trees and in the pollen of the date palm. The significance of these findings remain to be explained (40,45).



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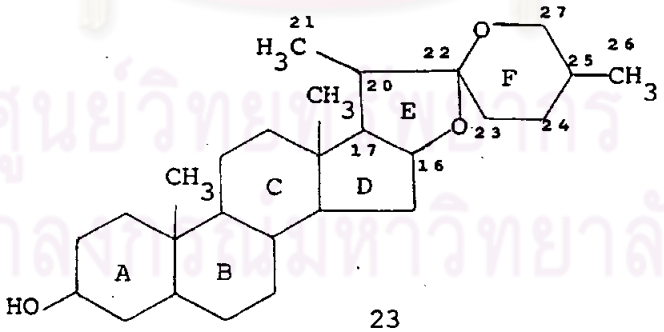
1.2.3 Steroid Saponins

The saponins (Latin *Sapo*, soap) are generally glycoside. They are powerful surface active agents which cause foaming when shaken with water and in low concentration often produce hemolysis of red blood cells. In very dilute solution, they are quite toxic to fish, and plants containing them have been used as fish poisons for hundreds of years. They have also been implicated as a contributing cause of bloat in cattle on some forage crops. Certain saponins have become important in recent years because they may be obtained in good yields from some plants and are used as starting material for the synthesis of steroid hormones to be used in medicine. The saponins have no known function in plants but have been shown to stimulate the growth of pea embryo (46).

Two types of saponins are recognized, steroidal saponin and triterpenoid saponin. Both types are soluble in water and ethanol but insoluble in ether. Their aglycones, called sapogenins, are prepared by acid or enzymatic hydrolysis (46). In this part, the steroidal saponins were briefly reviewed but triterpenoid saponins will be reviewed in detail.

The basic structure of steroid sapogenins is shown in structure 23. They are internal ketal of 16,27-dihydroxy-22-ketosteroids with 27 carbon atoms and contain two heterocyclic rings. Ring E is a five-membered (furan) and ring F is a six-membered (pyran) oxyene heterocyclic ring. The rings are joined at C-22 in spiroketal. Conformational formula of a steroid saponin has a A/B *trans* junction, but A/B *cis*-fused sapogenins are also common. The C-3 hydroxyl group is almost invariably β-oriented. The structure formula of tigogenin 24 and sarsasaponin 25 will illustrate the presentation of conformational relations. The corresponding conformational formula shows that the 3β-hydroxyl group is equatorial in the A/B *trans* steroids, such as tigogenin 26 but axial in the A/B *cis* steroids, such as sarsasapogenin 27. The equatorial methyl group at C-25 of tigogenin is indicated by a broken line in formula 26, and the axial methyl group at C-25 of sarsasapogenin is indicated by a full line in formula 27 (40,42).



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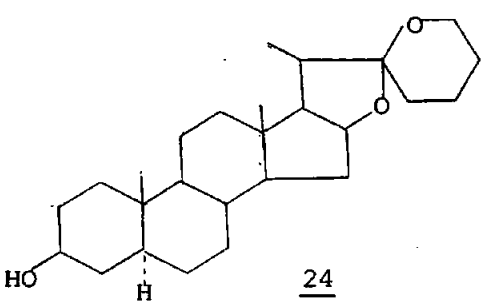

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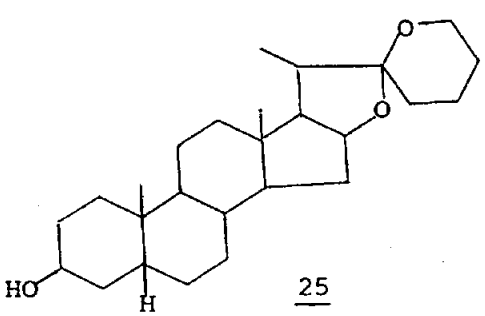

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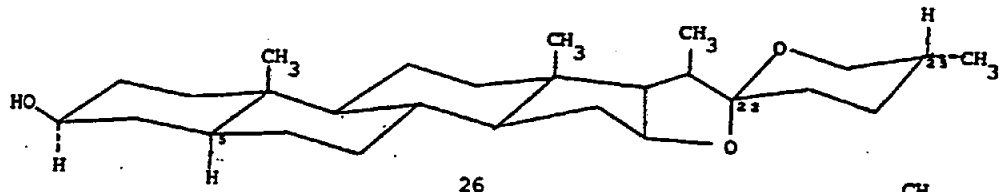

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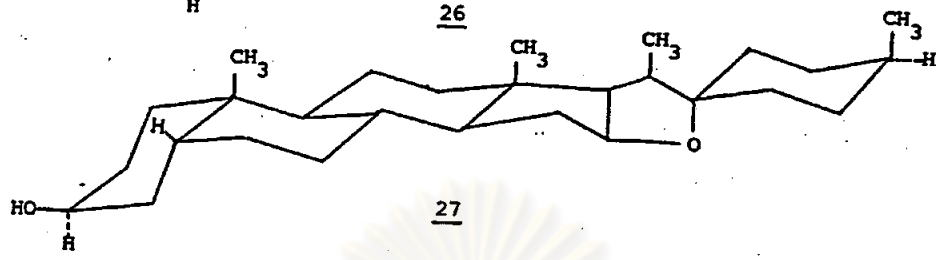

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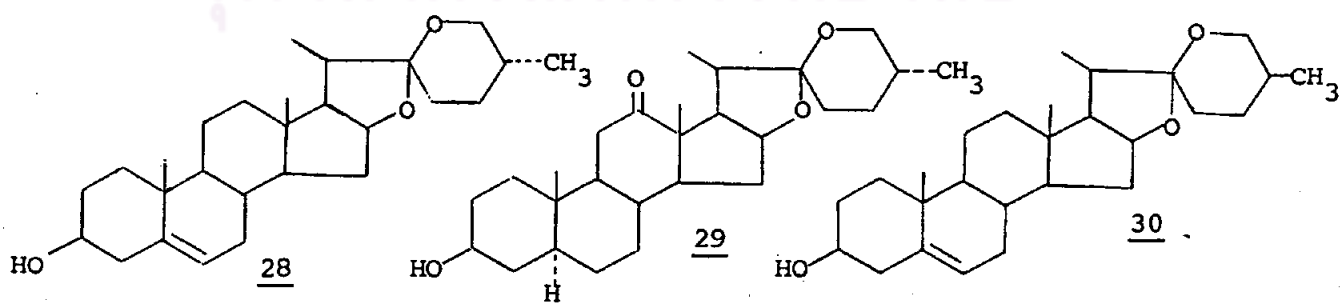
Plants synthesize steroid sapogenin from cholesterol and sitosterol by way of such intermediates in which the side chain is kept open by a sugar (40). A number of sapogenins are not used as therapeutic agents directly but they are served as useful starting materials for the chemical synthesis and the practical production of a number of steroid hormone substances which are medicinally important agents. Successful chemical techniques for high-yield conversion of sapogenins to steroid hormone substances not only resulted in phenomenal increase in availability but also dramatic decrease in the cost of these hormone as medicinal agent. Among the sapogenins which have been found to be the most useful as starting materials for chemical conversion to medicinal hormone substances are diosgenin 28, hecogenin 29 and yamogenin (as 25β epimer of diosgenin) 30 (47).



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The steroidal saponins are rather widely distributed among higher plants than animals or fungi. They occur in the Liliaceae family in *Yucca*, *Trillium* and *Smilax*; in the Amaryllidaceae family in *Agave*; in the Dioscoreaceae family in *Dioscorea*; in the Scrophulariaceae family in *Digitalis*; and in the Solanaceae in *Solanum*, *Lycopersicon* and *Cestrum* (40).

1.2.4 Steroid Alkaloids

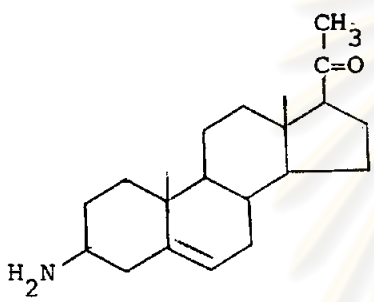
Steroid alkaloids are compounds possessing the basic or modified steroidal skeleton with nitrogen incorporated as an integral part of molecule either in the ring or in the side chain (48).

According to Sato (48), steroidal alkaloids are divided into two general types :- (a) C_{21} alkaloids and (b) C_{27} alkaloids.

