CHAPTER II

HISTORICAL

1. CHEMICAL CONSTITUENTS OF MICHELIA SPP.

The compounds commonly found in the genus *Michelia* are sesquiterpene lactones, alkaloids, steroids and volatile oils.

Lists of the compounds found in various species of *Michelia* are shown in Table 1.

Table 1. Chemical investigations of *Michelia* spp.

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Michelia alba DC.	Trunk Bark,	Ushinsunine	Alkaloid	9
(*M. longifolia* Bl.)	Root		(Aporphine Type)	
	Trunk Bark,	Oxoushinsunine	Alkaloid	9
	Root	(Liriodenine)	(Oxoaporphine Type)	
	Trunk Bark,	Salicifoline	Alkaloid	9
	Root		(Phenethylamine Type)	
	Trunk Bark,	Michelalbine	Alkaloid	9
	Root		(Aporphine Type)	
	Flower	Acetaldehyde	Miscellaneous	30
	"	*iso*-Aristolene	Sesquiterpene	11
	"	Allocimene	Monoterpene	30
	"	Benzyl Acetate	Miscellaneous	20
	"	Benzyl Benzoate	Miscellaneous	20
	"	Benzaldehyde	Miscellaneous	20

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Michelia alba DC.)	Flower	*beta*-Bisabolene	Sesquiterpene	11
(*M. longifolia* Bl.)	"	Butyric acid methyl ester	Miscellaneous	10, 30, 21
	"	Butyric acid,2-methyl-methyl ester	Miscellaneous	11
	"	Butyric acid, 3-methyl-methyl ester	Miscellaneous	10
	"	Butyric acid, alpha-methyl methyl ester	Miscellaneous	10, 30
	"	*iso*-Butyric acid, methyl ester	Miscellaneous	10
	"	Buthyl pentanoate	Miscellaneous	30
	"	*delta*-Cadinene	Sesquiterpene	11
	"	Camphene	Monoterpene	11
	"	Car-3-ene	Monoterpene	11
	"	*trans*-Carveol	Monoterpene	11

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Michelia alba DC.)	Flower	*cis*-Caryophyllene	Sesquiterpene	11
(*M. longifolia* Bl.)	"	1, 3-Cineol	Monoterpene	11
	"	*alpha*-Cubebene	Sesquiterpene	11
	"	*beta*-Cubebene	Sesquiterpene	11
	"	*ortho*-cymene	Monoterpene	10, 30
	"	Ethanol	Micellaneous	10, 30
	"	Ethyl-α-methyl Butyrate	Micellaneous	30
	"	Eugenol Methyl Ether	Lignan	11
	"	*iso*-Eugenol Methyl Ether	Lignan	11
	"	Hydroxy Citronellol	Monoterpene	20
	"	Limonene	Monoterpene	11, 20, 30
	"	Linalool	Monoterpene	11, 20, 21
	"	*cis*-Linalool oxide	Monoterpene	11
	"	*trans*-Linalool oxide	Monoterpene	11

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Michelia alba DC.	Flower	Methyl Acetate	Miscellaneous	30
(*M. longifolia* Bl.)	"	Methyl Benzoate	Miscellaneous	30
	"	Methyl-2-pentenoate	Miscellaneous	30
	"	Methyl hexanoate	Miscellaneous	30
	"	*beta*-Myrcene	Monoterpene	11
	"	Nerol	Monoterpene	20
	"	Ocimene	Monoterpene	11, 30
	"	*alpha*-Phellandrene	Monoterpene	11
	"	*beta*-Phellandrene	Monoterpene	30
	"	*beta*-Pinene	Monoterpene	11
	"	Propionic acid methyl ester	Miscellaneous	10, 30
	"	*beta*-Selinene	Sesquiterpene	11
	"	Terpenolene	Monoterpene	30
	"	Undecane agarol	Monoterpene	30

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Michelia alba DC. (*M. longifolia* Bl.)	Flower	*alpha*-Ylangene	Sesquiterpene	11
M. cathcartii Hook f & Thoms.	Trunk Bark	Lanuginosine	Alkaloid (Oxoaporphine Type)	12
	Trunk Bark	Liriodenine	Alkaloid (Oxoaporphine Type)	12
	Trunk Bark	Sitosterol	Steroid	12
M. Champaca L.	Trunk Bark	Oxoushinsunine (Liriodenine)	Alkaloid (Oxoaporphine Type)	16, 25
	Trunk Bark	Magnoflorine	Alkaloid (Aporphine Type)	16
	Trunk Bark	Ushinsunine	Alkaloid (Aporphine Type)	16

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Michelia champaca L.	Trunk Bark	β-sitosterol	Steroid	25
	Root Bark	Costunolide	Sesquiterpene Lactone	15
	Root Bark	Parthenolide	Sesquiterpene Lactone	15
	Root Bark	Dihydroparthenolide	Sesquiterpene Lactone	15
	Root Bark	Micheliolide	Sesquiterpene Lactone	15
M. compressa Sarg.	Trunk Bark	Oxyacanthine	Alkaloid (Biscoclaurine Type)	17
	Trunk Bark	Berberine	Alkaloid (Protoberberine Type)	17
	Trunk Bark	Palmatine	Alkaloid (Protoberberine Type)	17
	Trunk Bark	Jatrorrhizine	Alkaloid (Protoberberine Type)	17
	Trunk Bark	Magnoflorine	Alkaloid (Aporphine Type)	17

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Michelio compressa Sarg.	Trunk Bark	Michepressine	Alkaloid (Aporphine Type)	18
	Root Bark	Michelenolide	Sesquiterpene Lactone	6
	Root Bark	Micheliolide	Sesquiterpene Lactone	6
	Root Bark	Compressanolide	Sesquiterpene Lactone	6
	Root Bark	Dihydroreynosin	Sesquiterpene Lactone	6
	Root Bark	Parthenolide	Sesquiterpene Lactone	6
	Root Bark	Dihydroparthenolide	Sesquiterpene Lactone	6
	Root Bark	Costunolide	Sesquiterpene Lactone	6
	Root Bark	Lanuginolide	Sesquiterpene Lactone	6
	Root Bark	Reynosin	Sesquiterpene Lactone	6
	Root Bark	Santamarine	Sesquiterpene Lactone	6
	Root Bark, Heartwood	Liriodenine (Micheline B)	Alkaloid (Oxoaporphine Type)	6, 26

Tabel 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Michelia compressa Sarg.	Heartwood	Micheline A	Alkaloid (Aporphine Type)	26
M. excelsa Bl. (*M. doltsopa* Buch-Ham ex DC.)	Trunk Bark, Root Bark	Liriodenine	Alkaloid (Oxoaporphine Type)	12
	Trunk Bark, Root Bark	Sitosterol	Steroid	12
	Fruit	Lanuginolide	Sesquiterpene Lactone	14
	Fruit	Dihydroparthenolide	Sesquiterpene Lactone	14
	Fruit	11, 13-Dehydrolanuginolide	Sesquiterpene Lactone	14
M. figo Spreng	Leaves	Magnolamine	Alkaloid (Bisbenzylisoquinoline Type	27
	Leaves	γ-Sitosterol	Steroid	27
	Flower	*iso*-Butyl Acetate	Miscellaneous	22, 23

Table 1. (Continue)

Botanical Origin	Plant Part	Chemicla Substance	Category	Reference
Michelia figo Spreng	Flower	Ethyl Acetate	Miscellaneous	23
	Flower	Ethyl isobutyrate	Miscellaneous	23
	Flower	*iso*-Butanol	Miscellaneous	23
	Flower	8Z, 11Z, 14Z-8, 11,14-heptadecatrien-2-one	Miscellaneous	22
M. fuscata Bl.		Deacetyllanuginolide	Sesquiterpene Lactone	24
		Michefuscalide	Sesquiterpene Lactone	24
		Dehydrolanuginolide	Sesquiterpene Lactone	24
		Lipiferolide	Sesquiterpene Lactone	24
		Syringaresinol	Lignan	24
		Magnolamine	Alkaloid (Bisbenzylisoquinoline Type)	27
M. hedyosperma Law.	Volatile Oil	Safrole	Monoterpene	19

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Michelia hedyosperma Law.	Volatile Oil	Methyleugenol	Lignan	19
		Limonene	Monoterpene	19
		Linalool	Monoterpene	19
M. lanuginosa Wall.	Trunk Bark	Dihydroparthenolide	Sesquiterpene Lactone	12, 28, 29
	Trunk Bark	Lanuginolide	Sesquiterpene Lactone	12, 28, 29
	Trunk Bark	11, 13-Dehydrolanuginolide	Sesquiterpene Lactone	12, 13, 28
	Trunk Bark	Parthenolide	Sesquiterpene Lactone	12, 13, 28
	Trunk Bark, Leaves, Root Bark	Lanuginosine	Alkaloid (Oxoaporphine Type)	12
	Trunk Bark	Michelanugine	Alkaloid (Aporphine Type)	12
	Leaves, Root Bark	Liriodenine	Alkaloid (Oxoaporphine Type)	12

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Michelia	Leaves	Sitosterol	Steroid	12
lanuginosa Wall.	Trunk Bark	Sinapaldehyde	Miscellaneous	28

2. APORPHINE AND OXOAPORPHINE ALKALOIDS

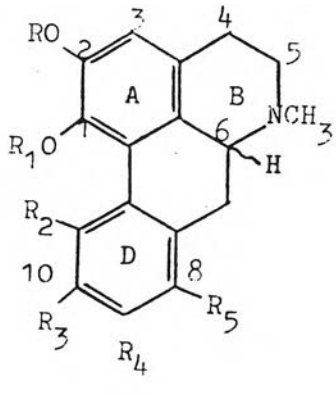
The alkaloids widely distributed in the plant of genus *Michelia* belong to aporphine and oxoaporphine alkaloids. These alkaloids are lanuginosine 1, michelanugine 2, liriodenine or oxoushinsunine 3, ushinsunine 4, magnoflorine 5, michepressine 6, and michelalbine 7,

Ushinsunine and oxoushinsunine were found to distribute commonly, especially in the genus *Michelia* (16).

2.1 Chemistry of Aporphine Alkaloids

Aporphine alkaloids are the largest group (over 100) of isoquinoline alkaloids and represented by the general structure 8. These alkaloids are distributed in at least 18 plant families, of which the most important are the Papaveraceae, Anonaceae, Lauraceae and Monimiaceae (48).

In terms of structure it can be derived from a benzylisoquinoline skeleton 9 by additional ring closure (49).



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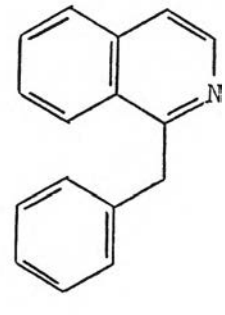


9

Benzylisoquinoline nucleus

The nitrogen atom is usually methylated; although some noraporphine are known, they are not very stable and are often characterized as their N-acetyl derivatives. Aporphines are known with the C-6a stereochemistry either α or β(48).

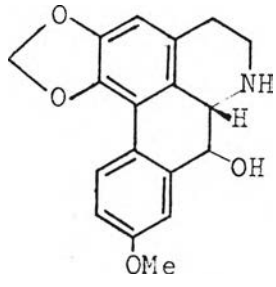
The most diverse structural feature of the aporphine is the oxygenation pattern. Position 1 and 2 are always oxygenated, either by hydroxy, methoxy or methylene dioxy groups. It is common to find further oxygen substituents at C-9, C-10 and C-11 and occasionally at C-8. It is rare to find oxygenation at C-7, except in the oxoaporphines, and even rarer to find any oxygenation in ring B. (48).

Aporphine alkaloids isolated from *Michelia* spp. are shown in Fig. 2.1.

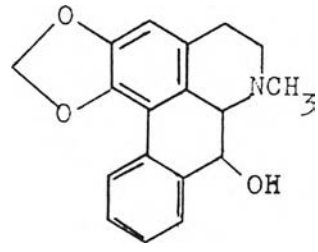
2.2 Chemistry of Oxoaporphine Alkaloids

The oxoaporphines represent the most highly oxidized state of the aporphine skeleton. They are widely distributed (at least nine plant families) and commonly cooccur with aporphine alkaloids. The numbering system is the same as that of aporphines and, like the aporphines, beyond the perennial 1, 2-dioxygenation, they exhibit a variety of oxygen substitution patterns. In this series however, there is a tendency toward 3-substitution and not 11-substitution (48). Oxoaporphines isolated from *Michelia* spp. are shown in Fig. 2.2.

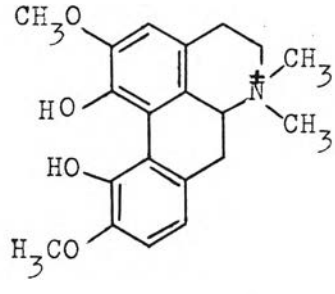
The first oxoaporphine to be isolated was liriodenine *3*, a bright yellow constituent of the heartwood of the tulip tree *Liriodendron tulipifera* L. (Magnoliaceae).



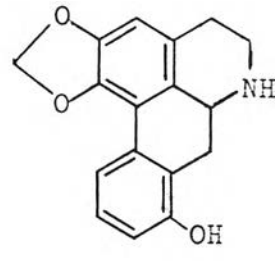
Michelanugine 2



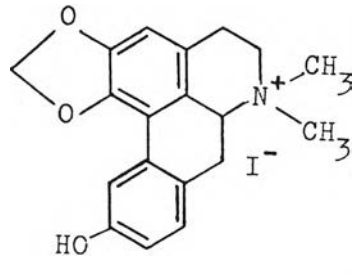
Ushinsunine 4



Magnoflorine 5



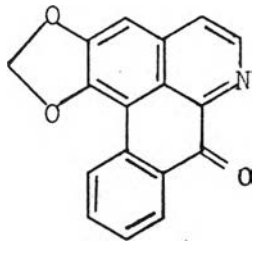
Michelalbine 7



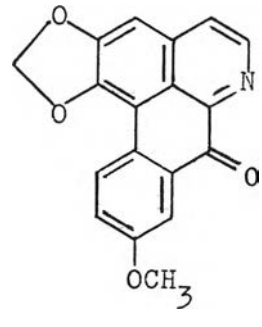
Michepressine Iodide 6

Figure 2.1 Aporphine alkaloids isolated from *Michelia* spp.

Liriodenine is the most widely distributed oxoaporphine and has been obtained from at least 22 genera in eight plant families.



Liriodenine 3



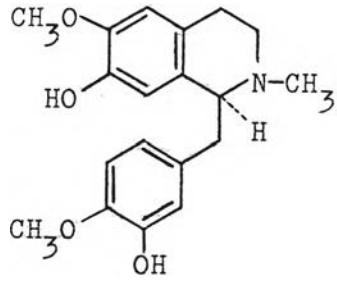
Lanuginosine 1

Figure 2.2 Oxoaporphine alkaloids isolated from *Michelia* spp.

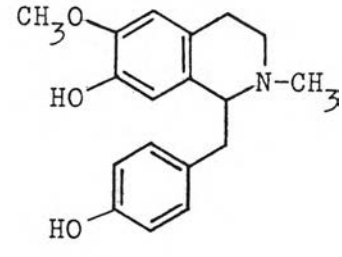
2.3 Biosynthesis of Aporphine Alkaloids

Aporphine alkaloids are usually optically active, and possess either the R or the S absolute configuration (55).

The common building blocks for the aporphine alkaloids are the tetrahydrobenzylisoquinolines reticuline and N-methylcoclaurine. The tetraoxygenated reticuline usually acts as a precursor to the aporphines when it is in the S absolute configuration which is dextrorotatory. On the other hand, the trioxygenated N-methylcoclaurine may function as a precursor in the dextro or the levorotatory form (55).



(+)-reticuline



N-Methylcoclaurine

The biogenetic pathways leading to isoquinoline alkaloids are derived from tyrosine. Tyrosine is first elaborated to a suitable hydroxylated and derivatised phenethylamine which is then combined with a second building block. This second building block can vary widely to give various types of isoquinolines (50). The proven or probable biogenetic loci for the formation of the isoquinoline alkaloids is shown in Figure 2.3 (56).