C_{21} alkaloids

A great number of C_{21} alkaloids have recently been isolated from Apocynaceae and Buxaceae. The Apocynaceae alkaloids, found mainly in *Holarrhena*, *Funtumia* and *Malouetia* species are derived from pregnenolone by amination at either C-3 or C-20, or both, and by modifications, such as subsequent methylation of the amino groups or reduction of the Δ^5-double bond. The *Holarrhena* alkaloids, or sometimes referred to as the 'Kurchi' alkaloids are synthesized by direct amination of pregnenolone such as holaphylamine 31 conessine 32. The pyrrolidine ring in conessine 32 is probably derived from a precursor in which C-18 is oxygenated, such as holarrhimine 33. Conessine, the most abundant C_{21} alkamine, is

a desirable starting material for the synthesis of certain hormones, such as aldosterone. Example of alkaloids in which the Δ^5-double bond has been reduced are funtuphyllamine A 34 and its methylated analogs in *Funtumia* and the bistrimethylammonium base, malouetine 35 in *Malouetia* (40,42).



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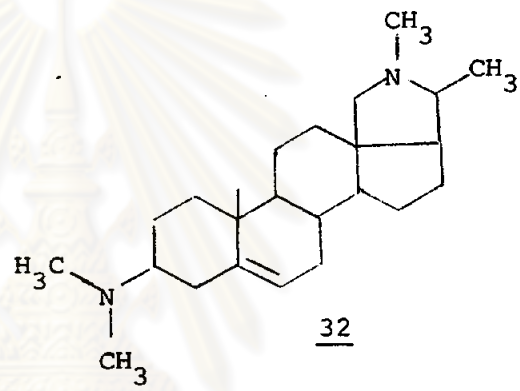

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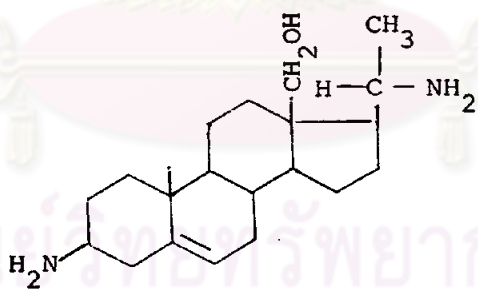

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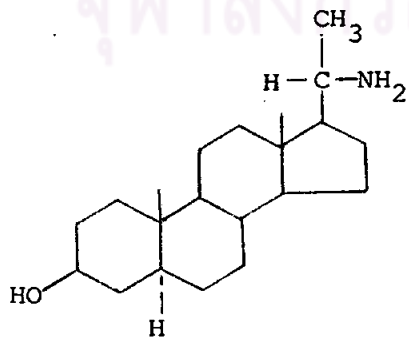

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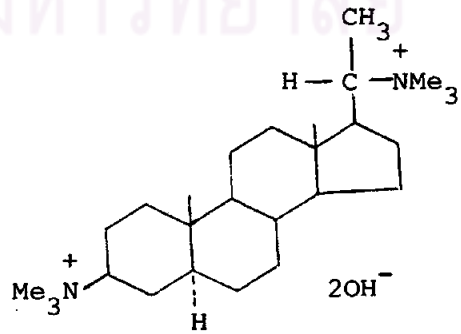

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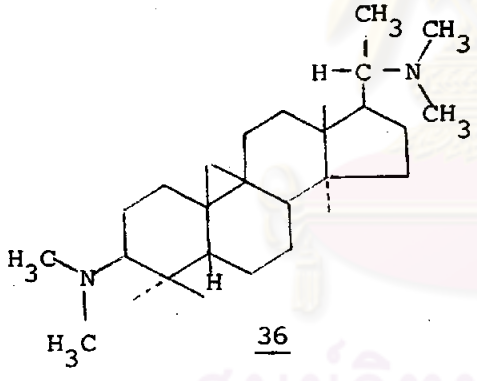
Alkaloids of the Buxaceae, in addition to the type of C_{21} alkaloids, are biogenetically related to cycloartenol and other steroid precursors. Cycloprotobuxin A 36, found in various *Buxus* species, may be regarded as prototype. Analogs with primary and secondary amino groups at C-3 and C-20 and with hydroxyl group at C-16 are known. Instead of the geminal methyl groups at C-4, there may be a methyl, methylene or hydroxymethyl group. At C-9 to C-10 in steroid skeleton of *Buxus* alkaloids have an additional cyclopropane ring, especially buxamines 37, in which the B ring is enlarged (39,40).



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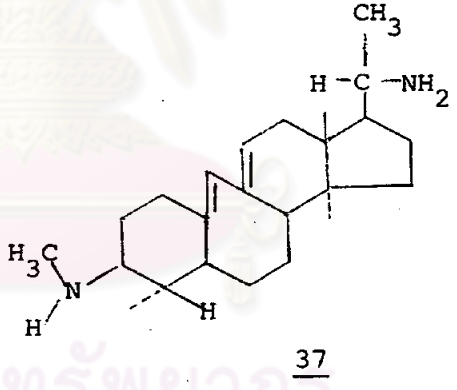


37

C_{27} alkaloids

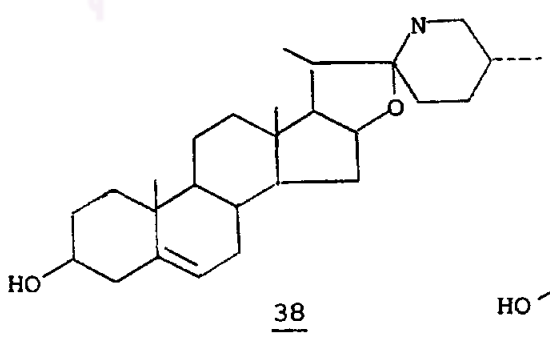
Many of these alkaloids, such as solasodine 38, are simply nitrogen analogs of the C_{27} sapogenin or 20-piperidylpregnane with steroid skeleton. In the form their glycosides, as glycoalkaloids, they are often found in the same plant and in combination with the same sugars as the analogous sapogenins. However, the distribution of C_{27} alkaloids is restricted to the genera *Veratrum* and *Fritillaria* (Liliaceae); *Solanum*, *Lycopersicon* and *Cestrum* (Solanaceae)(40). Alkaloids in *Solanum* species, conjugated with

sugars at C-3 can be divided into two groups.

The first group may be considered as nitrogen analog of the sapogenins with an NH group instead of an oxygen atom between C-22 and C-26 in ring F, such as solasodine 38 and tomatidine 39 (41). Solasodine has the same structure as diosgenin, except for the fact that NH is substituted for O in the F ring. Tomatidine is the nitrogen analog of neotigogenin. Tomatidine was first isolated in the course of an investigation of the antibiotic effect of tomato leaf extracts. It has some fungistatic and bacteriostatic effect but it is perhaps of greater interest as a potential raw material for steroid synthesis (42).

The second group of *Solanum* alkaloids have no cyclic oxygen atom but it has a condensed ring system and tertiary nitrogen, such as solanidine 39 and demissidine 40 (41).

Compounds of both groups have been obtained by partial synthesis from sapogenins (41). In animal tests, large doses of certain *Solanum* alkaloids produce parenchymatous nephritis and hemoglobinuria,followed by nervous paralysis and cardiac arrest (42).



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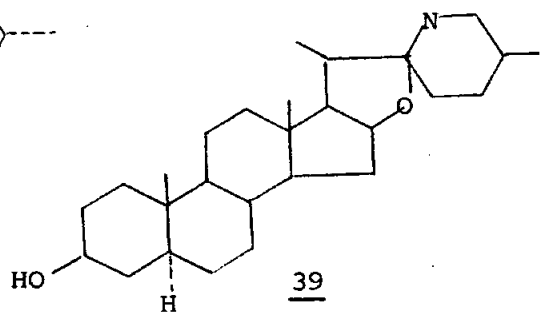

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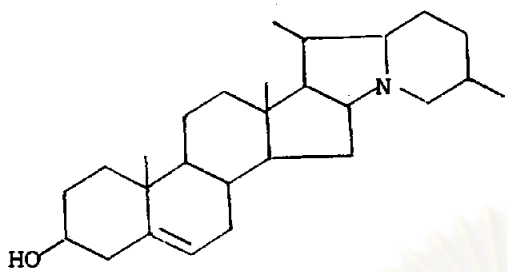

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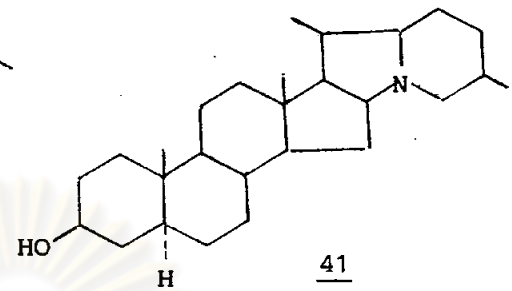
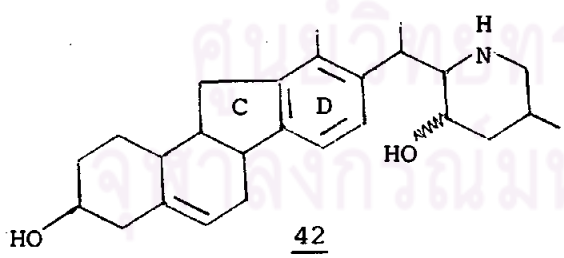


41

The alkaloids occur in *Veratrum* species are not steroids because they contain a five-membered C ring and a six-membered D ring. Veratramine 42 and jervine 43 are representatives of *Veratrum* alkaloids. Veratramine contains an aromatic D ring and a hydroxyl group at C-23, where as jervine contains a conjugated 11-keto group and a five-membered E ring, formed by the 17, 23-epoxide. *Fritillaria* alkaloids have structure similar to *Veratrum* alkaloids such as sipieimine which have been used as the Chinese medicine for a long time (41,42).



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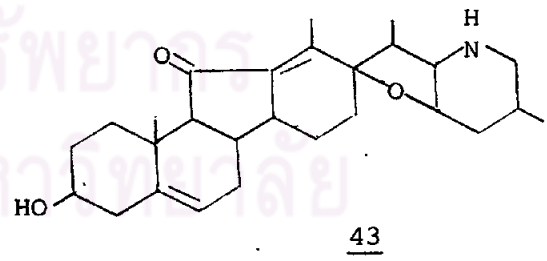


43

The *Veratrum* alkaloids stimulating the sensory nerve endings, and in contact with the skin, they produce a prickling sensation. They act on the mucous membranes of the nose to induce sneezing. Taken internally, *Veratrum* alkaloids and their derivative prolong the contraction of striated muscles and act on the pulmonary

stretch receptors to produce respiratory depression. The circulatory effect are of greatest interest. The alkaloids produce bradycardia and in large doses, cardiac arrhythmias and eventually ventricular tachycardia and fibrillation. Like the cardiac glycosides, *Veratrum* alkaloids increase the work performance of the failing heart. The hypotensive effect is mediated through the nervous system (reflex inhibition of vasomotor centers) and it is used in the treatment of essential and malignant hypertension. Kerosene solution of *Veratrum* alkaloids have also found some use as insecticides (42).

1.2.5 Cardiac Glycosides

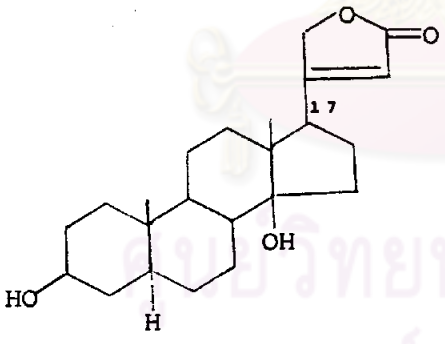
A number of cardiac glycosides with steroidal aglycone occur in various species of genera in several plant families, such as *Digitalis* in Scrophulariaceae, *Urginia* in Liliaceae and *Strophanthus* in Apocynaceae (47). About eleven plant families are known to contain cardiac glycosides (40).

The principal cardiac glycoside structure was divided into three component parts, a) steroid nucleus, b) unsaturated lactone ring and c) sugar(s).

a) Steroid nucleus :- In most cases the steroid portion has no double bond. The principle structure of these cardiac glycosides results in the pharmacological activities. The fusion of ring A and B is *cis*, with the hydrogen at C-5 having a β-configuration. The C/D ring-fusion is *cis*, with the hydrogen at C-8 is β. and at C-9 is α-configuration. These aglycones, all have hydroxyl groups at C-3 and C-14 (both having the β-configuration). The sugar-portion is linked through the hydroxyl group at C-3 of

the aglycone with the hydroxyl group at C-1 of the sugar. However the naturally occuring steroidal glycosides are known to have configuration different from these, for example with 3-α-hydroxyl group, or which an α-configuration at C-5 or with the A/B ring fusion has the *trans* configuration and C/D ring fusion has the *cis* configuration (47).

b) Unsaturated lactone ring :- In addition to this steroidal portion, there is an unsaturated lactone ring attached to C-17 of the steroidal carbon skeleton. On the basis of the lactone ring structure, these aglycones may be grouped into two groups, (a) the cardenolides 44 and (b) the bufadienolides 45 (which are also referred to as scilladienolides)(47).



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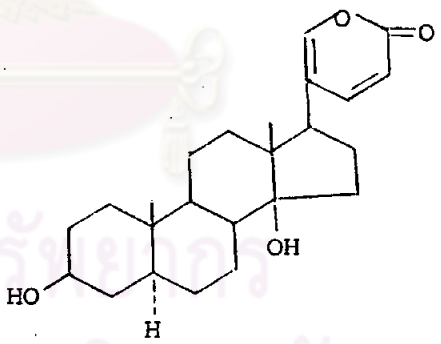
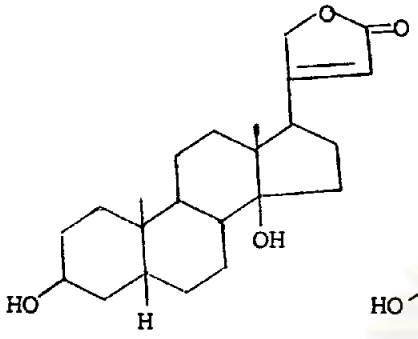


45

In the cardenolide (aglycone with 23 carbons), the 5-membered lactone ring attached to C-17 is a butenolide (4-carbon) which is also known as a $\Delta^{\alpha\beta}$-γ-lactone. Aglycone in this type was isolated from *Digitalis* and *Strophanthus* such as digitoxigenin 46, gitoxigenin 47 and strophanthidin 48 (40,47).



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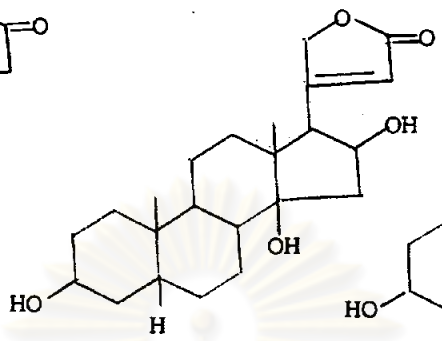

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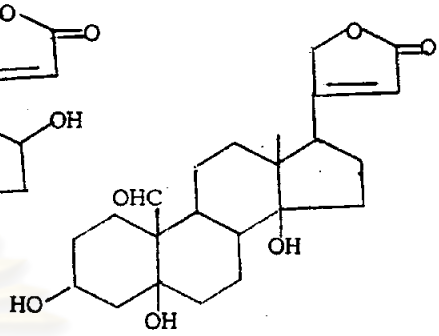
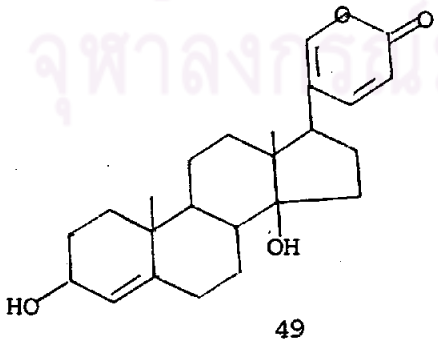


48

In the bufadienolide 45 (aglycone with 24 carbons), the 6-membered lactone ring attached to C-17 is a pentadienolide (5 carbons, with two double-bonds) which is also called a pentenolide, or otherwise known as a $\Delta^{\alpha\beta,\ \gamma\delta}$-δ-lactone. Bufadienolide type has been found in plant only in the buttercup family (Ranunculaceae) and lily family (Liliaceae). Scillarenin 49 is the cardiac genin found in the bulb of *Bowiea* and *Scilla* in Liliaceae, hellebrigenin 50 is a genin in the rhizomes of Helleborus which also been found in toad venom and named bufotalidin 49 (40,42,47).