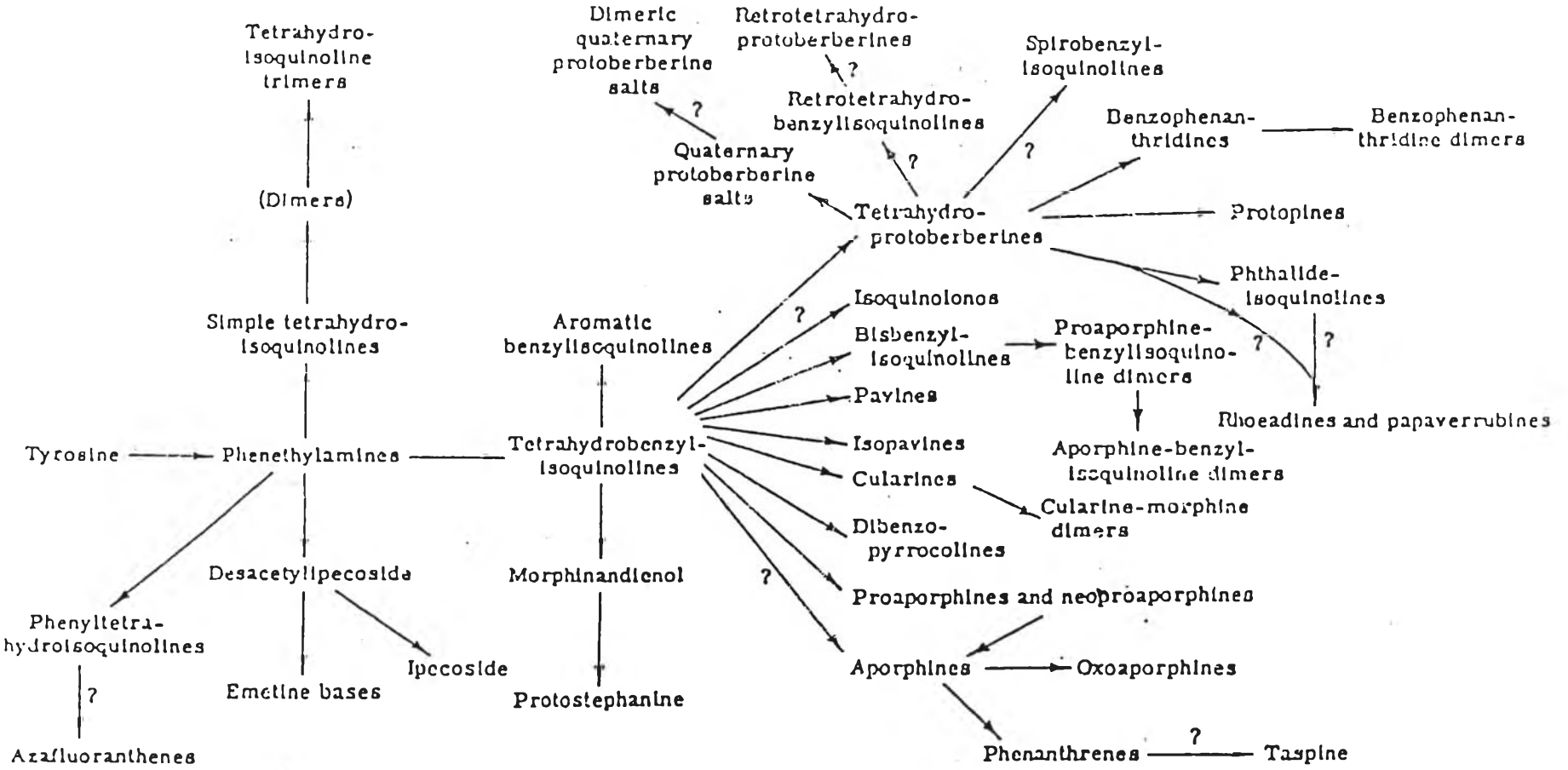


Figure 2.3 Proven or Probable Biogenetic Loci for the Formation of the Isoquinoline Alkaloids

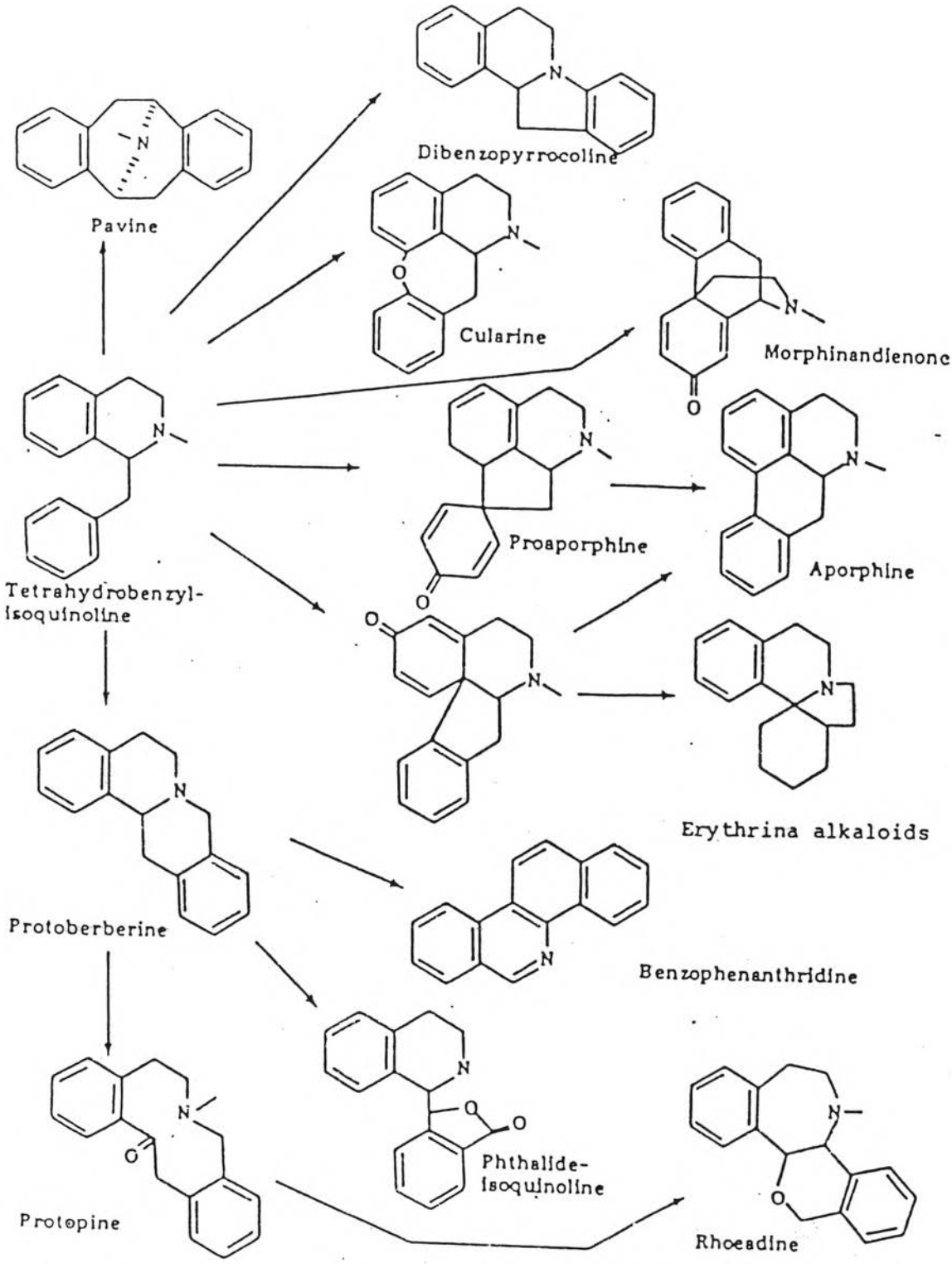


Figure 2.4 Biogenetic relationships of the major alkaloid groups derived from a tetrahydrobenzylisoquinoline precursor

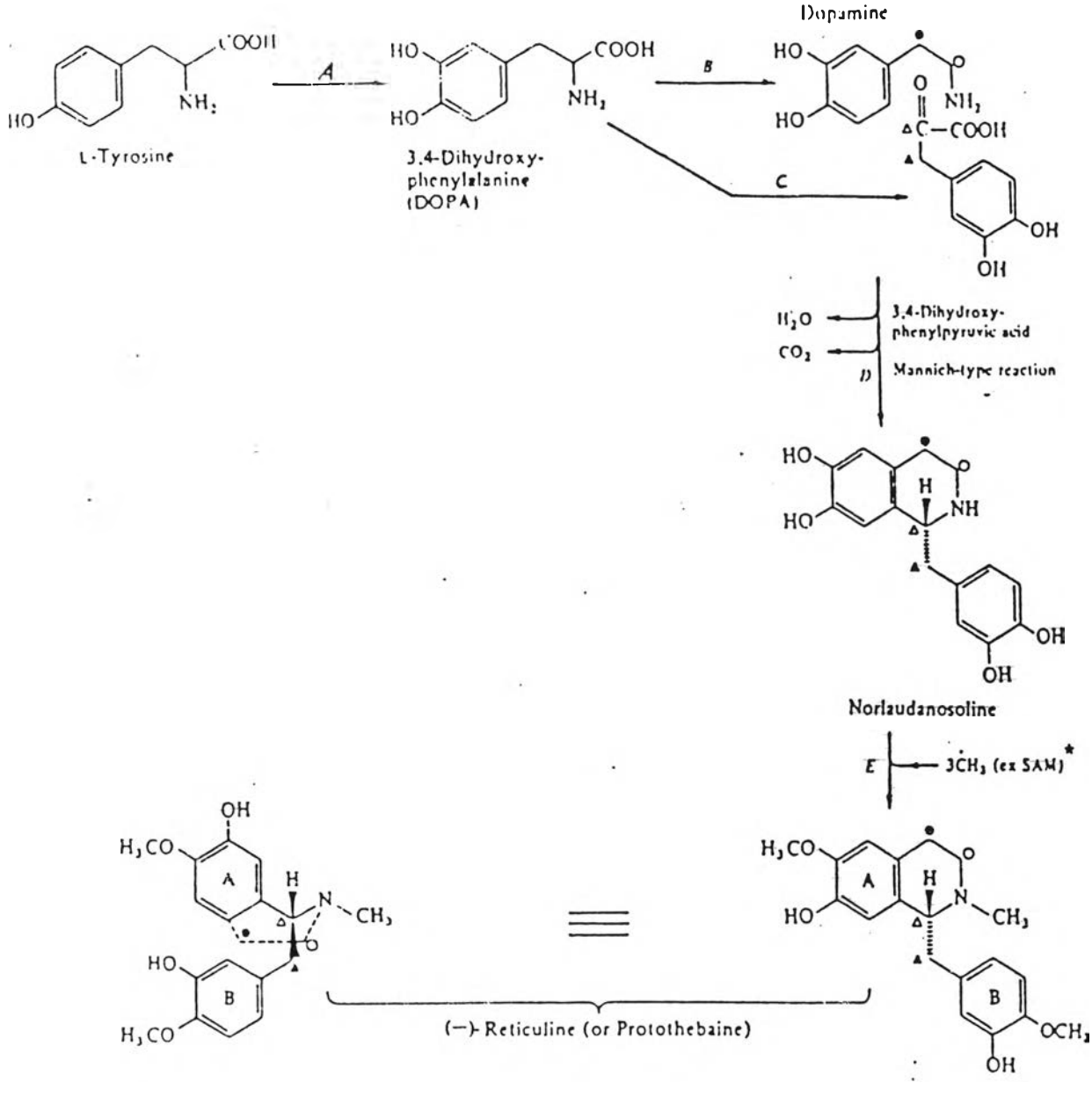


Figure 2.5 The formation of 1-benzyl-1, 2, 3, 4-tetrahydroisoquinolines

* SAM = S-adenosylmethionine

Benzylisoquinolines derive from two molecules of L-tyrosine. They occupy a paramount position in alkaloid chemistry because they act as *in vivo* precursors to so many of the other naturally occurring isoquinolines. Reticuline is regarded as the key intermediate in the biosynthesis of many alkaloids based on the benzylisoquinoline nucleus, and as a result of the study of these alkaloids much has been learned of the biosynthesis of reticuline. (57).

The example of the formation of reticuline in *Papaver somniferum* Linn. is shown in Figure 2.5 (51).

The first step (A) involves the conversion of two molecules of L-tyrosine into two molecules of DOPA. One molecule of DOPA is converted into dopamine (B) whilst the other is converted into 3,4-hydroxyphenyl pyruvic acid (C). These two compounds then combine with the elimination of water and carbon-dioxide in a Mannich-type reaction to yield a molecule of norlaudanosoline (D). O-and N-methylation (E) then lead to (-)-reticuline (51).

More recent experiments have definitely shown that benzyl-isoquinolines are natural precursors of aporphine alkaloids. Tritium-labeled N-methylcoclaurine fed to *Papaver dubium* was a good precursor of (+)-roemerine, the major alkaloid of this plant, Fig. 2.6 (49).

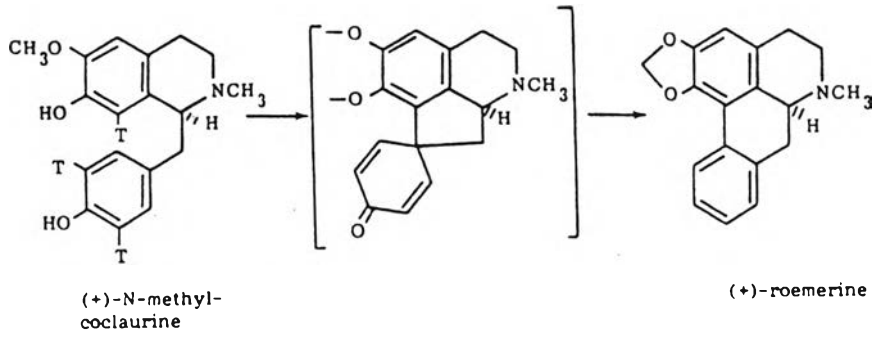
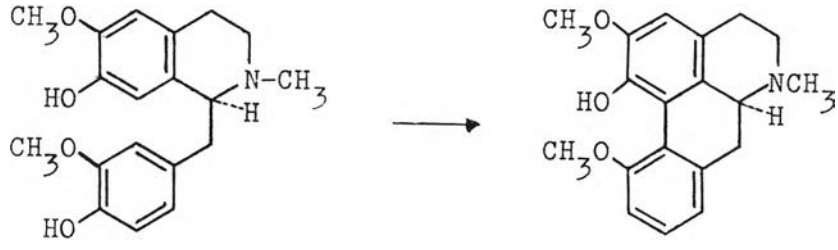


Figure 2.6 Biosynthesis of (+)-roemerine in *Papaver dubium*

Feeding tritium labeled (+)-and (-)-orientalines, (±)-orientaline-3-(^{14}C), or doubly labeled orientaline to *Papaver orientale* gave a preferential conversion of (+)-orientaline to isothebaine with no loss of methoxyl groups, Fig. 2.7 (49).



(+)-Orientaline (+)-Isothebaine

Figure 2.7 Biosynthesis of (+)-isothebaine in *Papaver orientale*

Other experiments have shown reticuline to be the precursor of some aporphine alkaloids (eg., magnoflorine, bulbocapnine and boldine) Fig.2.8, and norprotosinomenine to be the precursor of others. These various results, in which the only difference between precursor benzylisoquinolines is the pattern of O-methylation, illustrate an important principle, the positions that are methylated early in the sequence have a controlling influence on the future direction of the pathway (48, 49)

The discussion on the biogenetic synthesis of the aporphine alkaloids has raised a number of points concerning the biosynthesis of this group. In particular and depending on the orientation of phenolic and methoxy groups we might envisage any of at least five routes being in operation from a benzylisoquinoline precursor as shown in Figure 2.9 (48).

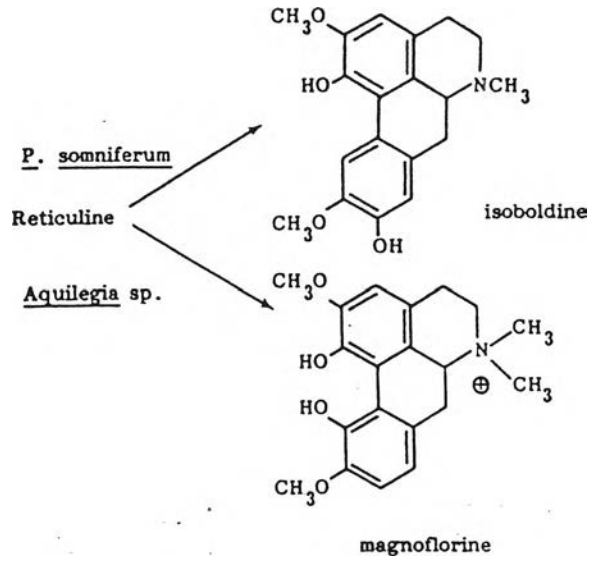


Figure 2.8 Biosynthesis of isoboldine and magnoflorine

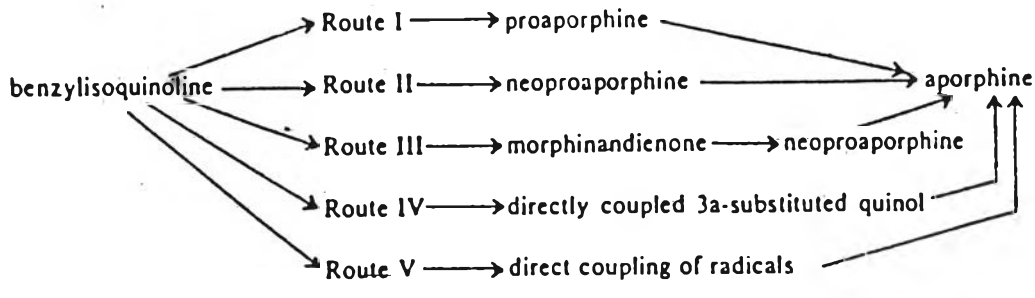


Figure 2.9 Biogenesis of aporphines from benzylisoquinolines

The structure of the aporphine alkaloids (+)-roemerine (Fig.2.6) and (+)-isothebaine(Fig.2.7) indicated that they are probably not derived from a tetrahydrobenzylisoquinoline by direct coupling.

Barton and co-workers investigated the formation of (+)-roemerine in *Papaver dubium* L. and found that tritium-labeled (+)-N-methylcoclaurine was well incorporated. The position of the hydroxy group in the precursor suggests that a proaporphine intermediate is involved (Figure 2.6) (48).

The biosynthesis of boldine in different plant species have been studied in detail, the quite different results were obtained as (a) and (b) (48).

(a) The biosynthesis of boldine in *Litsea glutinosa* (Lour.) C.B. Rob. *Var. glabraria* Hook (Lauraceae) has been studied by Kapil and co-workers. Of several variously methylated benzylisoquinoline precursors, only 4'-O-methylnorlaudanosoline, nor-reticuline and reticuline were precursors. In the latter case the (+)-reticuline was incorporated with considerable preference into boldine, Figure 2.10.

(b) The more definitive results have been obtained by Battersby and co-workers concerning the formation of dicentrine, corydine and glaucine in *Dicentra eximia* (Ker.) Torr. (Fumariaceae). Reticuline and orientaline were not precursors, but 4'-O-methylnorlaudanosoline and norprotosinomenine were effective precursors. 4'-O-methylnorlaudanosoline was converted into norprotosinomenine which must be incorporated into the three aporphine alkaloids by the way of the two neoproaporphine intermediates as indicated in Figure 2.11.

From Figure 2.10, norprotosinomenine was not a precursor of boldine in *Litsea glutinosa var. glabraria*, which contrasts with that of boldine in *Dicentra eximia* (Figure 2.11).

These results would suggest that boldine is produced by two different pathways in two different plants. If this is general trend, the biosynthesis of aporphine alkaloids may never be established. Clearly, this is an area in need of considerable further study, and at this time it is not clear which, or how many, of the possible biosynthetic routes may be operating in order to produce the various

aporphine alkaloids (48).

Benzylisoquinolines are not only the precursor of aporphines, but they are also the precursor of many other isoquinoline alkaloids which is summarized in Figure 2.4.

2.4 Biological Activities of Aporphine Alkaloids

Ushinsunine showed strong bacteriostatic against *Staphylococcus* and strong bactericidal action against *Shigella, Mycobacterium* and *Bacillus subtilis* (52).

Liriodenine (Oxoushinsunine) showed significant inhibitory activity against human carcinoma of the nasopharynx test system (53) and exhibited quite a wide range of antibacterial and antifungal activity *in vitro* (48, 54).

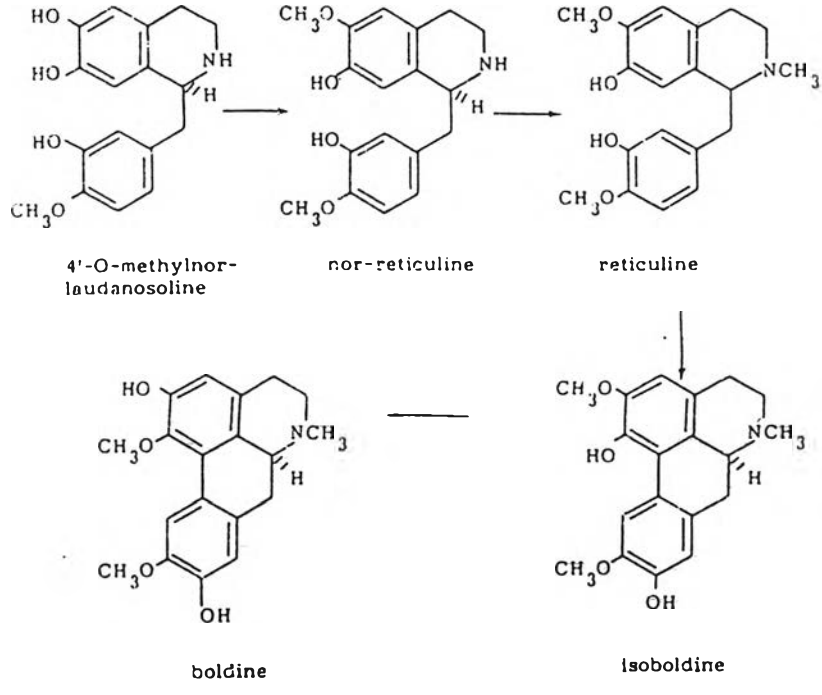


Figure 2.10 Biosynthesis of boldine in *Litsea glutinosa* var. *glabraria*.

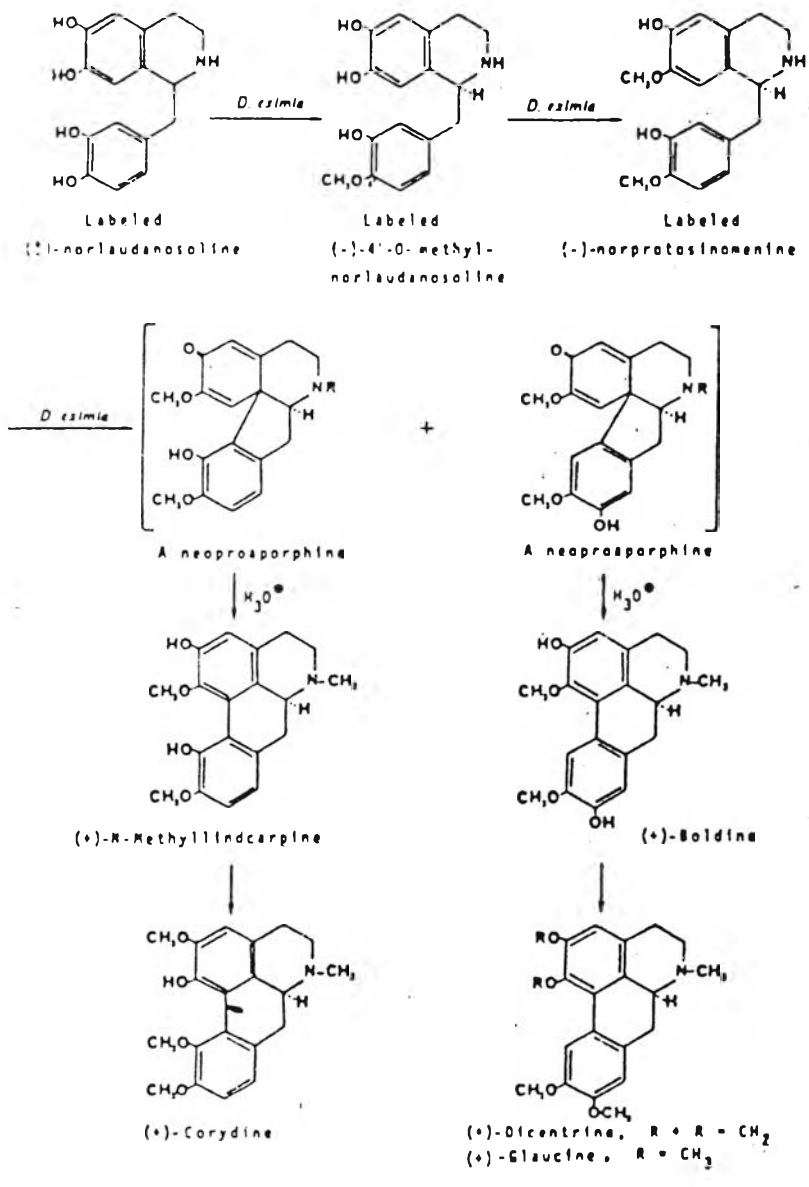


Figure 2.11 Biosynthesis of Corydine, Dicentrine and Glaucine in *Dicentra eximia* (Ker.) Torr.

3. SESQUITERPENE LACTONES

During the past 20 years over 500 sesquiterpene lactones have been isolated and identified from many species of higher and lower plants, chiefly from species of Compositae (58).

Sesquiterpene lactones were also isolated from various species of of the genus *Michelia* (Magnoliaceae) as shown in Table 1.

Two reasons can be given for the strongly increasing interest in this group of natural products. First, sesquiterpene lactones have been successfully used as markers in biochemical systematic (chemotaxonomy) studies, mainly in the Compositae. Second, more recently a number of compounds received considerable attention due to their various biological activities such as anti-tumor, cytotoxic, antimicrobial and phytotoxic activities and they are also known to poison livestock, to act as insect feeding deterents and to cause allergic contact dermatitis in humans (58, 64).

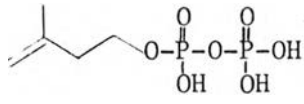
3.1 Chemistry of Sesquiterpene Lactones

Sesquiterpene lactones are a group of sesquiterpenoid, a class of terpenoid compounds, which possesses the functional group of lactone (cyclic keto ester).

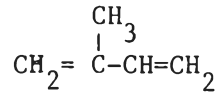
Terpenoids refer to the group of natural products which are derived biosynthetically from the 5-carbon compound, isopentenyl pyrophosphate which is also known as "activated isoprene". Isoprenoid compounds are also referred to as "terpenes" or "terpenoids" (63, 77).

Table 2. Natural products formed from isoprene units.

Class	Number of Isoprene Units	Occurrence
Hemiterpene (C_5)	1	Isoprene, emitted by the leaves of different species of higher plants.
Monoterpenes (C_{10})	2	Constituents of volatile oils, iridoid substances.
Sesquiterpenes (C_{15})	3	Constituents of volatile oils and resins.
Diterpenes (C_{20})	4	Constituents of volatile oils and resins, phytol, vitamin A.
Triterpenes (C_{30})	6	Squalene, steroids, pentacyclic triterpenes.
Tetraterpenes (C_{40})	8	Carotenes, xanthophylls.
Polyterpenes $(C_5)_n$	n	Rubber, gutta-percha, balata



Isopentenyl Pyrophosphate



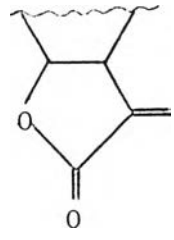
Isoprene

Terpenoids or isoprenoid compounds are classified according to the number of isoprene units contained in their molecules into monoterpenes, sesquiterpenes, diterpenes etc. Table 2. illustrates the variety of natural products formed from isoprene units.

Sesquiterpene lactones are colorless, bitter, relatively stable, lypophilic constitunets which are biogenetically derived from *trans-trans* farnesyl pyrophosphate following an initial cyclisation and subsequent oxidative modifications (58).

3.2 Classification of Sesquiterpene Lactones

Their classification is based on their carbocyclic skeleton in which the suffix "olide" refers to the lactonic function. In sesquiterpene lactones formed by oxidation of the "head" methyl group of farnesol, the lactonic function commonly represents an α-methylene γ-lactone moiety (1), or a biomodified functionality derived from (1) (64).



(1)

The majority of sesquiterpene lactones belongs to this category which can be considered biogenetic derivatives of the largest class, the germacranolides (2). The presently known structural classes and names of the various carbocyclic ring systems are shown in Figure 2.12 and the presumed biogenetic relationships are indicated by arrows (64). Structural modifications of the basic terpene skeleton involve the incorporation of an epoxide ring, hydroxyl groups (generally esterified) and or a 5-carbon acid, such as tiglic or angelic acid. Some sesquiterpene lactones also contain covalently bond halogen atoms. The α,β-unsaturated lactone is either *cis*- or *trans*-fused to the C_6-C_7 or C_8-C_7 positions of the carbocyclic skeleton, for the sake of simplicity, only the 7, 6-lactonized types are presented in Figure 2.12 (58, 64).

Other minor groups of sesquiterpene lactones, are formed by biosynthetic routes distinctly different from the above skeletal types (64).

The germacranolides represent the largest group of sesquiterpene lactones with nearly 300 known naturally occurring members. Recent recognition of configurationally isomeric germacranolides has led to a reclassification into four subgroups which are characterized by a cyclodecadiene skeleton with double bonds in the C-1.10-and C-4.5-

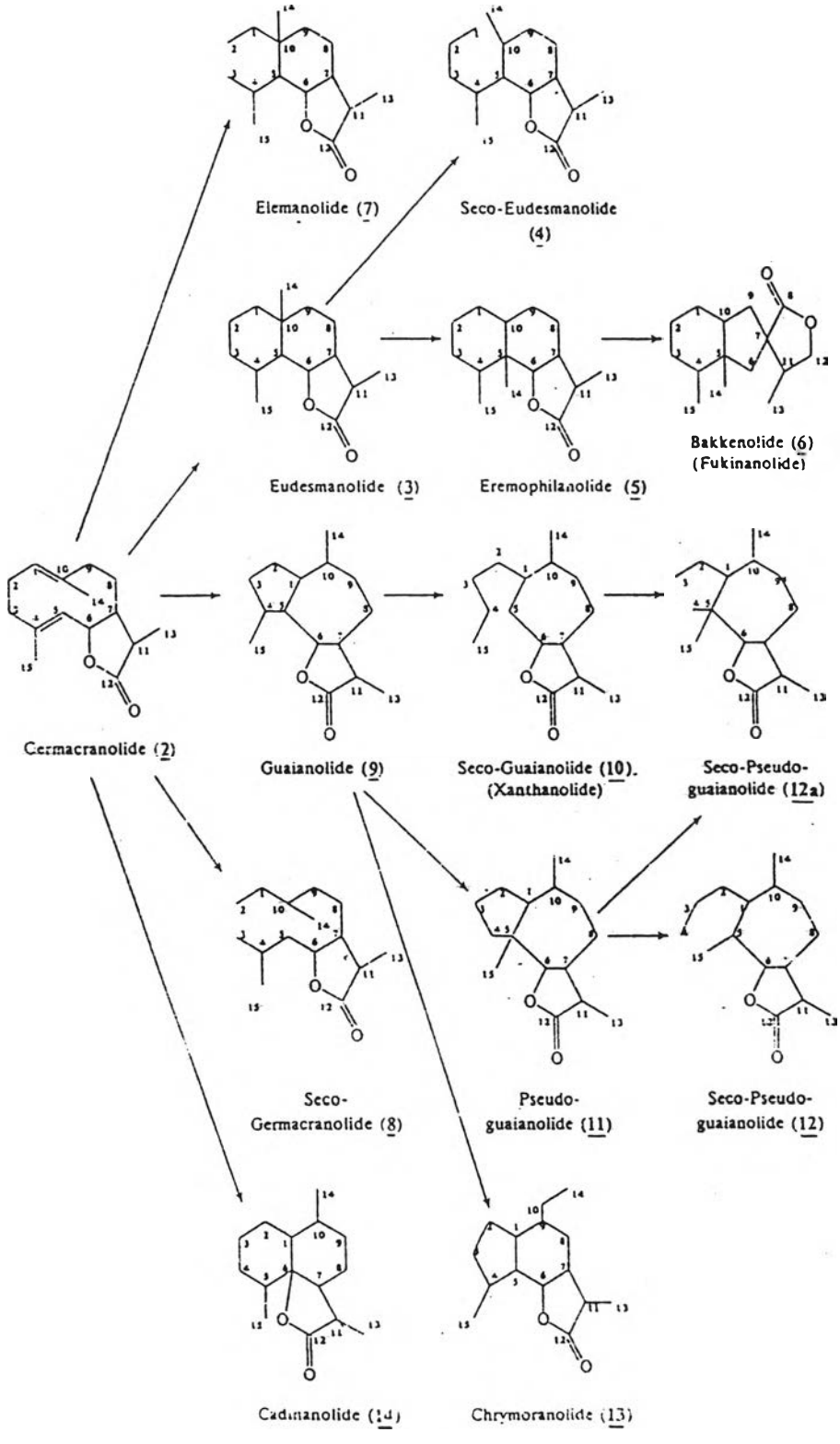


Figure 2.12 Types and biogenetic relationships of germacranolide-derived sesquiterpenes

positions as shown in Figure 2.13. Among the four subgroups, the majority is germacrolide type (64).

Structures of some sesquiterpene lactones isolated from various species of the genus *Michelia* (Magnoliaceae) were shown in Figure 2.14.

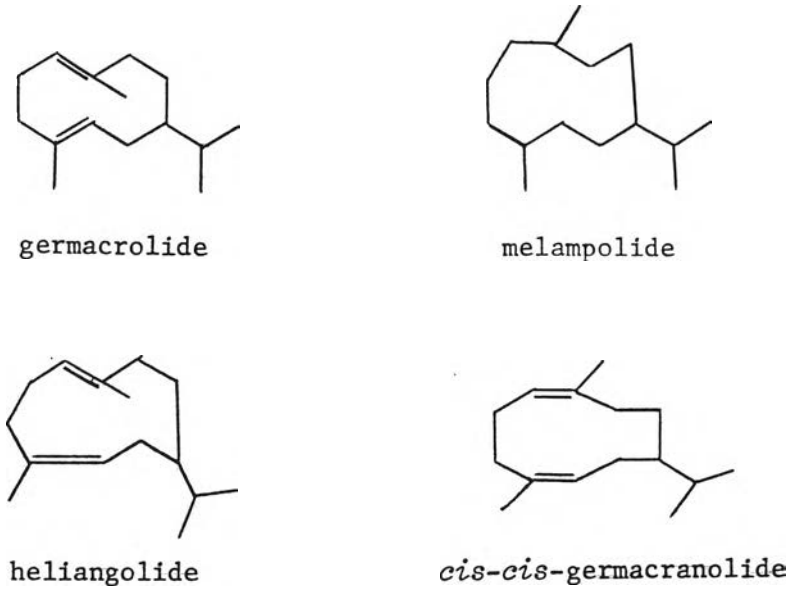


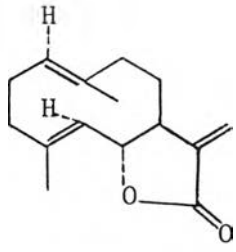
Figure 2.13 Configurational types of germacranolides.

3.3 Distribution of Sesquiterpene lactones

An individual plant species generally yields only one skeletal type, with oxidative variations on that skeleton. In genera having wide-ranging geographical distributions, a given species may exhibit considerable infraspecific variation in its sesquiterpene lactone structures. For example, the weedy annual, *Ambrosia confertiflora* elaborates as many as four different sesquiterpene lactone-types in populations derived from Maxico and central Texas. The highest concentration of lactones is found in the leaves and flowering heads (phyllaries). Large amounts are stored in glandular trichomes of the upper leaf surface, phyllaries and achenes of *Parthenium*

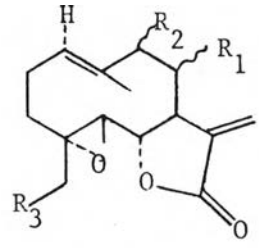
hysterophorus (58). The percentage of lactones obtained can vary quantitatively in a given species from 0.001-5 % , dry weight. In taxa of *Artemisia*, the lactone content may vary from winter to summer (60). Lactones are rarely found in stems and roots but eudesmanolides have been reported from the roots of *Liriodendron tulipifera* (Magnoliaceae) (61). and from the bark of numerous Brazilian species of *Eremanthus* (Compositae) (62).