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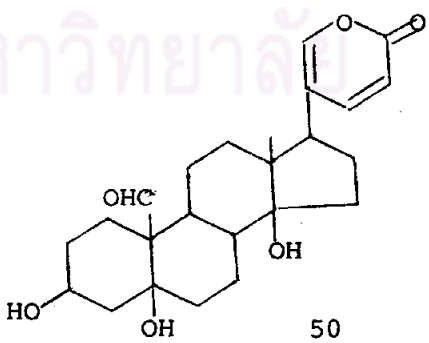
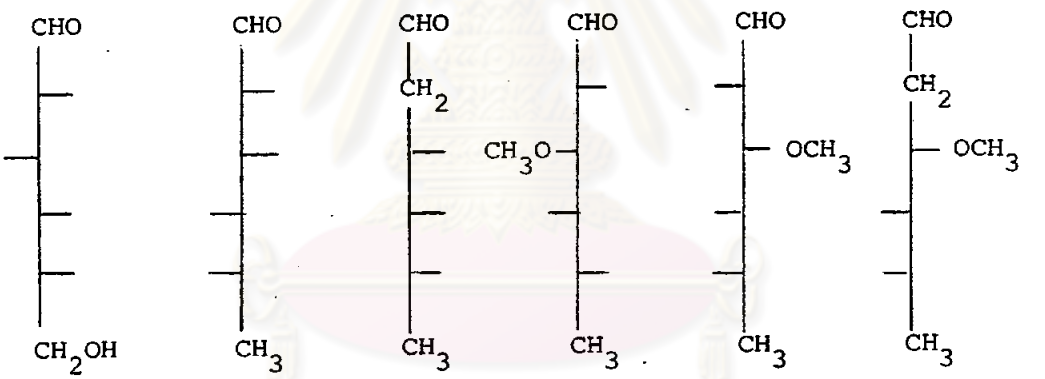


50

c) Sugar(s) :- Hydrolysation of cardiac glycosides, many kinds of sugars were found such as D-glucose 51, L-rhamnose 52 and deoxysugars which are sometime referred to as the "rare sugar". Deoxysugars which have not yet been found in other natural sources may be 2,6-deoxysugars such as D-digitoxose 53, 6-deoxy-3-methoxysugars such as D-digitalose 54 and L-thevetose 55 or 2,6-desoxy-3-methoxy-sugars such as L-oleandrose 56. The glycosidic linkage is always β in the case of D-sugars and α in the case of L-sugars. The structures of some of these sugars are shown below in the Fischer projection (42).



51 52 53 54 55 56

2. TRITERPENOIDS

2.1 Chemistry of triterpenoids

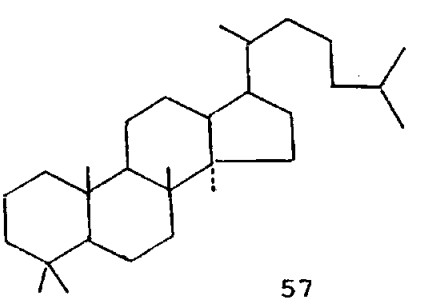
Triterpenes are C_{30} compounds, produced from two molecules of farnesyl pyrophosphate (FPP) condensed head-to-head to squalene. The majority of natural triterpenes are pentacyclic compounds. The next largest group is the tetracyclic triterpene. There is also a small number of triterpenes with various other cyclic structures. The only important acyclic triterpene is squalene (and its 2,3-oxide as a metabolic intermediate). Most triterpenoids are alcohols (3-OH), they are found free and as glycosides (saponins) or esters. Free triterpenoids are often components of resins, latex, or cuticle. Saponins are powerful surface active agents and can cause lysis of red blood cells. Some of them have been used as fish poisons. There are probably upward of 500 naturally occuring triterpenes of known structure, new compounds and new structural types are still being discovered (49).

2.2 Classification of triterpenoids

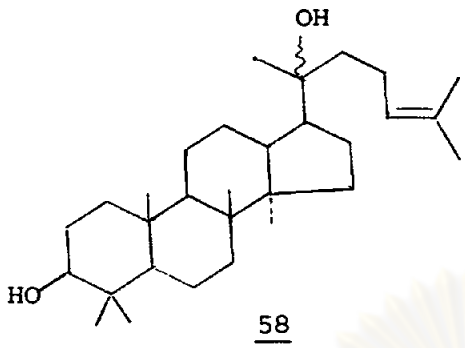
The triterpenes consist of two large groups, either tetracyclic or pentacyclic in form and miscellaneous groups.

2.2.1 Tetracyclic Triterpenes (50-52)

a) Damarane type 57 :- dammarenediol 58, dipterocarpol 59



57



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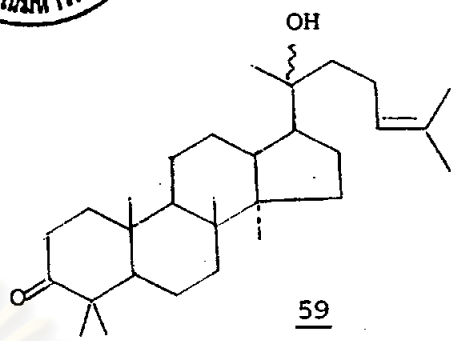
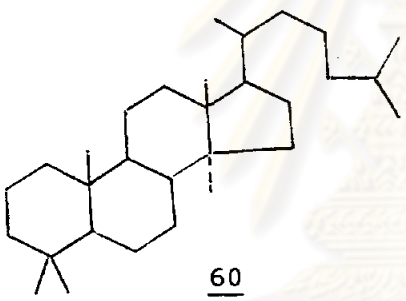


59

b) Lanostane type 60 :- Lanorterol 61



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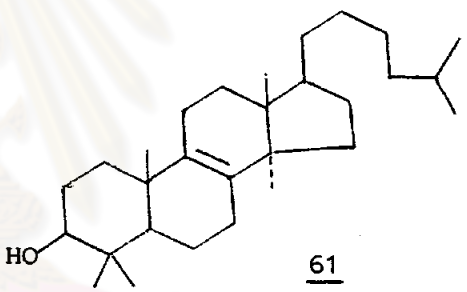
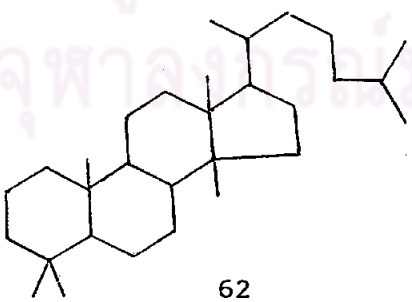


61

c) Euphane type 62 :- Euphol 63



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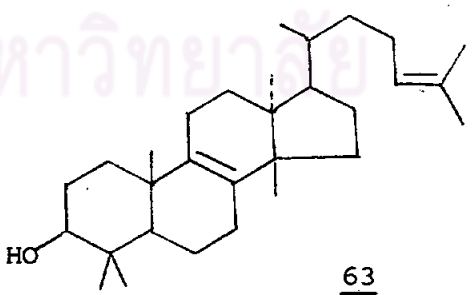
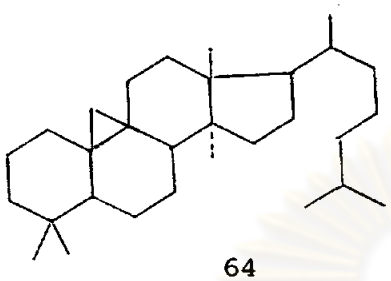


63

d) cycloartane 64 :- cycloartenol 65



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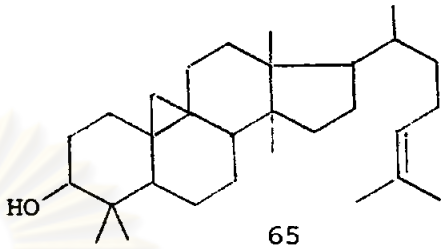
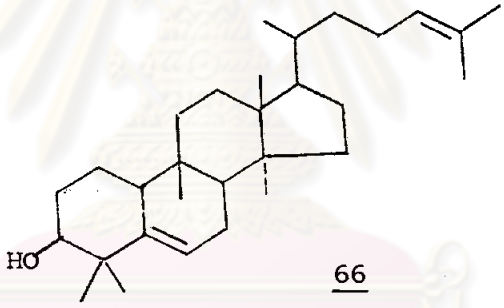