Sesquiterpene Lactones are common constituents of most genera of the Compositae with the exception of the evolutionary "advanced" tribe, the Tageteae. They have been reported to occur sporadically in genera of the Umbelliferae, Magnoliaceae, Lauraceae, Winteraceae, Illiciaceae, Aristolochiaceae, Menispermaceae, Cortiariaceae, Acanthaceae, Bursereae, Hepaticae, Amaranthaceae and Cannellaceae. Eudesmanolides, similar to those found in genus *Inula* (Compositae), are also present in the liverworts (Hepaticae) *Frullania dilatata*, *F. tamarisci* and *Diplophylum albicans* (58, 59).



Costunolide

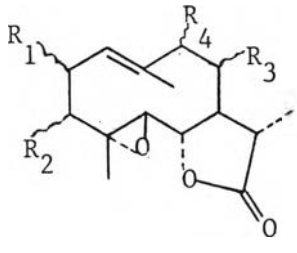
(Germacrolide Type)



Parthenolide; $R_1=R_2=R_3=H$

11,13-Dehydrolanuginolide; $R_1=\alpha$-OAc, $R_2=R_3=H$

(Germacrolide Type)

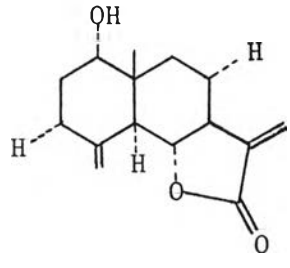


Dihydroparthenolide;

$R_1=R_2=R_3=R_4=H$

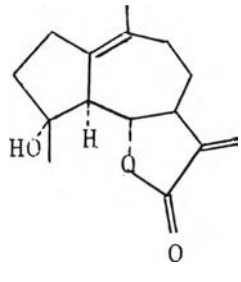
Lanuginolide; $R_1=R_2=R_4=H, R_3=\alpha$-OAc

(Germacrolide Type)



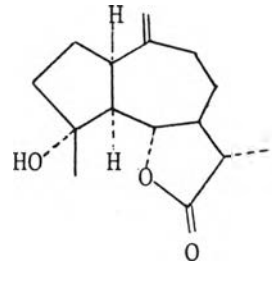
Reynosin

(Eudesmanolide Type)



Micheliolide

(Guaianolide Type)



Compressanolide

(Guaianolide Type)

Figure 2.14 Structures of some sesquiterpene lactones isolated from *Michelia* spp.

3.4 Biosynthesis of Sesquiterpene Lactones

There is general acceptance of the view that terpenoid compounds, from terpene to polyterpene and steroids, are the end products of a metabolic pathway that can be described in the following general terms :

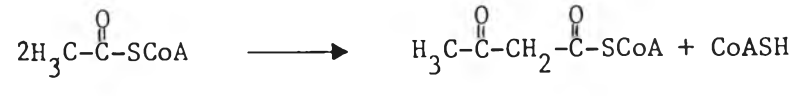
acetate ⟶ mevalonate ⟶ isopentenyl pyrophosphate ⟶ geranyl pyrophosphate ⟶ farnesyl pyrophosphate ⟶ $(C_5)_x$ compounds.

Biosynthesis of sesquiterpenoids involves modification and/or cyclisation of pyrophosphate esters of *trans, trans*-farnesol, *cis, trans*-farnesol or nerolidol (64, 65, 66)

Therefore, the proposed biosynthetic pathways of sesquiterpene lactones are involved the following steps :

3.4.1 Conversion of Acetyl-CoA to Acetoacetyl-CoA.

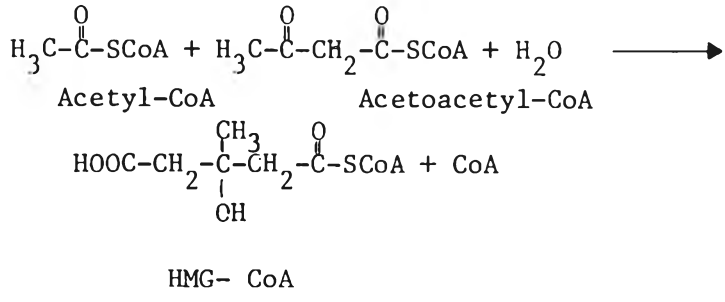
Acetoacetyl-CoA thiolase catalyzes the condensation of two molecules of acetyl-CoA to form Acetoacetyl-CoA (67).



Acetyl-CoA Acetoacetyl-CoA

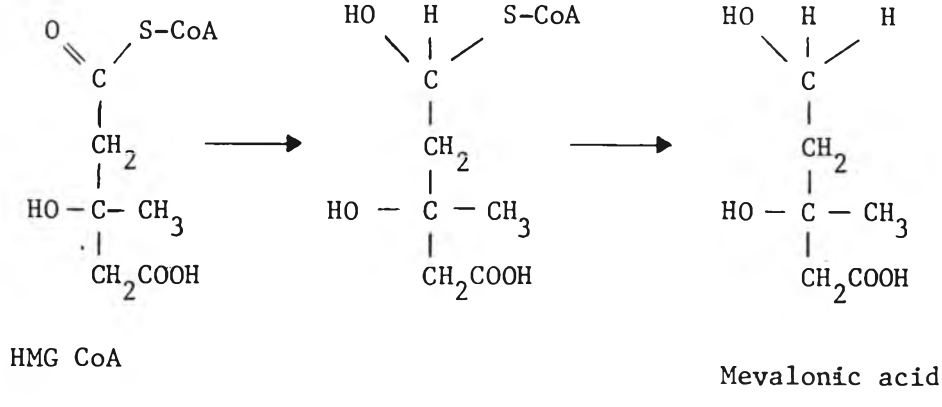
3.4.2 Conversion of Acetoacetyl-CoA to β-Hydroxy-β-Methylglutaryl-CoA (HMG-CoA)

β-Hydroxy-β-methylglutaryl-CoA synthase catalyzes the condensation of acetoacetyl-CoA with acetyl-CoA to form HMG-CoA (68).



3.4.3 Conversion of HMG-CoA to Mevalonic Acid

β-Hydroxy-β-methylglutaryl coenzyme A reductase catalyzes the reduction of D-HMG-CoA by NADPH to form mevalonic acid (69).

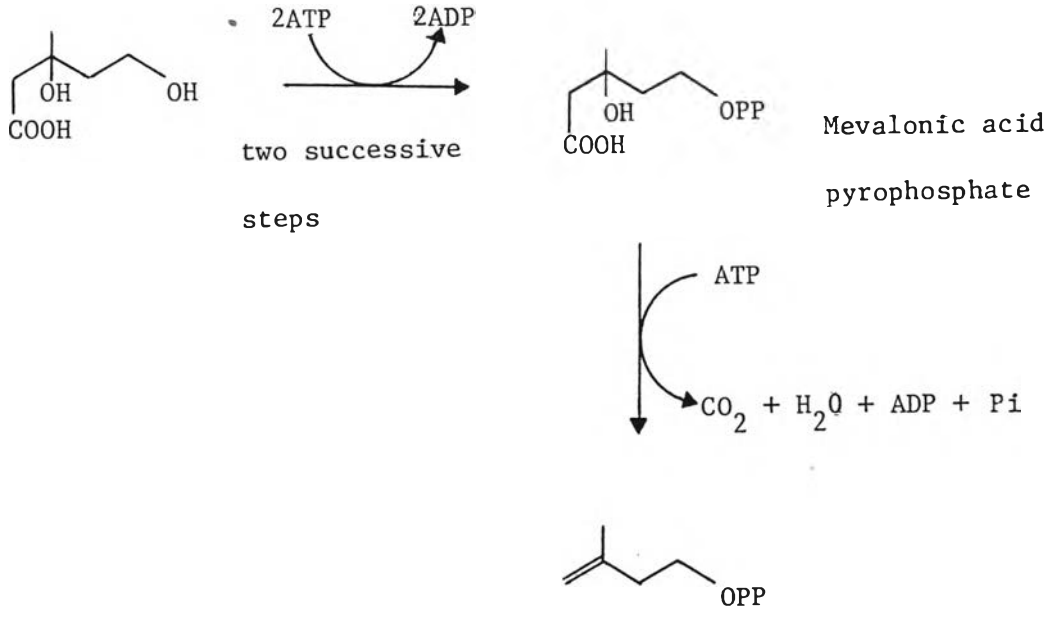


The two-step reduction of HMG-CoA to mevalonic acid. Mevaldic hemithioacetal is the intermediate formed in this reaction.

3.4.4 Conversion of Mevalonic Acid to Isopentenyl Pyrophosphate (IPP)

The formation of IPP from mevalonate involves two consecutive phosphorylations at position 5 to form mevalonic acid pyrophosphate. One mole of ATP is required for each phosphorylation reaction. Isopentenyl pyrophosphate is obtained from mevalonic acid

pyrophosphate by decarboxylation and elimination of a molecule of water. The reaction requires the presence of ATP and results in the production of ADP and inorganic phosphate. The exact mechanism of this reaction is unknown (70, 77).



The overall reactions for the conversion of acetyl-CoA to isopentenyl pyrophosphate were performed in the Fig. 2.15 (77).

The biosynthesis of isopentenyl pyrophosphate is widespread in living organisms such as bacteria, yeast, higher plants and mammals and it is this compound that is converted enzymatically to the wide variety of polyisoprenoid compounds (70).

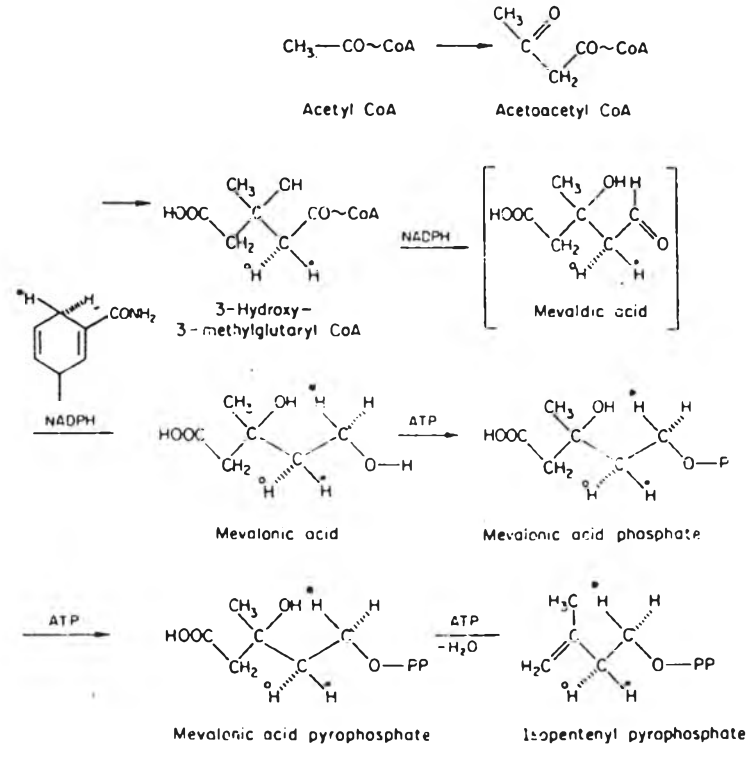
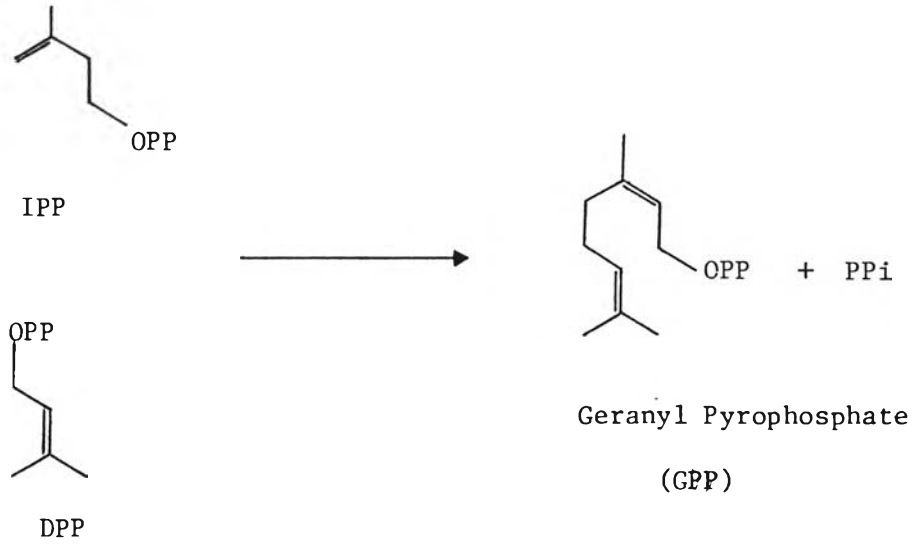


Figure 2.15 Biosynthesis of isopentenyl pyrophosphate from acetyl CoA.

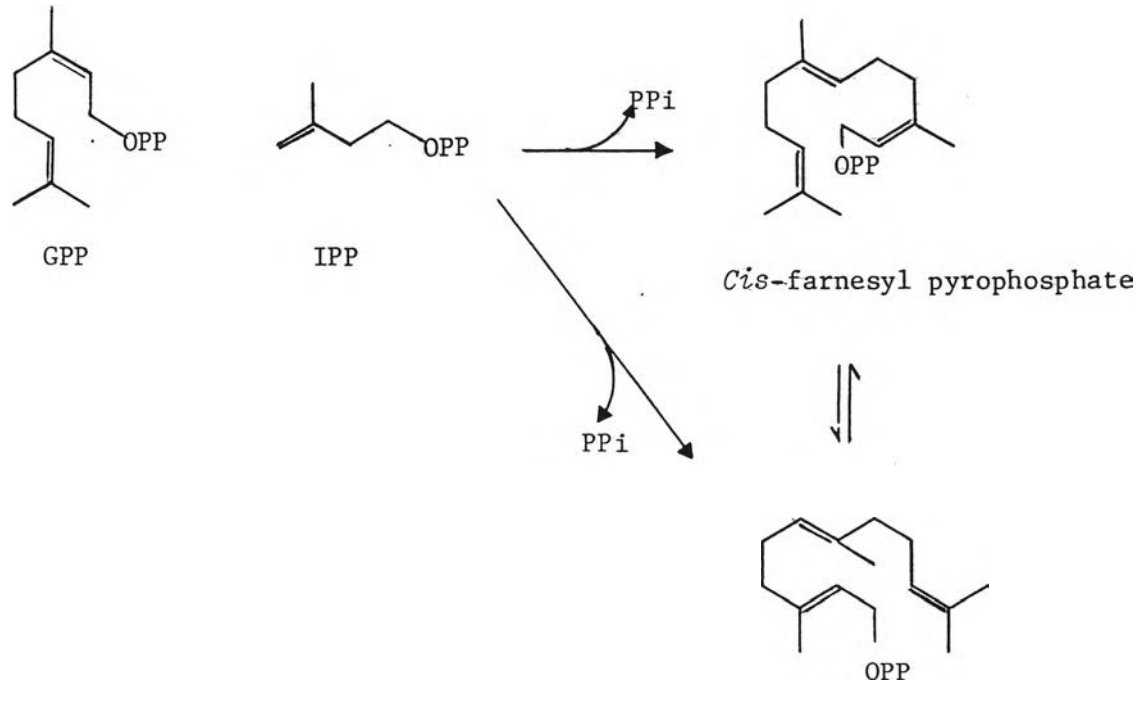
3.4.5 Isomerization of Isopentenyl Pyrophosphate (IPP) to Dimethylallyl Pyrophosphate (DPP) (72).

IPP DPP

3.4.6 Formation of Geranyl Pyrophosphate (GPP) from Isopentenyl Pyrophosphate (IPP) and Dimethylcellyl Pyrophosphate (DPP). (72).



3.4.7 Formation of Farnesyl Pyrophosphate from Geranyl Pyrophosphate (GPP) and Isopentenyl Pyrophosphate (IPP.) (72).



Both *cis*-and *trans*-farnesyl pyrophosphate are the precursors of sesquiterpenoid compounds with different carbon-skeletal types.

3.4.8 Formation of Germacradiene and the Lactone Ring

As outlined in Fig. 2.16, cyclization of *trans*, *trans*-farnesyl pyrophosphate (15) result in the *trans*, *trans*-germacradiene intermediate (16) which by enzymatic oxidative modifications provides the germacranolides represented by its simplest member, costunolide (17). From the germacradiene the different other skeletal types of sesquiterpene lactones shown in Fig. 2.12 can be derived (64, 65).

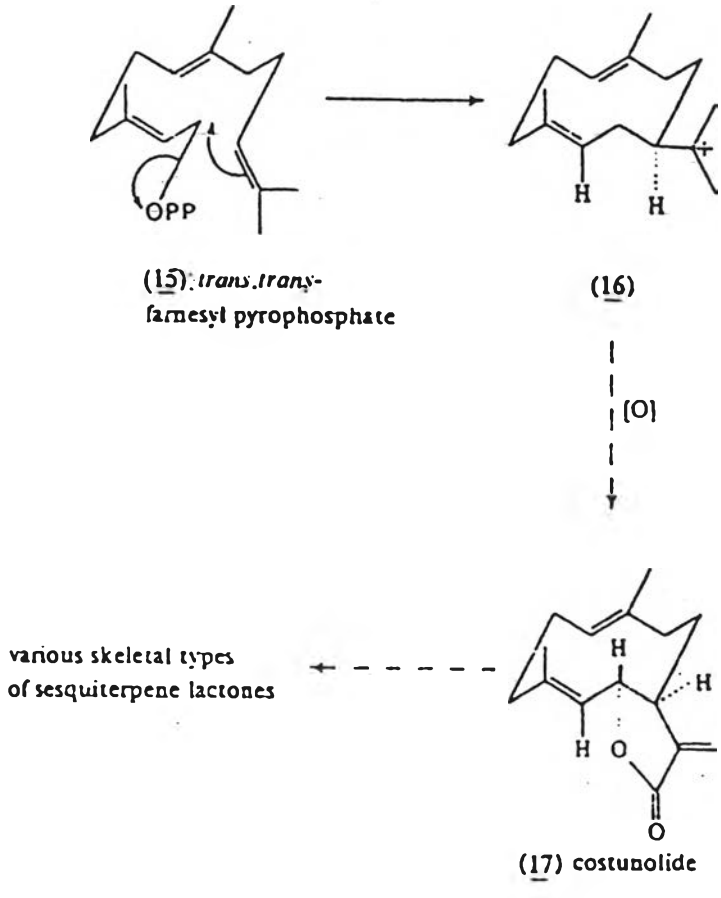


Figure 2.16 Biogenesis of the germacranolide skeleton.

Two possible biogenetic routes have been suggested for the formation of the lactone ring of these sesquiterpenoids. The various schemes of formation of the α,β-unsaturated γ-lactone of type (1) have been discussed by Geissman and Herz. Possible steps involved the biogenesis of costunolide (17) and inunolide (24) are outlined in Fig. 2.17. The overall process requires oxidative modifications at C-12 amd C-6 or C-8, respectively (64).

One hypothetical intermediate en route from cation (16) to lactone (17) and (24) is germacrene A (18), a naturally occurring hydrocarbon in which all non-olefinic carbons are allyllically activated for hydroxylation except C-8. Introduction of an oxygen function at C-12 in (18) to give alcohol (21) could either proceed via epoxide intermediate (19) or could involve the hydroperoxide (20), the latter being formed by an enzymatically-mediated reaction mimicking the reaction of singlet oxygen with olefins. In either case the process involves migration of a double bond from what was originally C-11, C-13 to C-11, C-12. Further oxidative modifications of (21) via aldehyde (22), acid (23) and hydroxylation at C-6 or C-8 would after lactonization give costunolide (17) or inunolide (24), respectively. Although, the question regarding the sequence of oxidations and the detailed mechanism remains open, the general routes outlined in Fig. 2.17 appear reasonable, since sesquiterpenes with oxidation patterns of the isopropenyl side chain indicated in (21) to (23) occasionally accompany the lactonic plant constituents (64).

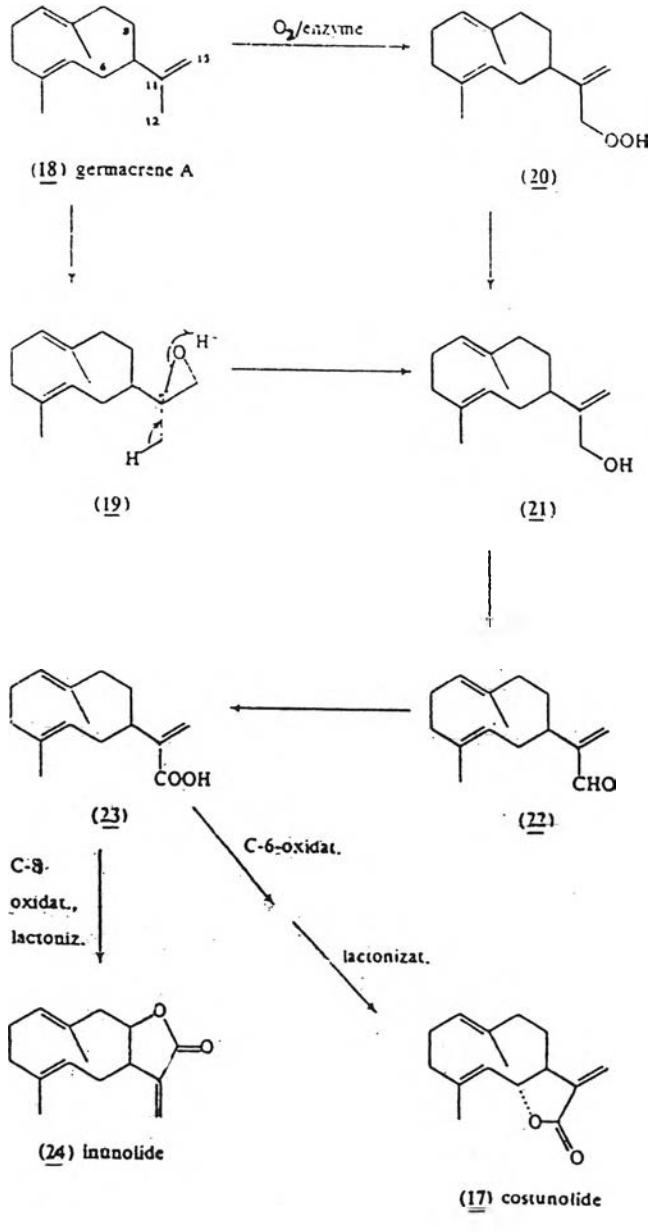
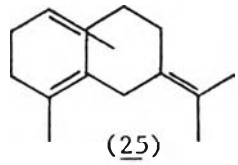


Figure 2.17 Biogenesis of the lactone ring

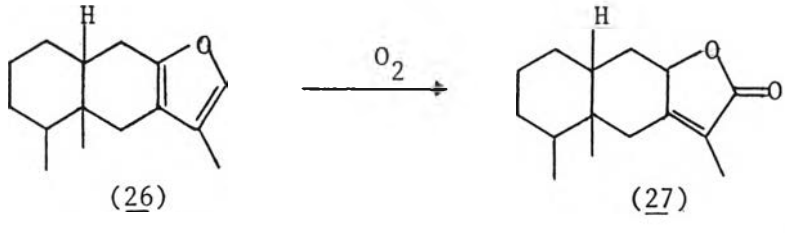
Germacrene B (25) is a possible precursor in lactone biosynthesis, since C-8 hydroxylation in a sesquiterpene lactone precursor of type (25) would now be favored due to allylic activation of C-8.



(25)

Furthermore, C-6 in (25) represents a doubly allylic carbon center favoring hydroxylation at this position over all other allylic carbons. This could possibly be the reason for predominant formation of C-6-oxygenated sesquiterpenoids (64).

Sesquiterpene lactones of type (27) commonly cooccur with and are derived from furanosesquiterpenes (26) by autoxidation suggesting that the lactones are also biogenetically derived from the furan ring as shown in Fig. 2.18.



(26) (27)

an eremophilanolide

Figure 2.18 Biogenesis of the lactone ring *via* furanosesquiterpenes.

3.5 Biological Activities of Sesquiterpene Lactones

Sesquiterpene lactones are a group of natural products which exhibit various interesting biological activities which will be described further.

3.5.1 Anti-tumor and Cytotoxic Activity

In a review of actineoplastic agents from plants, over 50 sesquiterpenes were evaluated for their growth-inhibitory potential against numerous tumor models. It was found that all the known cytotoxic sesquiterpenes contained a lactone function; all but one was α ,β-unsaturated and the α-ethylenic linkage was exocyclic in every case (58).

In a further study of the structure-activity relationships among sesquiterpene lactones, it was noted that the presence of a C_{11}-C_{13} exocyclic double bond conjugated to γ-lactone was essential for cytotoxicity (Fig. 2.19). Furthermore, it has been shwon that, in contrast to α-methylene-γ-lactones, which react rapidly with cysteine to form stable adducts, endocyclic α ,β-unsaturated-γ-lactones react slowly with cycteine, to form unstable adducts. These marked chemical difference coupled with the fact that elephantopin-bis (cycteine) adduct, which contains an endocyclic $\Delta^{\alpha,\beta}$ -lactone, is inactive led us to believe that the endocyclic lactone does not contribute significantly to the cytotoxic activity of the parent compound, elephantopin (58, 73).

In a recent study of bakkenolides from *Petasites alba*, *P. fragrans* and *P. hybridus*, it was noted that bakkenolide-A (Fig. 2.20) a β-methylene-γ-lactone (which does not have an $O{=}C{-}C{=}CH_2$ system) gave results against cells derived from human carcinoma (H. Ep-2, Table 3) similar to that reported for other sesquiterpene lactones. This recent finding suggests that other structural parameters must be taken into consideration when evaluating the cytotoxic potential of sesquiterpene lactones and absolute purity of test samples is obviously a prerequisite for precise study.

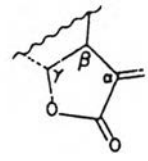


Figure 2.19 α-Methylene-γ-lactone, a major functional group for biological activity in diverse compounds

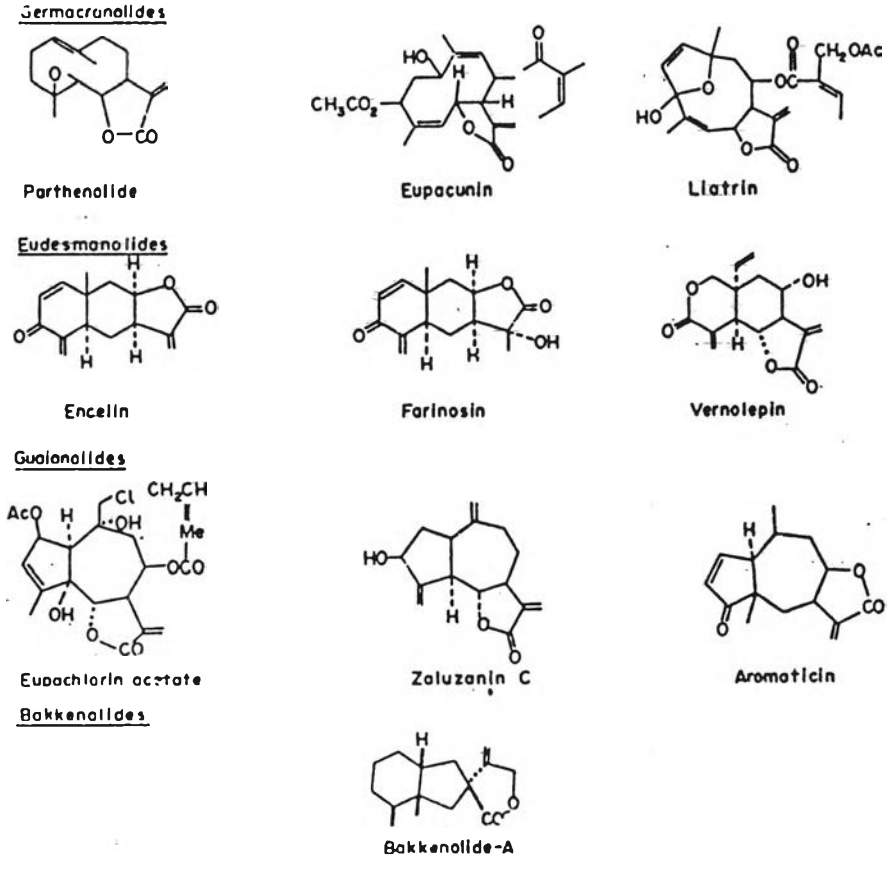


Figure 2.20 Structures of sesquiterpene lactones exhibited antitumor activity

Table 3 includes those sesquiterpene lactones that exhibit antitumor activity. As noted by numerous workers, the structures and reactivities of these sesquiterpene lactones (Fig. 2.20) may be associated with selective alkylation of nucleophilic groups in enzymes (eg. sulphydryl enzymes) which control cell division. Vernolepin, a eudesmanolide, has been shown to inhibit phosphofructokinase, an enzyme which has many -SH group (58).

3.5.2 Microbial growth-inhibitors (antibiotics)

Some sesquiterpene lactones have been shown to possess antibacterial, antifungal or anthelmintic properties. The germacranolides, mikanolide and dihydromikanolide (Fig. 2.21) from *Mikania monagasensis* inhibit the growth in culture of a bacterium, *Staphylococcus aureus* and also of a yeast, *Candida albicans*. Helenalin, a helenanolide common in species of *Helenium*, was shown to exhibit activity against the human pathogenic fungi, *Trichophyton mentagrophytes*, *T. acriminatum* and *Epidermophyton* sp. Parthenin, the major lactone from

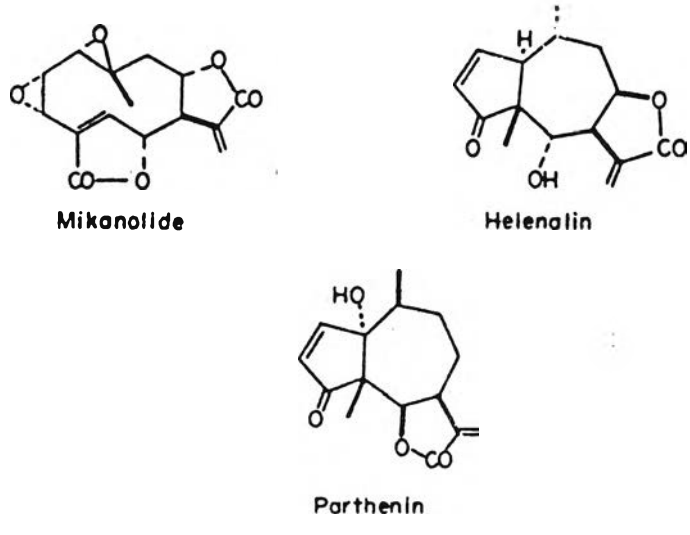


Figure 2.21 Structures of antimicrobial sesquiterpene lactones

Parthenium hysterophorus was reported to inhibit sporangial germiannation and zoospore mobility in *Sclerospora graminicola;* such activity against the conidial development of *Aspergillus flavus* was lacking (58).

3.5.3 Chemoprophylaxis by lactones in schistosomiases

The wood oils of the Brazilian trees, *Eremanthus elaegnus, Vanillosmopsis erythropappa* and *Mosquinea velutiva* (Compositae) contain lactones that inhibit skin penetration by cercariae of the trematode, *Schistosoma mansoni.* Analysis of the wood oils indicated that the sesquiterpene lactones, eremanthine, costunolide and β-cyclocostunolide (Fig 2.22) were the active principles. Dihydro-α-cyclocostunolide which lacks an exocyclic methylene group on the lactone ring was found to be inactive (62).

Recently a novel germacranolide, goyazenolide, isolated from *Eremanthus goyazensis* was also shown to have schistosomicidal properties (74). It was suggested that the activity of the schistosomicidal lactones may be related inhibition of sulphydryl groups in cercarial enzymes (58).

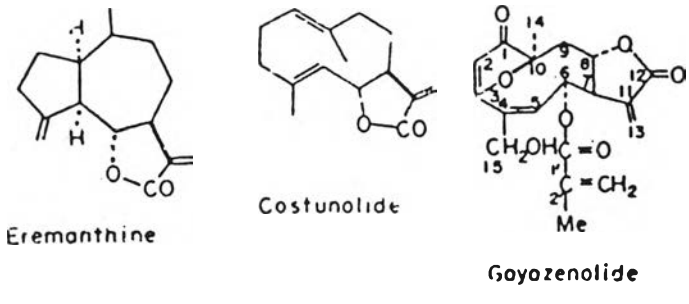


Figure 2.22 Structures of sesquiterpene lactones exhibited schistosomicidal activity

3.5.4 Allergic contact dermatitis in man.

Sesquiterpene lactones from species of the Compositae, Lauraceae and Magnoliaceae and from the liverwort, *Frullania* (Jubulaceae) have been shown to be a major class of allergens causing allergic contact dermatitis in humans. Over 80 sesquiterpene lactones were used in patch test to determine their allergenic potential, and the presence of an α-methylene group, exocyclic to the γ-lactone, was shown to be the principal immunochemical requisite for the production of dermatitis. One of the allergenic lactones tested was the pseudoguianolide, parthenin (Fig 2.21), the major allergen in *Parthenium hysterophorus* (58).

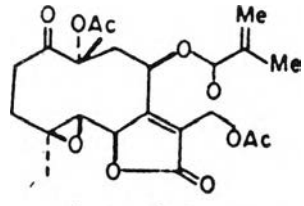
All the known allergic sesquiterpene lactones contain an exocyclic α-methylene function which may conjugate with sulphydryl groups of proteins in cells by a Michael-type addition to form complete antigens capable of producing cell-mediated contact allergic reactions (58).

Table 4 demonstrates the structures of some sesquiterpene lactones reported to cause allergic contact dermatitis in humans.

3.5.5 Insect feeding deterrents

Experimental evidence that sesquiterpene lactones provide resistance to insect feeding has been demonstrated by a study of the Compositae and *Vernonia*. Larval feeding experiments were conducted on *Spodoptera eridania, S. frigiperda, S. ornithogalli, Diacrisia virginia and Trichoplusia ni* to determine the feeding preference of the 6 species was noted to the presence of glaucolide-A (Fig.2.23).

Supplementing the agar medium with glaucolide-A, the major lactone in numorous species of *vernonia*, resulted in greatly reduced larval feeding; feeding was inversely proportional to the concentration of glaucolide-A in the medium. It was suggested that, in the course of evolution, these compounds have been selected quantitatively and qualitatively in resistance to herbivore pressure (58).

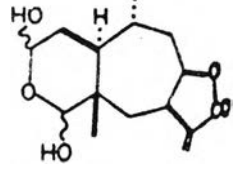


Glaucolide-A

Figure 2.23 Insect feeding deterrent sesquiterpene lactone

3.5.6 Vertebrate poisoning

Livestock-poisoning from foraging on bitter tasting plants of the compositae is well documented in agricultural literature. For example, *Hymenoxys odorata* (bitterweed) is an important livestock toxicant that affects primary sheep and goats. Recent chemical studies on *Hymenoxys odorata* have shown that hymenoxin (Fig 2.24), the major sesquiterpene lactone in populations of *H. odorata* from Texas, is the toxin involved in the death of sheep. It was suggested that the sesquiterpene lactone toxicant may alter the microbial composition of the rumen and thus affect vital metabolic functions (58).



Hymenoxin

Figure 2.24 Vertebrate poisoning sesquiterpene lactone

3.5.7 Plant-growth inhibitors (phytoxins)

A variety of sesquiterpene lactones of different skeletal types has been reported to show plant growth regulatory activity (75). Heliangine, the major germacranolide of *Heleanthus tuberosus*, inhibits the elongation of *Avena* coleoptile sections but promotes adventitious root formation of *Phaseolus* cuttings. Promotion of adventitious root formation was reduced by the addition of cysteine or by hydrogenation of the exocyclic methylene group (75).