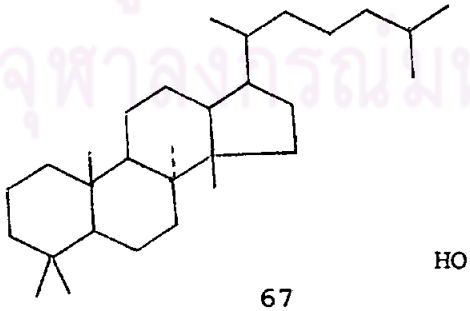
65

e) Cucurbitacin 66



66

f) Protostane type 67 :- Protosterol 68



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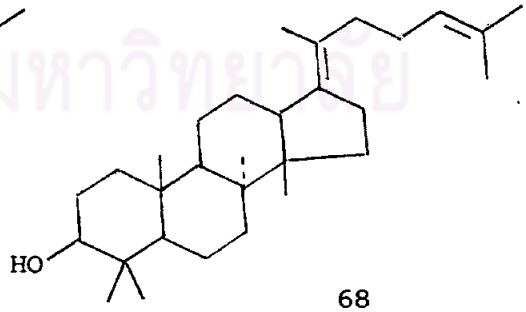
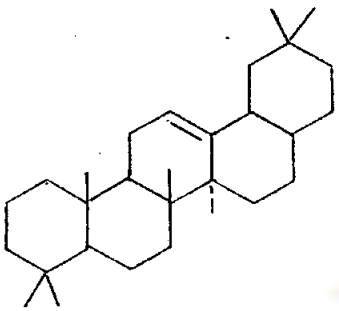


68

2.2.2 Pentacyclic triterpenes (50-52)

a) Oleanene type 69 :- β-amyrin 70, oleanolic acid 71



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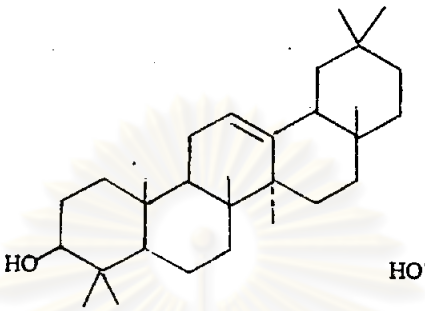

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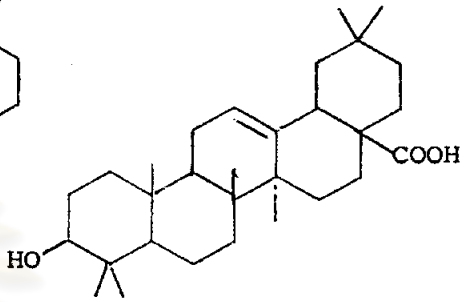
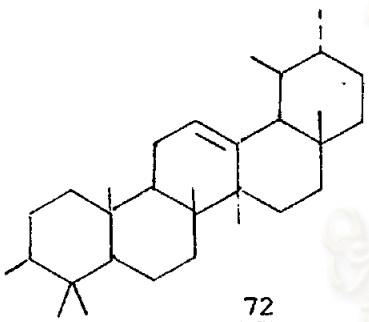


71

b) Ursene type 72 :- α amyrin 73, Ursolic acid 74



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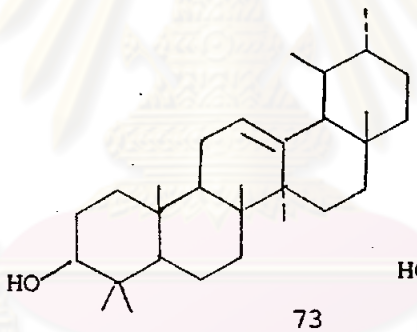

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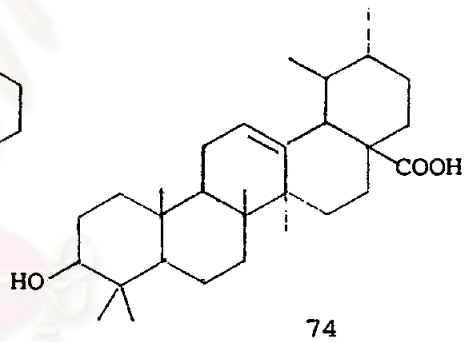
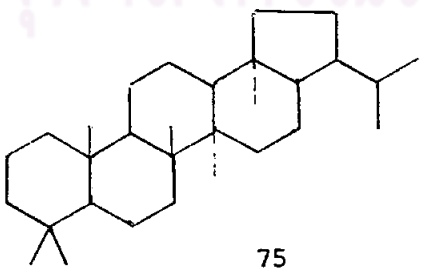


74

c) Hopane type 75 :- hopene 76



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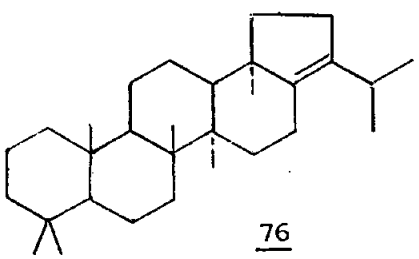
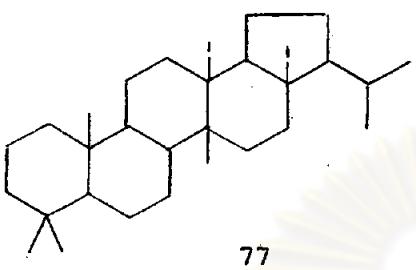


76

d) Fernane type 77 :- Fernene 78



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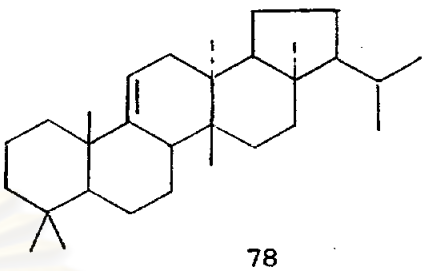
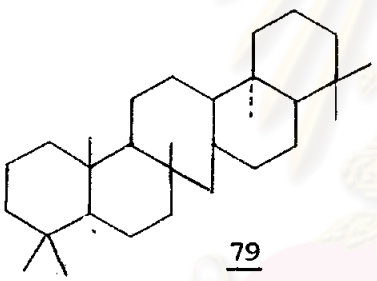


78

e) Serratane type 79 :- serratene 80



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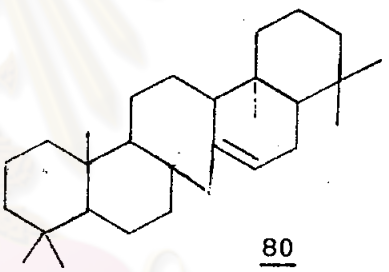
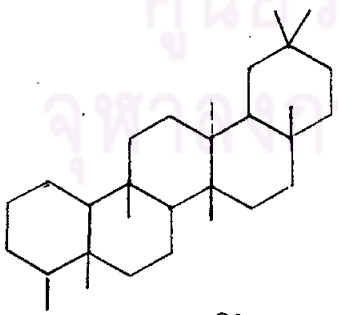


80

g) Friedelane type 81 :- Friedelin 82



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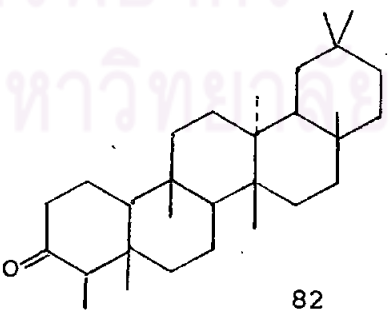
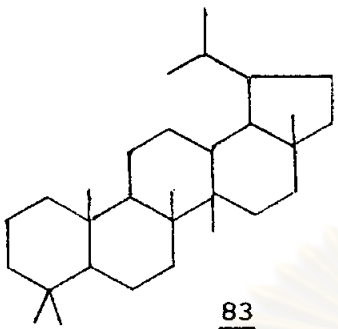


82

h) Lupane type 83 :- Lupeol 84



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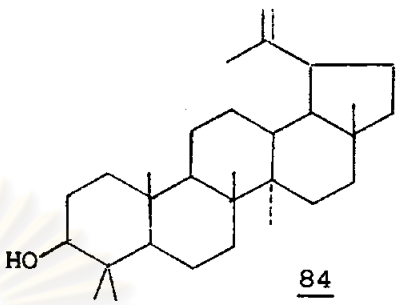
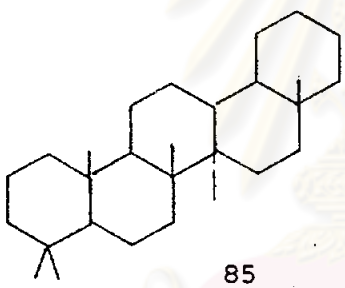


84

i) Germanicane type 85 :- germanicol 86



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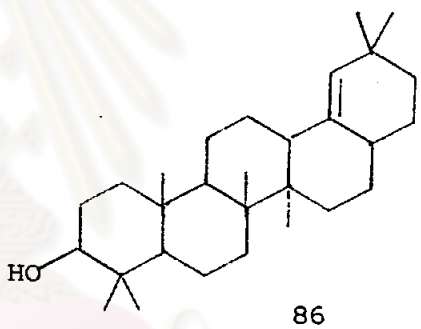
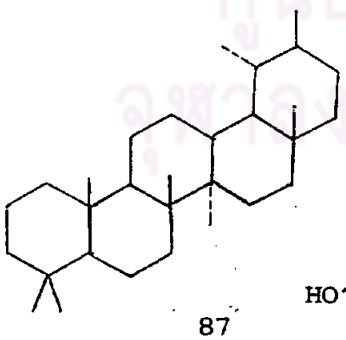


86

j) Taraxasterane type 87 :-γ-taraxasterol 88, taraxasterol 89



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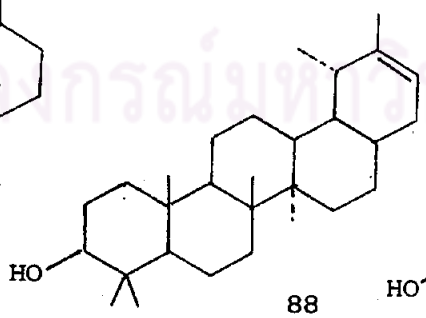

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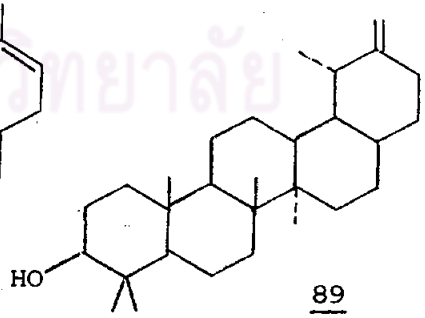

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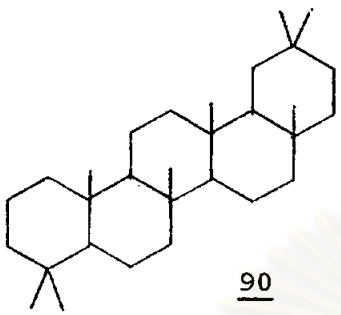
k) Taraxerane type 90 :- taraxerol 91



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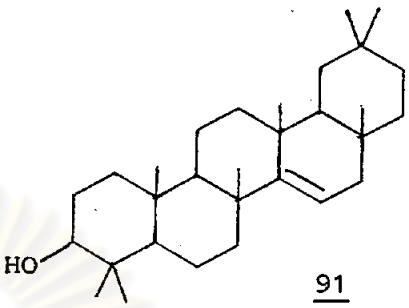
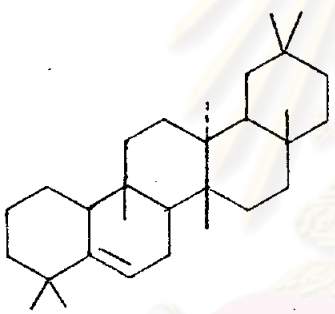


91

l) Glutinane type 92 :- Glutinone 93



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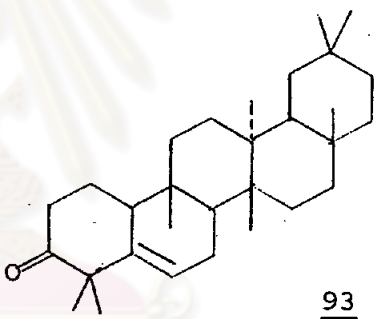
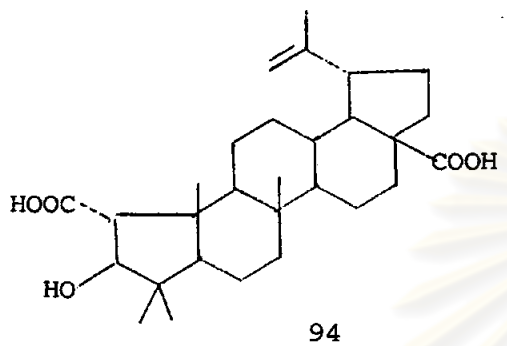


93

2.2.3 Miscellaneous

This group is some examples of unusual variations in the major types of triterpenes to be known. Ceanothic acid 94 is a member of the lupane type in which ring A has been contacted to five carbons. Nyctanthic acid 95 is a member of the oleanane group in which ring A has been opened. The seven membered ring of buxenine-G 96 are especially interesting. The limonins (or meliacins) are the bitter principle of *Citrus* species. An example of these is limonin 97 itself. The basic unit from which they are believed to be derived is tetracyclic triterpene euphol. Believed to be related to these by the distribution of methyl groups and general stereo-

chemistry are an additional group known as the quassins. These also are believed to be derived, biogenically, from euphol. A partly cyclized squalene is illustrated by ambrein 98, onocerin 99 and lansic acid 100 (3,4).



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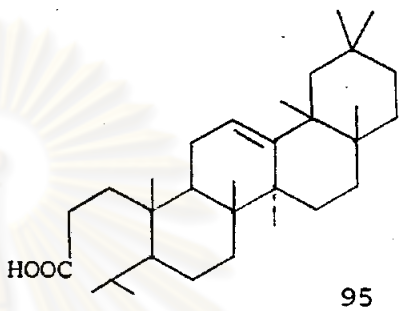

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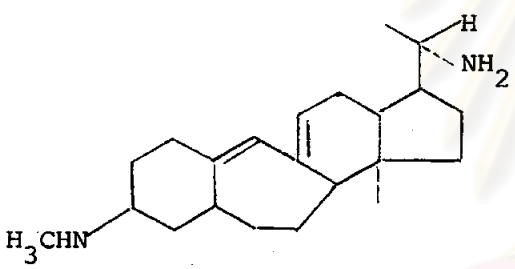

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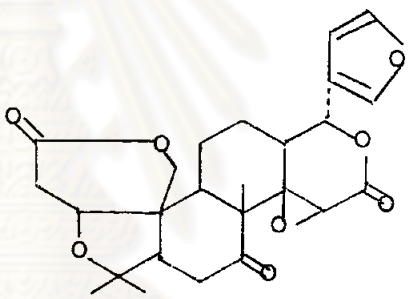

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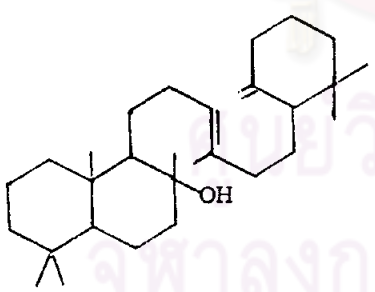

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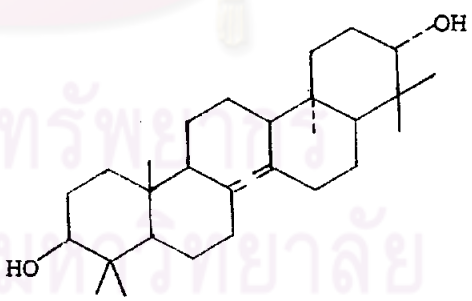

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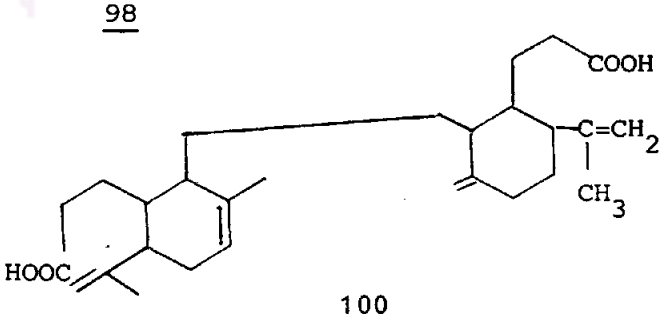

100

3. Biosynthesis of Steroids and Triterpenoids

3.1 Biosynthesis of Plant Sterols (39,42)

The formation of plant sterols especially the sitosterol-stigmasterol pathway occurs through three principal stages :

a) Squalene Biosynthesis (Figure 5, p. 55)

The formation of squalene is through the familiar pathway of acetyl CoA 101, acetoacetyl CoA 102, 3-hydroxymethylglutaryl CoA 103, mevalonic acid 104, mevalonic acid pyrophosphate 105, isopentenyl pyrophosphate 106, 3,3-dimethylallylpyrophosphate 107, geranyl pyrophosphate 108, farnesyl pyrophosphate 109 and squalene 110.

b) Squalene Cyclization (Figure 6, p. 56)

The cyclization of squalene to form the cyclopentanophenanthrene ring system is squalene-2,3-oxide 111 which is also the intermediate during cyclization. Cyclization is initiated by cation OH^+ attach at the squalene position which gives rise to C-3 of the sterol molecule. The epoxidase, which converts squalene to the 2,3-oxide, is microsomal in nature and requires NADPH and molecular oxygen, and addition of the sterol inhibitor, tri (2-diethylaminoethyl) phosphate, results in an accumulation of squalene-2,3-oxide. Formation of the tetracyclic steroid ring system is through molecular rearrangement, a migration of two hydrogen atoms and two 1,2-methyl shifts from C-8 to C-14 and from C-14 to C-13. The 3-β hydroxyl is derived from atmospheric oxygen and not from water. The conversion of squalene-2, 3-oxide to cycloartenol 112 requires the cyclase enzyme. It is generally accepted that cycloartenol is the first cyclic product in plants.

c) Conversion of the first cyclic intermediate (cycloartenol) to the sterol products.