An examination of sesquiterpene lactones that exhibit growth inhibitory properties indicated that the following structural configurations are at least the principal requirements for biological activity:

a) the presence of an exocyclic methylene conjugated to a γ-lactone;

b) the presence of a functional group, such as an epoxide, hydroxyl, chlorohydrin, unsaturated ketone or O-octyl adjacent to the α-CH_2 of γ-lactone which can enhance the reactivity of the conjugated lactone toward biological nucleophiles (58,75).

As noted previously, the inhibitory action of sesquiterpene lactones results from the presence of highly electrophilic functional groups. These selectively alkylate by Michael-type addition to sulphydryl proteins, specifically thiol groups in preference to other nucleophiles.

Biological activity of sesquiterpene lactones should be studied further in order to complete the previous information.

Table 3 Sesquiterpene lactones demonstrated to have antitumor and cytotoxic activity*

Compound +	Plant source	Tumor-system‡ assayed
Germacranolides		
Ridentin	*Artemisia* sp.	WI/H.EP-2
Parthenolide, Tamaulipin-A, Tamaulipin-B	*Ambrosia confertiflora*	WI/H.EP-2
Chamissonin diacetate	*Ambrosia chamissonis*	KB
Eupacunin	*Eupatorium cuneifolium*	KB
Liatrin	*Liatris* sp.	KB
Elephantopin, Elephantin	*Elephantopus elatus*	KB/OS/WM
Eupatolide	*Eupatorium formosanum*	H.EP-2
Molephantin	*Elephantopus millis*	H.EP-2
Phantomolin	*Elephantopus millis*	H.EP-2
Deoxyelephantopin	*Elephantopus carolinianus*	WI
Guaianolides		
Eupachlorin acetate	*Eupatorium* sp.	KB
Deacetoxymatricarin	*Achillea lanulosa*	WI/H.EP-2/W-18Va2
Canin	*Artemisia cana*	WI/H.EP-2/W-18Va2
Arteglasin-A	*Artemisia douglasiana*	WI/H.EP-2/W-18Va2
Zaluzanin-C	*Zaluzania robinsonia*	PS
Pseudoguaianolides		
Helenalin	*Helenium autumnale*	KB/PS/H.EP-2
Helenalin derivatives		KB/PS/H.EP-2

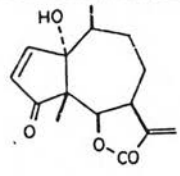
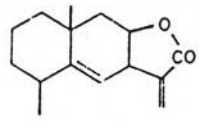
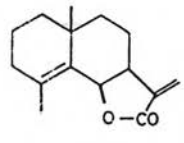
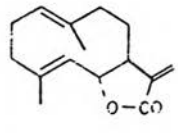
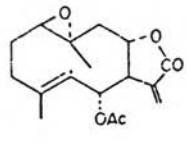
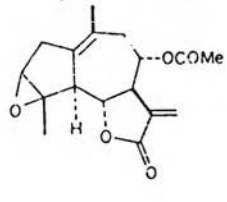
Table 3 (Continue)

Compound[+]	Plant source	Tumor-system[++] assayed
Aromaticin	*Helenium aromaticum*	KB
Mexicanin I	*Helenium mexicanum*	KB
Plenolin	*Baileya pleniradiata*	H.EP-2
Augustibalin	*Balduina angustifolia*	H.EP-2
Hymenoflorin	*Hymenoxys gradiflora*	LZ/PS
Ambrosin	*Hymenoclea salsola*	PS
Eudesmanolide		
α-Santonin	*Artemisia* spp.	WI/H.EP-2/W-18Va2
Vulgarin	*Artemisia vulgaris*	WI/H.EP-2/W-18Va2
Ludovicin	*Artemisia ludoviciana*	WI/H.EP-2/W-18Va2
Encelin	*Encelia farinosa*	WI/H.EP-2/W-18Va2
Farinosin		WI/H.EP-2/W-18Va2
Vernolepin	*Vernonia hymenolepsis*	KB
Bakkenolides		
Bakkenolide-A	*Petasites albus*	H.EP-2
	P. hybridus	
	P. fragrans	

Note : [*] Sesquiterpene lactones known to be actineoplastic agents and reviewed by Hartwell and Abbott (1969) are not included in this table. [+] Refer to Fig. 2.20 for structures. [++] Code for tumor system assayed follows that of Hartwell and Abbott (1969) : H.Ep -2 -Human epidermoid carcinoma of larynx; KB-Human epidermoid carcinoma of the nasopharynx. Cell cultures;

PS-P-388 lymphocytic leukemia. Mouse; WI- Walker carcinosarcoma 256. Ascites. Rat; WA- Walker carcinosarcoma 256. Rat; WM- Walker carcinosarcoma 256, Intramuscular; WI- 38-Human diploid fibroblast; W-18Va2-Simian virus 40-transformed cell of human origin; LZ-Leukemia L-210. Mouse (subcutaneous).

Table 4 Some sesquiterpene lactones reported to cause allergic contact dermatitis in humans (58).

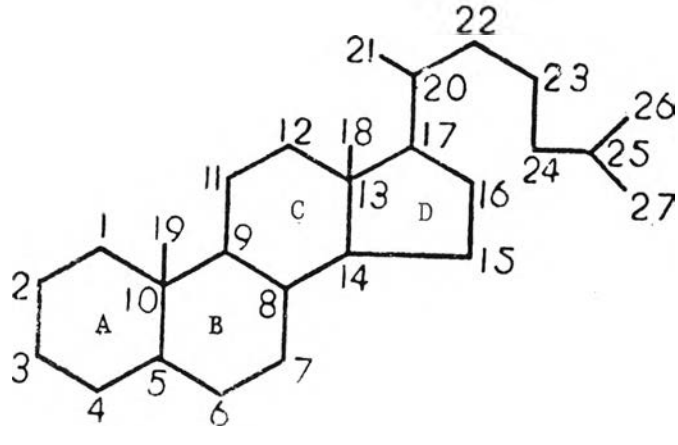
Compound	Plant source
 Parthenin	*Parthenium hysterophorus*
 Alantolactone	*Inula helenium* *I. racemosa*
 Frullanolide	*Frullania tamarisci*
 Costunolide	*Saussurea lappa* *Laurus nobilis*
 Pyrethrosin	*Chrysanthemum* spp.
 Arteglasin-A	*Chrysanthemum indicum*

4. STEROIDS

4.1 Chemistry of Steroids

Steroids are the most important tetracyclic triterpenes occurring in nature. They originate from cyclopentanoperhydrophenanthrene (sterane), and in most cases carry a hydroxyl group at position 3 and are often substituted by methyl groups at position 10 and 13 and by a side chain at position 17. In addition, further methyl groups, hydroxyl groups, double bonds etc. may be present (77, 78).

Steroid numbering system is as follows :-



4.2 Distribution of Steroids

Steroids are probably synthesized by all living organisms. Even bacteria and blue - green algae, long believed to be free of steroids, have now been found to contain cholesterol and other sterols. Certain representatives often occur in plants as well as animals. The plant steroids, however, are mostly glycosides while those in animals occur almost extensively in the free form (77, 78).

The plant steroids include sterols with 27 or more carbon atoms, sapogenins and alkaloids with 27 carbons, neutral and basic C_{21} steroids, cardiac aglycones with 23 or 24 carbons, and derivatives of androstane (C_{19}) and estrane (C_{18}) (78).

However, it is true that the distribution of some steroids is limited to a few plant families. At the same time it is becoming evident that many steroids occur in both plants and animals. In fact, the only classes of plant steroids not encountered in animals so far are the alkaloids with 21 and 27 carbons (78).

It is quite likely that many steroids in animals come from a plant diet, e.g., various C_{28} and C_{29} sterols, the C_{27} cholegenins and the C_{23} cardenolides. Some classes of animal steroids have not been found in plants so far, notably the bile acids and alcohols and the C_{24} alkaloids (78, 80).

4.3 Classification of Steroids

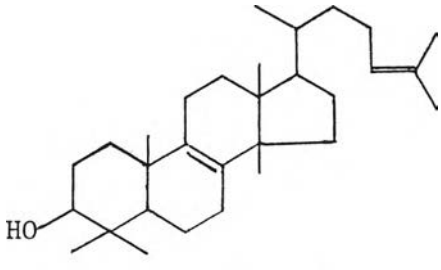
Steroids which are based on the cyclopentanoperhydrophenanthrene ring system, can be divided into at least five groups of compounds : sterols, steroid hormones, steroid saponins, steroid alkaloids and cardiac glycosides.

4.3.1 Sterols

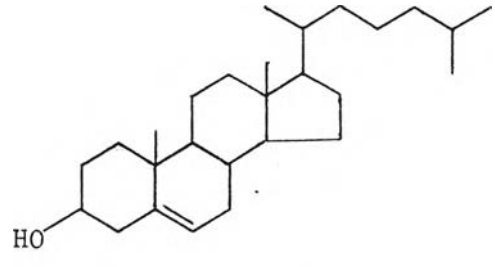
Steroids which are characterized by a long isoprenoid side chain at carbon atom 17 are called sterols. These compounds usually consist of 27 to 29 carbon atoms (81). Fundamental steroid nucleus is the same as that of lanosterol and other tetracyclic triterpenoids, but only two methyl groups are attached to the ring system, at position 10 and 13. The eight-carbon side chain found in lanosterol is also present

in many sterols, especially from animal sources; but most plant sterols have one or two additional carbon atoms. Most higher plant sterols have an α-24 alkyl group (79). The structures of lanosterol and a sterol are shown in Fig. 2.25.

The name "sterol" applies specifically to steroid alcohols; but since practically all plant steroids are alcohols with a hydroxyl group at C-3, they are frequently all called sterols (79).



Lanosterol



Cholesterol

Figure 2.25 Structures of lanosterol and cholesterol (a C_{27} sterol)

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 :

4.3.1 (a) C_{27} Sterols

Cholesterol, once believed to be the typical animal sterol, has recently been found to be rather widely distributed among plants.

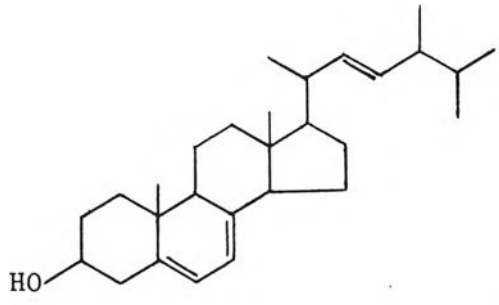
So far, cholesterol has been identified in various algae; in the pollen of many plants, including the date palm, cottonwood,

sunflower, dandelion, cat's-ear and mustard, in the seeds of many plants, including the soybean, peanut, oat, apple, avocado and oil palm; in the epigeous parts of *Dioscorea spiculiflora* and other *Dioscorea* species, tobacco, beans, corn, spinach, *Digitalis canariensis* and *D. purpurea;* in the needles and bark of pine trees; in the bark of *Erythrina superba* and in the roots of the cactus, *Wilcoxia viperina* (78).

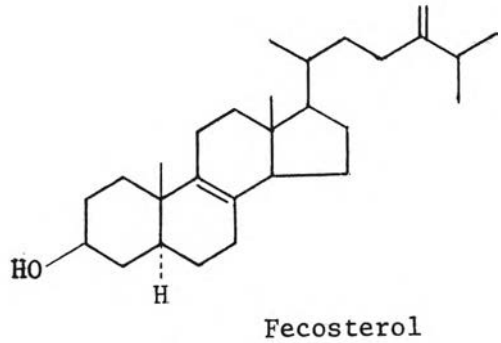
Cholesterol has a very important function in plants where, as in animals, it, or one of its precursors, serves as the starting material for the biosynthesis of all other steroids (80, 81), such as molting hormones and related products; C_{27} sapogenins and alkaloids; various pregnane derivatives, including progesterone and the C_{21} alkaloids; cardiac aglycones; and probably also the sex hormones (78).

4.3.1 (b) C_{28} Sterols

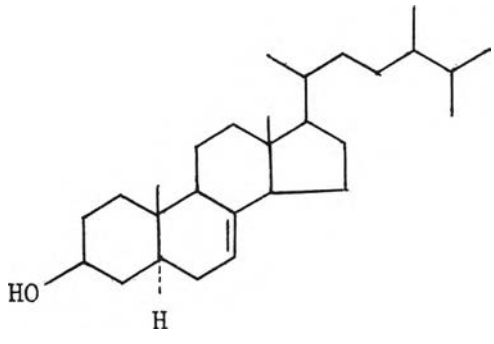
The C_{28} sterols derived from the C_{27} sterols and from either the carboxyl or methyl carbon of acetate for C-28 (87). The most important C_{28} sterol is ergosterol (Fig. 2.26) which was first isolated from ergot (87, 88) but also occurs in yeast and in most fungi, but more recently it has also been discovered in higher plants (78). Irradiation with ultraviolet light converts ergosterol to vitamin D_2 (ergocalciferol), so it is quite possible to find vitamin D_2 in plants (78). Fecosterol, fungisterol and 5-dihydroergosterol (Fig. 2.26) are biogenetically related to ergosterol and also occur in yeasts and other fungi (87).



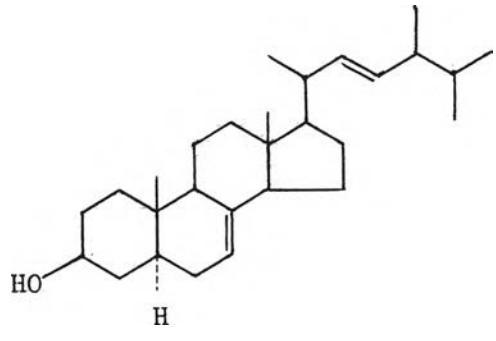
Ergosterol



Fecosterol



Fungisterol

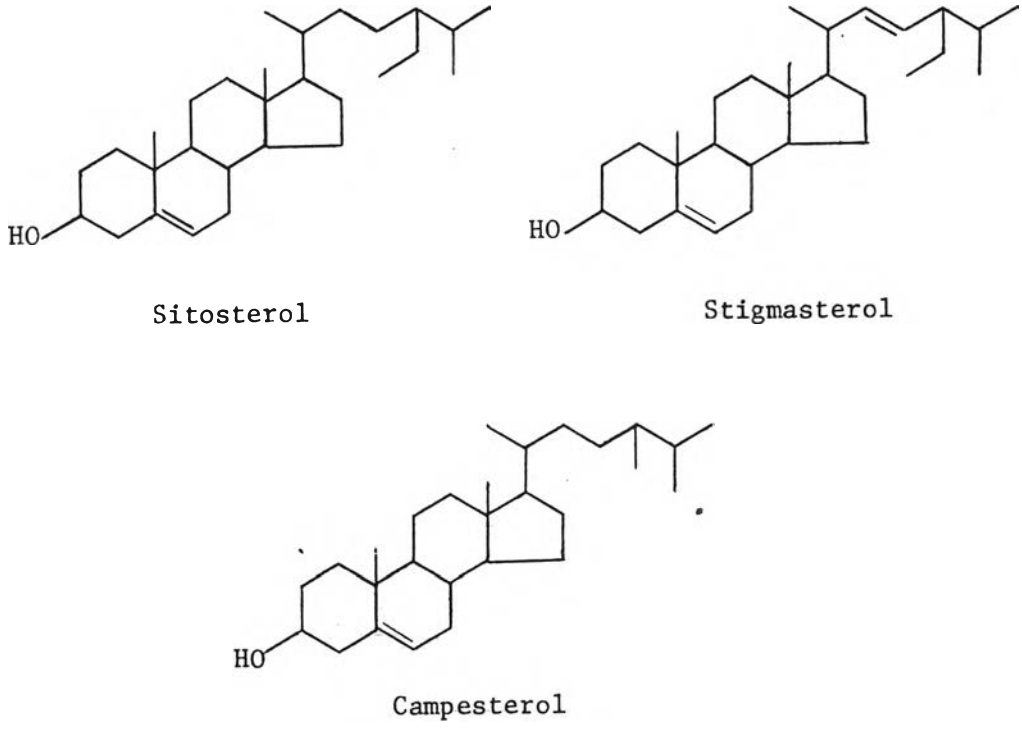


5-dihydroergosterol

Figure 2.26 Some C_{28} Sterols

4.3.1 (c) C_{29} Sterols

The most widely distributed sterols in higher plants are sitosterol, stigmasterol and campesterol (Fig. 2.27) which are called Phytosterols. These common sterols occur both free and as simple glycosides (78, 87, 89). The sterol most often isolated from plants is sitosterol but stigmasterol and campesterol are also quite common (90).



Sitosterol

Stigmasterol

Campesterol

Figure 2.27 Some plant sterols (Phytosterols)

Sitosterol is generally called β-sitosterol, but since both α and γ -sitosterols have turned out to be mixtures, the designation sitosterol is now unequivocal (78). β-sitosterol is the most widely distributed plant sterol (86). 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 sterol in marine vertebrates and was called clionasterol before its identity with γ-sitosterol was recognized. Toads also secrete α-sitosterol through their skin glands (87).

Stigmasterol 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 and other steroid hormones. The 24-epimer of stigmasterol occurs in various marine invertebrates. 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 differentation (78, 87).

4.3.2 Steroid Hormones

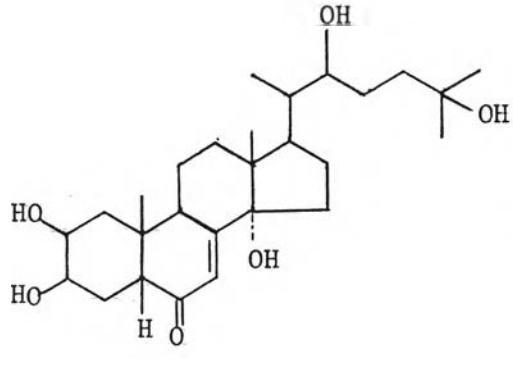
There are a large number of steroid hormones in nature. They are classified into two groups according to the number of carbon atoms to be steroid hormone with 27 to 29 carbon and 18 to 21 carbon atoms.