To form the major phytosterols from cycloartenol, an alkylation at C-24 is probably the first step and this occurs through transmethylation involving S-adenosyl methionine which is the product in this step is 24-methylene cycloartanol 113, a 4,4-dimethyl sterol. Demethylation at C-4 is probably the next step, producing cycloeucalenol 114 the first 4-methyl sterol. The next step, the 9β, 19β-cyclopropane ring can be opened most efficiently to form obtusifoliol 115 and 31-norlanosterol 117 by C-14 demethylation to occur, a $\Delta^{8(9)}$ bond. The most generally accepted pathway is through 24-methylene cycloartanol → cyclocucalene → obtusifoliol but the sequence cycloartenol → 31-norcycloartenol 116 → 31-norlanosterol 117 obtusifoliol 115 has also been indicated. From obtusifoliol to 24-methylene lophenol 118 occurs through molecular rearrangement by migration of double bond. The formation of 24-ethylidene lophenol 119 occurs by the second alkylation of C-28. Methionine is again the methyl donor for the second alkylation. During this process, a cationic site at C-24 of the steroid molecule is created which is stabilized through the loss of a hydrogen atom from C-28. The removal of the second C-4 methyl group from 24-ethylidene lophenol 119 is also through oxidative decarboxylation and this product is Δ^{7}-avenasterol 120. The conversion of Δ^{7}-avenasterol 120 to the major phytosterols, sitosterol and stigmasterol, appears that the pathway involves a reduction of $\Delta^{24(28)}$ and the rearrangement of the double bond in ring B to form avenasterol 122. Formation of sitosterol from avenasterol requires hydrogenation of $\Delta^{24(28)}$ and

reduction of the 24-ethylidene. Formation of stigmasterol is assumed to occur through sitosterol by the enzyme 22,23-dehydrogenase. Formation of sitosterol and stigmasterol may also be Δ^7-avenasterol 120 → stigmasta-5, 7, 24(28)-trien-3β-ol 121 → stigmasta-5, 7-diene-3β-ol 123 → sitosterol 124 or stigmasterol 125. This sequence would not involve avenasterol.

Another pathway for the biosynthesis of major higher plant sterols is first reduction of $\Delta^{24(28)}$ of Δ^7-avenasterol to form stigmasta-7-en-3β-ol 126. This reaction must be through a $\Delta^{24(25)}$ intermediate since, the C-25 hydrogen atom is lost. Next, stigmata-7-en-3β-ol goes through the $\Delta^{7(8)} \rightarrow \Delta^{5,7} \rightarrow \Delta^{5(6)}$ rearrangment to form sitosterol. For stigmasterol formation can be through spinasterol 127 → 7-dehydrostigmasterol 128.

It is quite possible that all of the discussed pathways operate in plants,and depending upon species and environmental conditions.

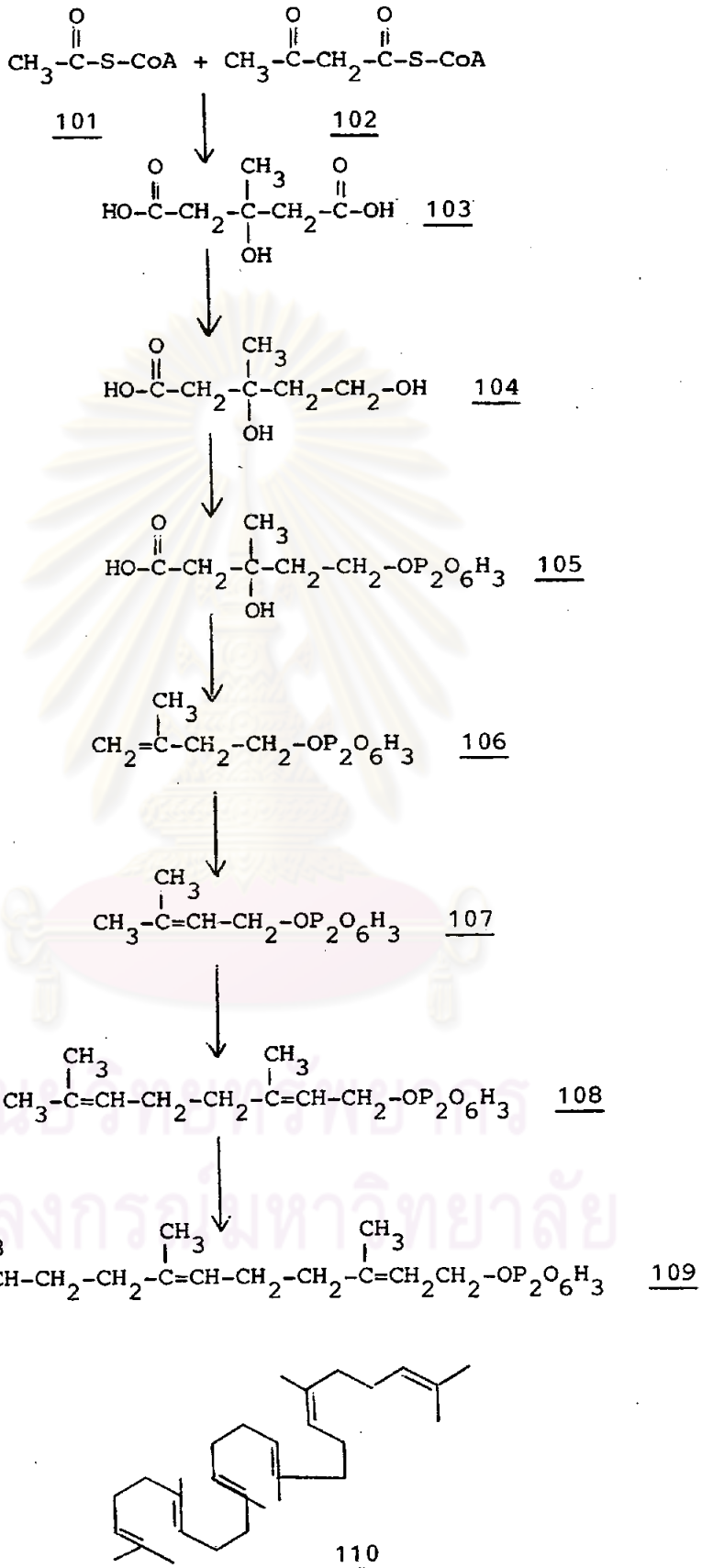

Figure 5 Squalene Biosynthesis

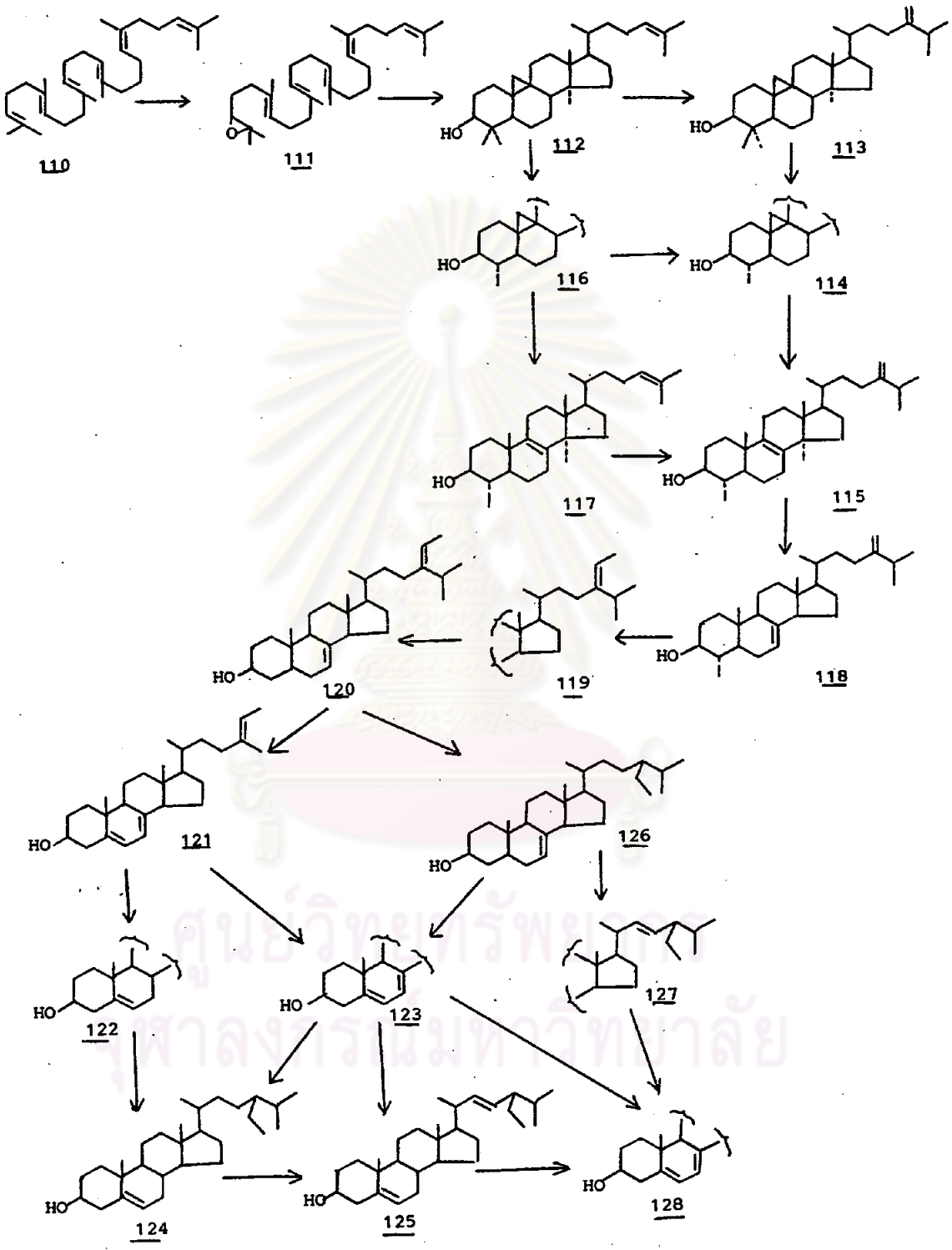

Figure 6 Biosynthesis pathway of plant sterols

3.2 Biosynthesis of Steryl Glycosides (39)

In the glycosides, the 3β-hydroxyl of the steryl moiety is linked to the hydroxyl of the C-1 position of the carbohydrate. Steryl glycoside formation was first demonstrated with cell particulate preparations from immature soybeans and has since been confirmed with several other species. All 4-demethyl sterols are incorporated at about the same rate and the glycoside bond is mediated through nucleotide sugars. Uridine diphosphate-glucose is the most active glycosyl donor. Steryl glycoside formation is stimulated by ATP, Ca^{2+} and Mg^{2+}. The optimum medium pH is critical and depends upon the tissue-generally it is near the neutral point.

3.3 Biosynthesis of Pentacyclic triterpenes (The Amyrins) (39,40,50,51)(Figure 7, p. 59).

The amyrins are derived biosynthetically from the acyclic C_{30} hydrocarbon, squalene. The formation of squalene follows the same pathway as the plant sterol which have been discussed elsewhere in squalene biosynthesis (Figure 5, p. 55). The evidence is not repeated here.

Cyclization of a squalene 129 chain in the amyrins has squalene-2, 3-oxide 130 as an intermediate between squalene and triterpene, its conversion to lanosterol 131. Lanosterol can rearrange by migration of the C-16 methylene group to the structure 132. The migrations during the pause at the tetracyclic stage result in a rearrangement of the carbon backbone of squalene such that C-16 is now joined to C-18 in pentacyclic triterpenes. The terminal isoprene unit is folded as a boat, its cyclization onto the tetracyclic

nucleus already for product intermediate 133. A carbon skeleton rearrangement by C-20 methylene migration from C-21 to C-22 will produce a new intermediate 134. From intermediate 134 can serve as the origin for a variety of 1,2 shifts and proton losses. Migration of hydride and a methyl group gives β-amyrin 135. If the migration of the intermediate 132 is followed by a hydride migration from C-21 to C-22 to yield the intermediate 136 and there then occurs the indicated series of 1,2-shifts, α-amyrin 137 will arise.

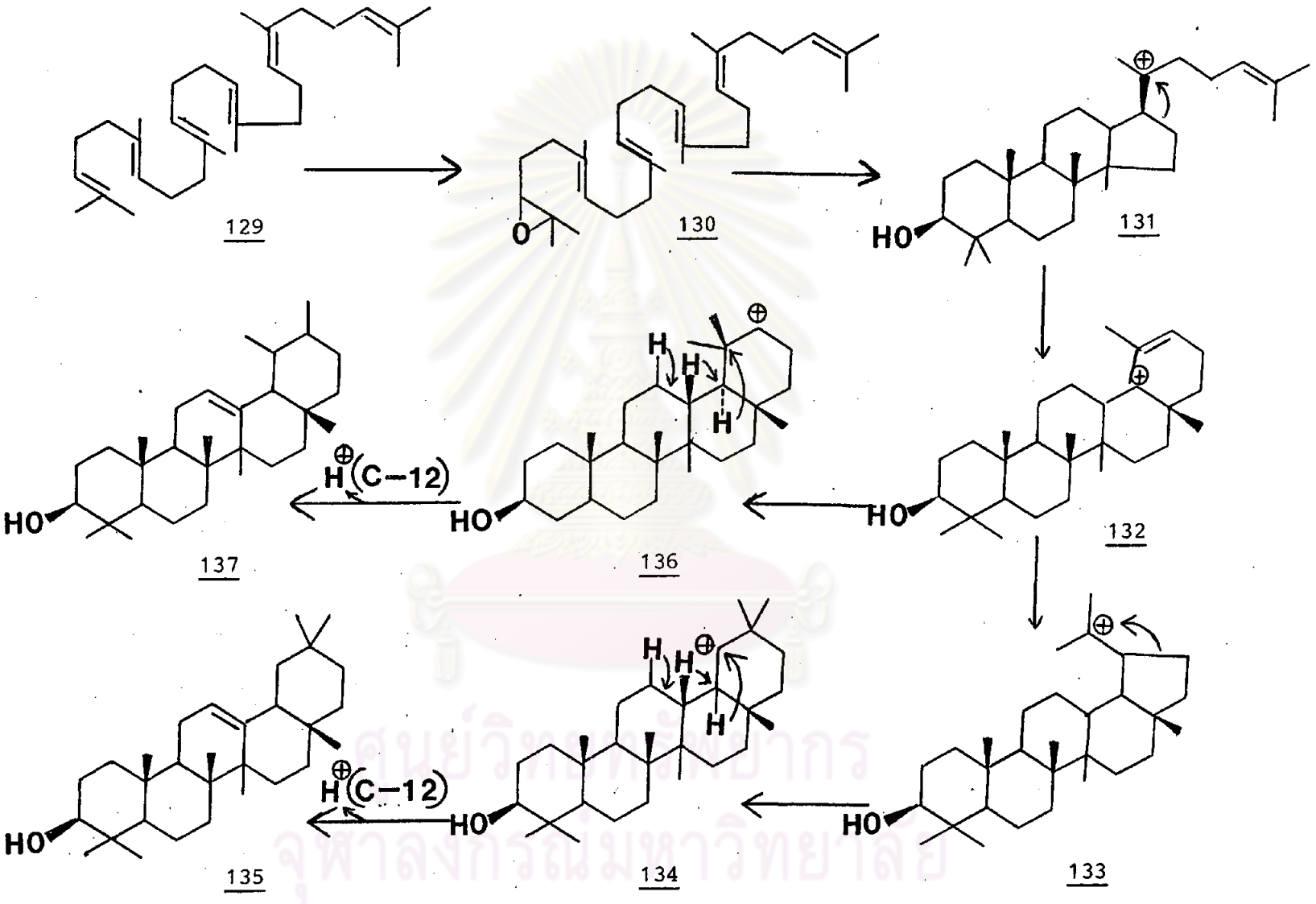

Figure 7 Biosynthesis pathway of the amyrins (α-amyrin 137, β-amyrin 135)