4.3.2 (a) C_{27} to C_{29} Steroid Hormones

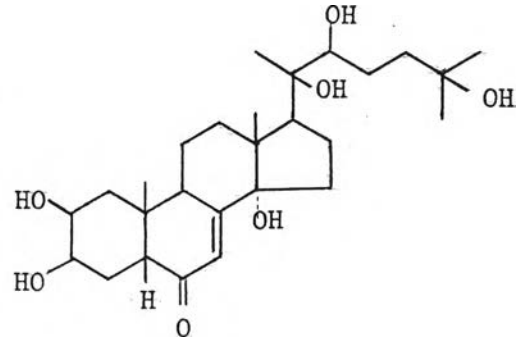
This group is the most insect-molting hormones. Insect-molting hormones in plants are sometimes referred to as phytoecdysones because the structures of the C_{27} hormones are analogous to that of ecdysone (Fig 2.28) which had earlier been isotated from insect and call α-ecdysone. Their common features are the 14α-hydroxyl group and the Δ^{7}-6-keto group. Other hydroxyl group may be attached to various positions (78).

Insect-molting hormones are water-soluble in contrast to other sterols (78, 85). The hydroxyl groups are in positions rarely substituted in natural sterols.

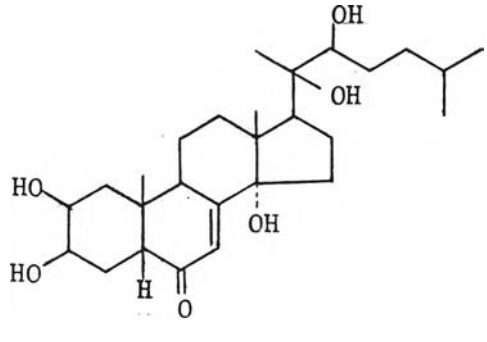
The most widely distributed representative of this group is ecdysterone or the 20-hydroxyecdysone and formerly also called β-ecdysone. Ecdysterone (Fig 2.28) has been found in various ferns, yews, *Podocarpus* and *Achyranthes* species and in mulberry and *Vitex megapotamica* leaves. So far, over twenty insect-molting hormones have been isolated from plants (78), some examples were shown in Fig 2.28. They often occur in much larger amounts and greater variety in plants than they do in insects, and the plant compounds are often more potent in activity than those found in insects (80).



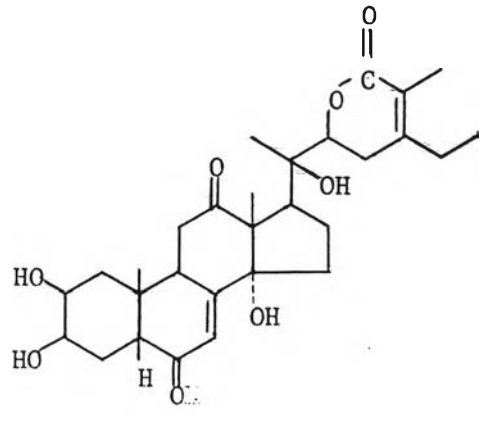
Ecdysone



Ecdysterone



Ponasterone A



Ajugalactone

Figure 2.28 Insect-molting hormones isolated from plants.

4.3.2 (b) C_{18} to C_{21} Steroid Hormones

The degradation of cholesterol to pregnenolone and its conversion to progesterone (Fig 2.29) which is the key reaction in the biosynthesis of steroid hormones in animals is also observed in higher plants (80,82). Other steroids may undergo analogous degradation in plants. Sitosterol is similarly converted to progesterone by *Digitalis* plants, which further convert it to the adrenocortical hormone, deoxy-corticosterone (Fig. 2.30) (82). Tomatoes carry out the same degradation of tomatidine to allopregnenolone (Fig 2.30) as the basic step in the partial synthesis of steroid hormones (80). Microorganisms are also known to be capable of converting sapogenins to C_{21} and C_{19} steroids (82).

In animal, the C_{19} and C_{18} steroids are formed from C_{21} by successive degradation steps (Fig 2.29). Although this reaction sequence has been observed in microorganisms, it is unable to demonstrate in higher plants. Fig. 2.31 shows C_{19} steroids identified in plants (82).

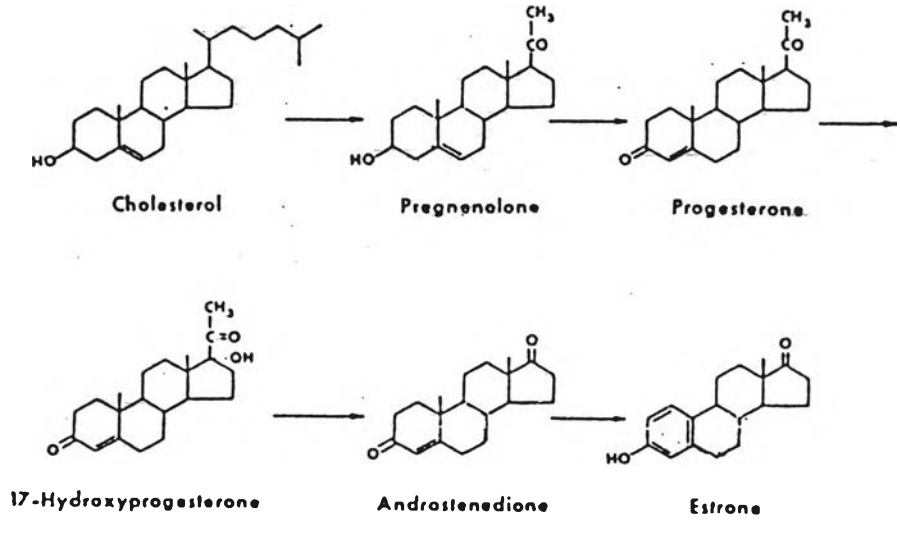


Figure 2.29 Biogenesis of esterone

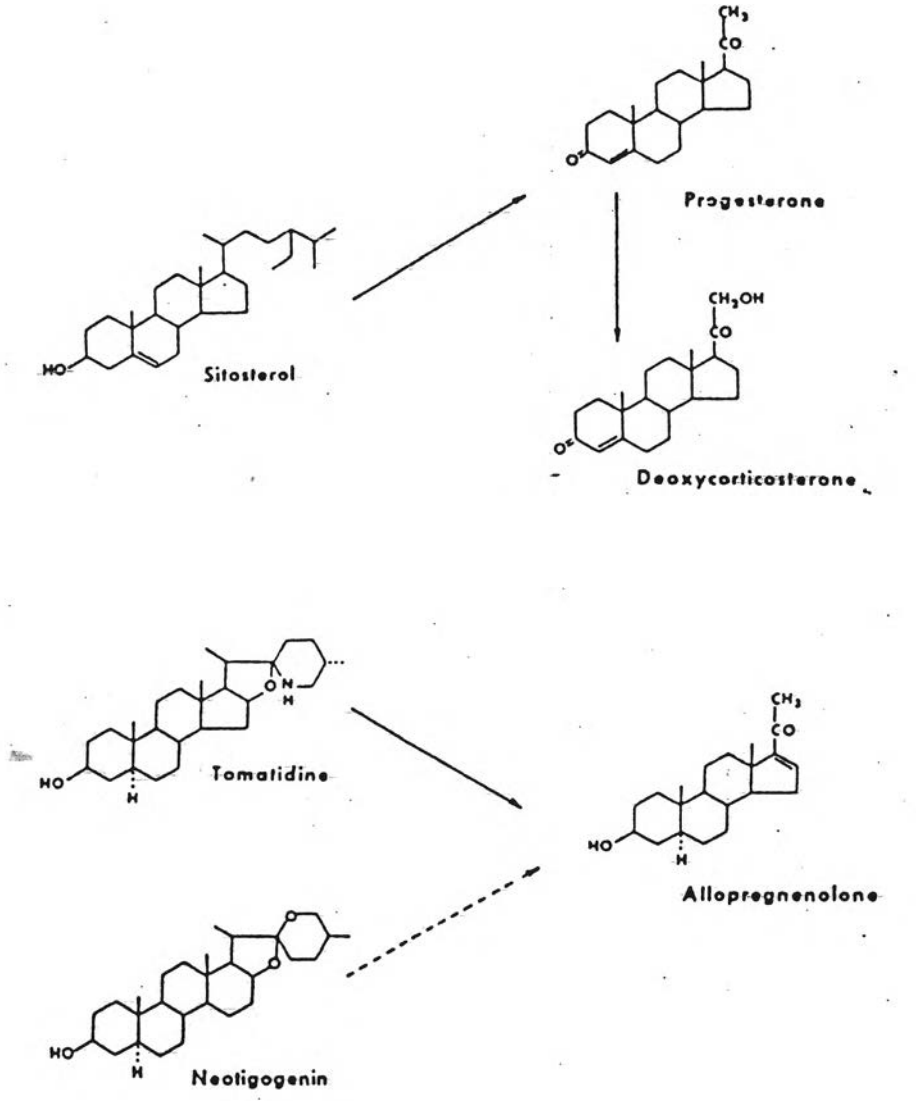
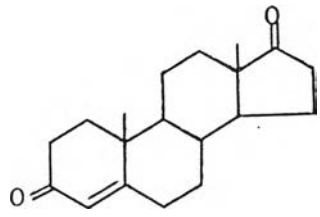
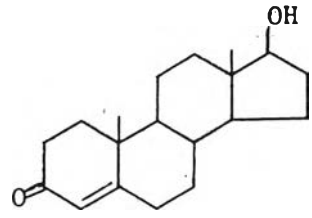


Figure 2.30 Biogenesis of C_{21} steroids



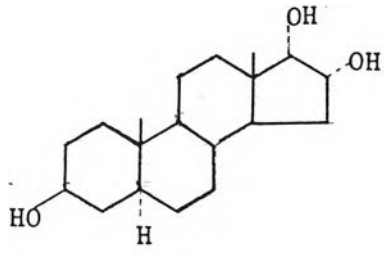
Androst-4-ene-3-, 17-dione

(Pinus sylvestris)



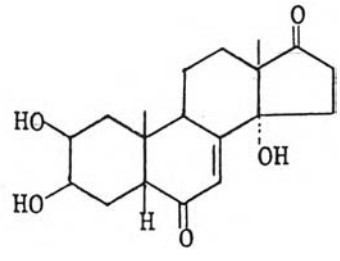
Testosterone

(Pinus sylvestris)



5α-Androstane-3β,16α,17α-triol

(Haplopappus heterophyllus)

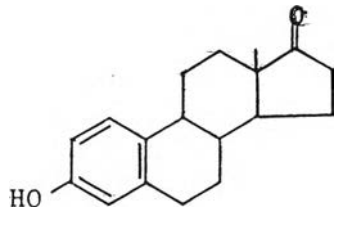


Rubrosterone

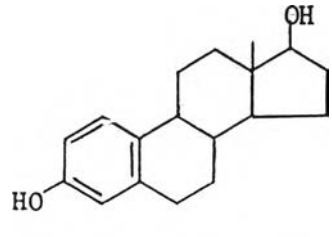
(Achyranthes rubrofusca)

Figure 2.31 C_{19} Steroids in higher plants

Steroidal estrogens have been identified in plants, such as estrone, a C_{18} steroid hormone (Fig 2.32), occurs in palm seeds, pollen of the date palm and pomegranate seeds; and estradiol (Fig. 2.32) in apricot seeds (82).



Estrone



Estradiol

Figure 2.32 C_{18} Estrogens in higher plants

4.3.3 Steroidal Saponins

A group of plant glycosides known as saponins share, in varying degrees, two common characteristics : (a) they foam in aqueous solution; and (b) they cause haemolysis of red blood cells. The aglycones of the saponins are collectively referred to as sapogenins (91). They are toxic to poikilotherms, but not to homoiothermic animals (80).

Two types of saponins are recognized : steroidal saponins and triterpenoid saponins. Steroidal saponins are glycosides of a particular steroid structure described as having a spiroketal side chain (Fig. 2.33). Ring E and F contain the same basic carbon skeleton as common animal steroids but lack the extra carbon atoms found in most plant sterols. It is possible that at least in some instances the spiroketal structure is an artifact formed by ring closure of an open chain precursor. In plants, steroidal sapogenins occur in the form of their glycosides, the saponin. Glycosylation is generally at C-3 (78, 80).

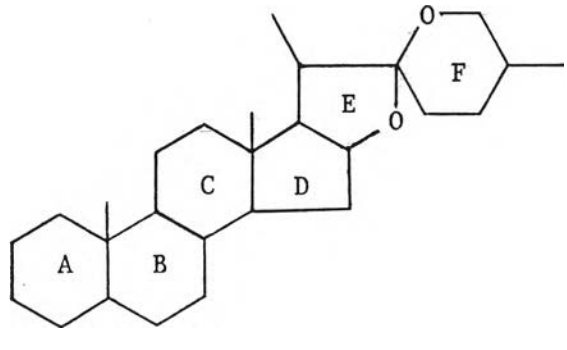
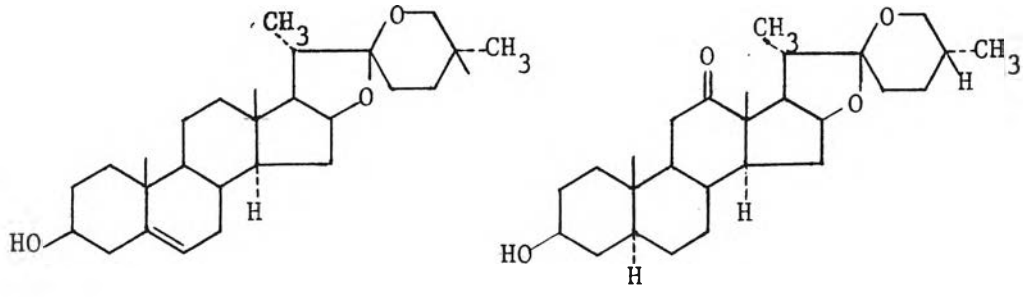


Figure 2.33 Structure of spiroketal steroid nucleus.



Diosgenin Hecogenin

Figure 2.34 Steroidal sapogenins

A number of steroidal sapogenins, while they are in themselves not used as therapeutic agents, serve as useful starting materials for the chemical synthesis and the practical production of a number of steroidal hormone substances which are medicinally important agents. Among the sapogenins which have been found to be the most useful as starting materials for chemical conversion to medicinal hormone substances are diosgenin, hecogenin, botogenin and their stereoisomers (Fig. 2.34). They are most common in the families Liliaceae, Amaryllidaceae and Dioscoreaceae. The discovery and isolation of these plant sapogenins, accompanied by advances in the chemical technique have greatly increased the availability and decreased the cost of the steroidal hormone substances used in medicines (86, 91)

4.3.4 Cardiac Glycosides

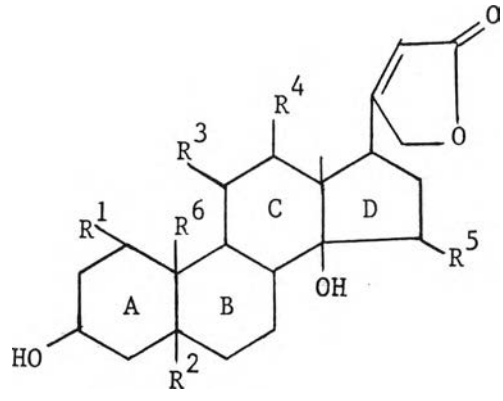
The structures of cardioactive glycosides are composed of three important portions :-

1) Steroidal Portion

The aglycones of these cardioactive glycosides possess the steroidal structure with the tetracyclic carbon skeleton which is, largely, saturated. In addition to this tetracyclic (steroidal) portion, there is an unsaturated lactone ring attached to C-17 of the steroidal carbon skeleton (92).

In the aglycones of these steroidal cardioactive glycosides, 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*. The hydrogen at C-8 is *beta* and the hydrogen at C-9 is *alpha* in configuration. The groups attached to C-10 and C-13 (that is, C-18 and C-19) both have the *beta* configuration. These aglycones have the hydroxyl groups at C-3 and C-14(both having the *beta*-configuration). In a number of these cardioactive glycosides, the aglycones have additional hydroxyl groups at other positions as well. In some, the group at C-19 is a methyl group, while in others, an aldehyde group or an hydroxymethyl (alcoholic) group. The sugar-portion (with one or more monosaccharide units) is linked through the hydroxyl group at C-3 of the aglycone with the hydroxyl group at C-1 of the sugar (92).

The steric features noted above apply to the great majority of the naturally occurring steroidal glycosides. However, a few glycosides, which are not used in medicine, are known to have configurations different from these (86, 92).



	R^1	R^2	R^3	R^4	R^5	R^6
Diginatigenin	H	H	H	OH	OH	CH_3
Digitoxigenin	H	H	H	H	H	CH_3
Digoxigenin	H	H	H	OH	H	CH_3
Gitaloxigenin	H	H	H	H	-O.CH=O	CH_3
Gitoxigenin	H	H	H	H	OH	CH_3
Ouabagenin	OH	OH	OH	H	H	$-CH_2OH$
Strophanthidin	H	OH	H	H	H	-CH=O
Strophanthidol	H	OH	H	H	H	$-CH_2OH$

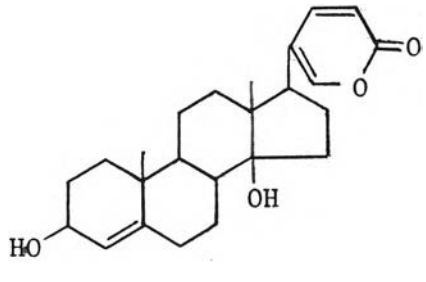
Figure 2.35 Structures of some aglycones of cardenolide group.

2) Lactone Rings of The Aglycones

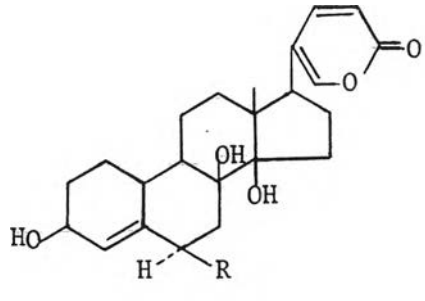
On the basis of the lactone-ring structure, these aglycones may be grouped into two groups (92):-

a) The cardenolides (aglycone with 23 carbons), the lactone ring attached to C-17 is a butenolide (4 carbons) which is also known as a $\Delta^{\alpha\beta}$-γ-lactone. The structures of this aglycone group are shown in Fig. 2.35.

b) The scilladienolides or bufadienolides (aglycone with 24 carbons), the 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. The structures of this aglycone group are shown in Fig. 2.36.



Scillarenin



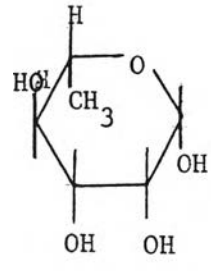
Scillirubrosidin R = H

Scillirosidin R = -O-C-CH_3 (O)

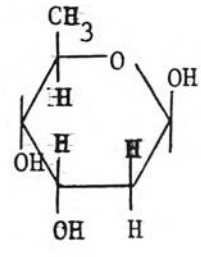
Figure 2.36 Structures of some aglycones of scilladienolide group.

3) Sugar Portion

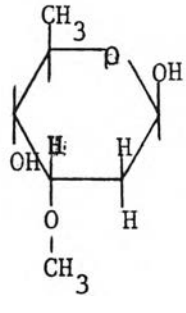
The different cardioactive glycosides may have one, two, three or four monosaccharide units in the sugar portion of the molecule. Apart from glucose, the other sugars (rhamnose, digitoxose, digitalose, cymarose) are 6-deoxyhexoses (5-methyl pentoses), Fig 2.37. These are sometimes referred to as the "rare sugar" (92).



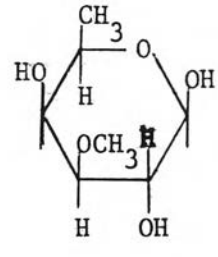
α-L-Rhamnose



β-D-Digitoxose



β-D-Cymarose



β-D-Digitalose

Figure 2.37 Sugar portion in cardiac glycosides

A number of cardioactive glycosides with steroidal aglycones occur in wide variety of plant species in several plant families. It may be noted that the glycosides occur principally in the seed in the *Strophantus* species, in the root in the *Apocynum* species, in the scales of the bulb in the *Urginea* species, in the leaf in the *Digitalis* species. Certain glycoside also occur principally in

Table 5. Some plant sources and the structural relatives of some cardioactive glycosides (92).

Plant species	Glycosides	Component Part (Aglycone and sugars)
Digitalis purpurea L. Fam. Scrophulariaceae	Digitoxin	Digitoxigenin-$(Digitoxose)_3$
Digitalis lanata Ehrh. Fam. Scrophulariaceae	Digoxin	Digoxigenin-$(Digitoxose)_3$
Strophantus gratus Wall. et Hook. Fam. Apocynaceae	Ouabain (Strophanthin-G)	Oubagenin-Rhamnose
Urginea burkei Baker Fam. Liliaceae	Scillaren A	Scillarenin-Rhamnose-Glucose

the seed in certain *Digitalis* species (92).

Some plant sources and the structural relatives of some cardioactive glycosides are shown in table 5.

4.3.5 Steroidal Alkaloids

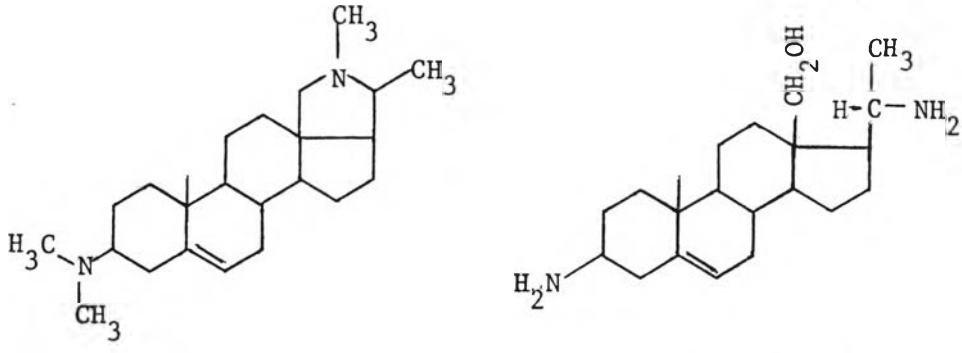
Steroid alkaloids are compounds possessing the basic or modified steroidal skeleton with nitrogen incorporated as an integral part of molecule either in the ring or in the side-chain (93).

According to Sato (93), steroidal alkaloids are divided into two general types :- C_{21} alkaloids and C_{27} alkaloids.

4.3.5 (a) C_{21} Alkaloids

Alkaloids of this group are the pregnane derivatives. A great number of C_{21} alkaloids have recently been isolated from Apocynaceae and Buxaceae. The Apocynaceae alkaloids, found mainly in *Holarrhena* and *Funtumia* species, are undoubtedly produced 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. Tracer experiments have, in fact, shown that the *Holarrhena* alkaloids, holaphyllamine and holaphylline as well as conessine (Fig. 2.38) are synthesized by direct amination of labeled pregnenolone (78).

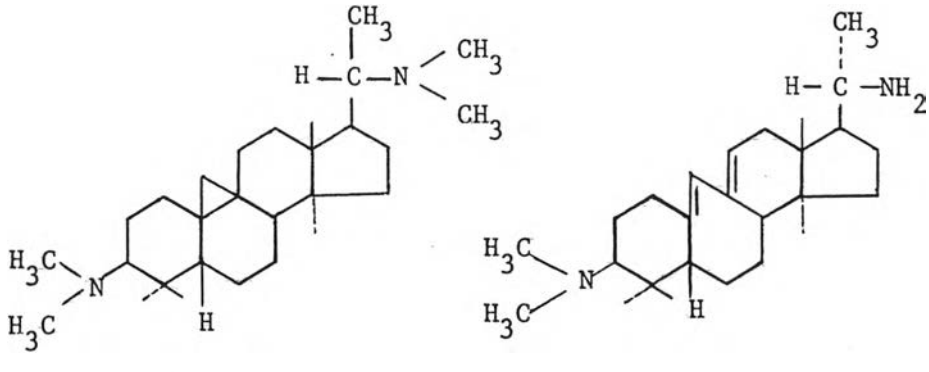
The pyrrolidine ring in conessine and other alkaloids of that type is probably derived from a precursor in which C-18 is oxygenated, such as holarrhimine (Fig 2.38). Conessine, the most abundant C_{21} alkamine, is a desirable starting material for the synthesis of certain



Conessine

Holarrhimine

Figure 2.38 Structures of some *Holarrhena* alkaloids (the C_{21} alkaloids)



Cycloprotobuxine A

Buxamine G

Figure 2.39 The structures of some *Buxus* alkaloids (the C_{21} alkaloids)

hormones, such as aldosterone (78).

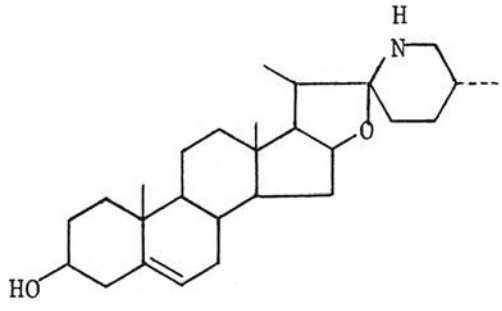
The alkaloids of the Buxaceae, in addition to the type of alkamines described above, are biogenetically related to cycloarternol and other steroid precursors. Cycloprotobuxine A, (Fig. 2.39) found in various *Buxus* species, may be regarded as a prototype. Analogs with primary and secondary amino groups at C-3 and C-20 and with a hydroxyl group at C-16 are known. In stead 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 there is an additional cyclopropane ring, especially one of the buxamines (Fig 2.39) in which the ring B is enlarged (78,90).

4.3.5 (b) C_{27} Alkaloids.

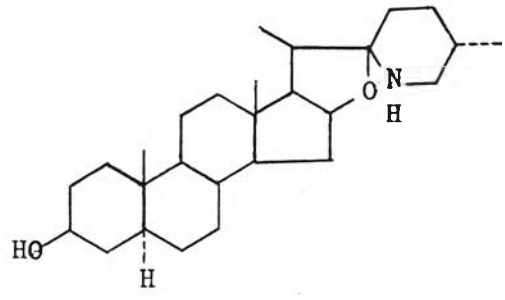
Many of these alkaloids, such as solasodine (Fig. 2.40), are simply nitrogen analogs of the C_{27} sapogenins. In the form of their glycosides, as glycoalkaloids, they are often found in the same plant and in combination with the same sugars as the analogous sapogenins. However, the distribution of C_{27} alkaloids is restricted to the genera *Veratrum* and *Fritillaria* (Liliaceae); *Solanum, Lycopersicon* and *Cestrum* (Solanaceae)(78).

Alkaloids in *Solanum* species, conjugated with sugars at C-3 can be divided into two groups.

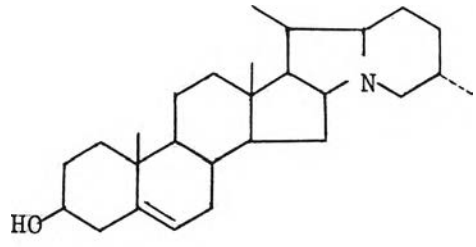
The first group may be considered as nitrogen analog of the sapogenin with an NH group instead of an oxygen atom between C-22 and C-23 in ring F, such as solasodine and tomatidine (Fig. 2.40) (83). Solasodine has the same structure as diosgenin, except for the fact



Solasodine

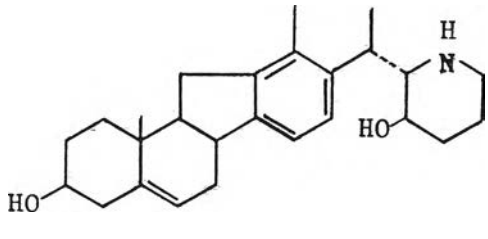


Tomatidine

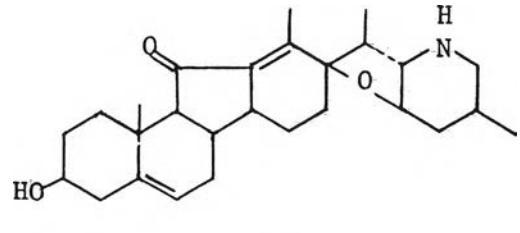


Solanidine

Figure 2.40 Structures of some *Solanum* alkaloids



Veratramine



Jervine

Figure 2.41 Structures of some *Veratrum* alkaloids

that NH is substituted for O in the F ring. Tomatidine is the nitrogen analog of neotigogenin. It has some fungistatic and bacteriostatic effect but it is perhaps of greater interest as a potential raw material for steroid hormone synthesis (78, 87).

The second group of *Solanum* alkaloids has no cyclic oxygen atom but it has a condensed ring system and tertiary nitrogen such as salanidine (Fig. 2.40) (83).

Both groups of solanum alkaloids are known to be synthesized by plants from cholesterol (78).

The alkaloids which occur in *Veratrum* species are not steroids because they contain a five-membered C ring and a six-membered D ring. Veratramine and jervine are representatives of *Veratrum* alkaloids (Fig. 2.41). *Fritillaria* alkaloids have structure similar to *Veratrum* alkaloids such as sipieimine which have been used as the Chinese medicine for a long time (83,87).

All C_{27} alkaloids are toxic to animals, and some of them are also toxic to fungi. The *Veratrum* alkaloids and their derivatives are used in medicine as hypotensive agents (78).

4.4 Biosynthesis of Steroids

As mentioned previously, steroids are a group of tetracyclic triterpenoids which is a group of isoprenoid compounds. All isoprenoid compounds originated from isopentenyl pyrophosphate which is synthesized from acetyl CoA in the same manner by both plants and animals as described in the previous section.

In the biosynthesis of steroids, the three successive steps are involved as follows :-

a) Formation of squalene from farnesyl pyrophosphate.

Although in the formation of most terpenoid compounds the isoprene groups are linked by means of the head-to-tail condensation discussed in an earlier section head-to-head condensation also occurs in certain cases, which have been most extensively investigated in the synthesis of squalene.

Squalene is formed by the condensation of two molecules of farnesyl pyrophosphate. The reaction proceeds stereospecifically, since a hydrogen atom at carbon atom 1 from one of the two farnesyl groups is replaced by a hydrogen atom originating from NADPH. The condensation may proceed according to the mechanism outline in Fig 2.42 (77).

b) Formation of cyclic triterpene ring system from squalene, Fig. 2.43 (77, 87, 90).

The cyclisation of squalene 1 to form the cyclopentano - phenanthrene ring system is squalene-2, 3-oxide 2 which is also the intermediate during cyclisation. Cyclisation is initiated by cation OH^+ attached to 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 shift 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 3 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 (77, 87, 90).

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 the product in this step is 24-methylene cycloartanol 4, a 4, 4-dimethyl sterol. Demethylation at C-4 is probably the next step, producing cycloeucalenol 5, the first 4-methyl sterol. The next step, the 9β , 19β-cyclopropane ring can be opened most efficiently to form obtusifoliol 6 and 31-norlanosterol 8 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 3 → 31-norcycloartenol 7 → 31- norlanosterol 8 → obtusifoliol 6 has also been indicated. From obtusifoliol 6 to 24-methylene lophenol 9 occurs through molecular rearrangement by migration of double bond. The formation of 24-ethylidene lophenol 10 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 second C-4 methyl group from 24-ethylidene lophenol 10 is also through oxidative decarboxylation and this product is Δ^{7} -

avenasterol 11. The conversion of Δ^{7}-avenasterol 11 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 13. Formation of sitosterol 15 from avenasterol requires hydrogenation of $\Delta^{24(28)}$ and reduction of the 24-ethylidene. Formation of stigmasterol 16 is assumed to occur through sitosterol by the enzyme 22, 23-dehydrogenase. Formation of sitosterol and stigmasterol may also be Δ^{7}-avenasterol 11→ stigmasta-5, 7, 24(28)-trien-3β-ol 12→ stigmasta-5, 7-diene-3β-ol 14→ sitosterol 15 or stigmasterol 16. 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 17. This reaction must be through a $\Delta^{24(25)}$ intermediate since the C-25 hydrogen atom is lost. Next, stigmasta-7-en-3β-ol goes through the $\Delta^{7(8)} \rightarrow \Delta^{5,7} \rightarrow \Delta^{5(6)}$ rearrangement to form sitosterol. For stigmasterol formation can be through spinasterol 18→ 7-dehydrostigmasterol 19.

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

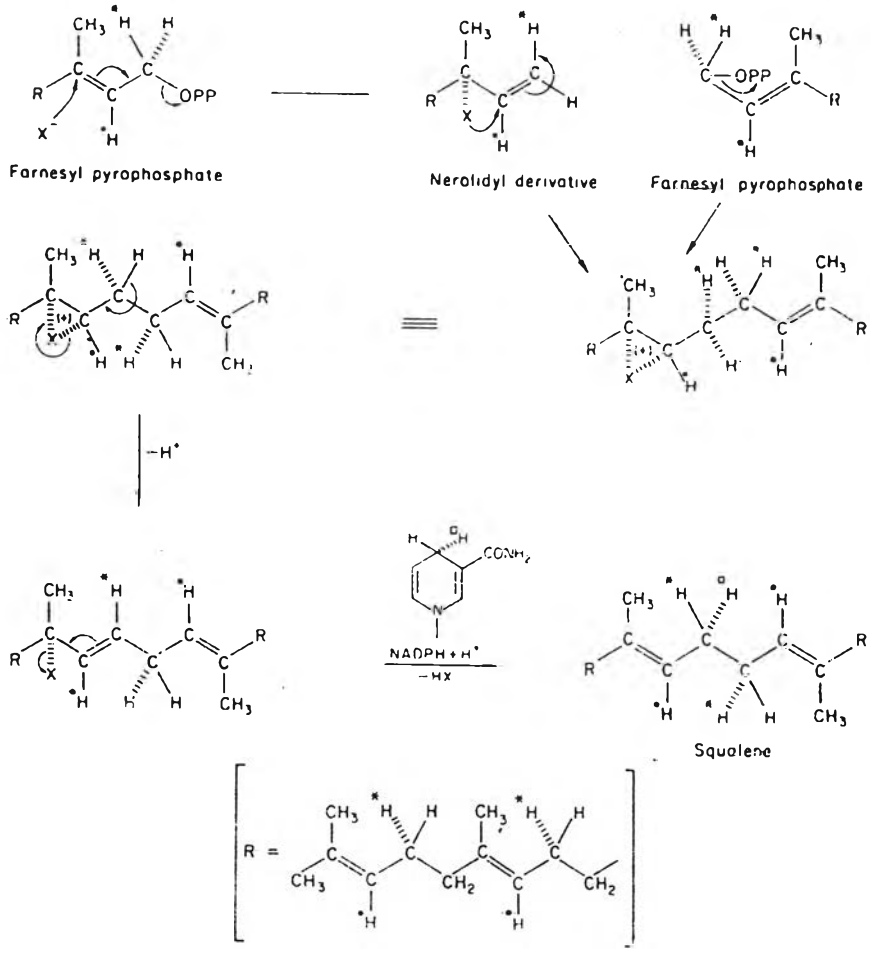


Figure 2.42 Formation of squalene from farnesyl pyrophosphate

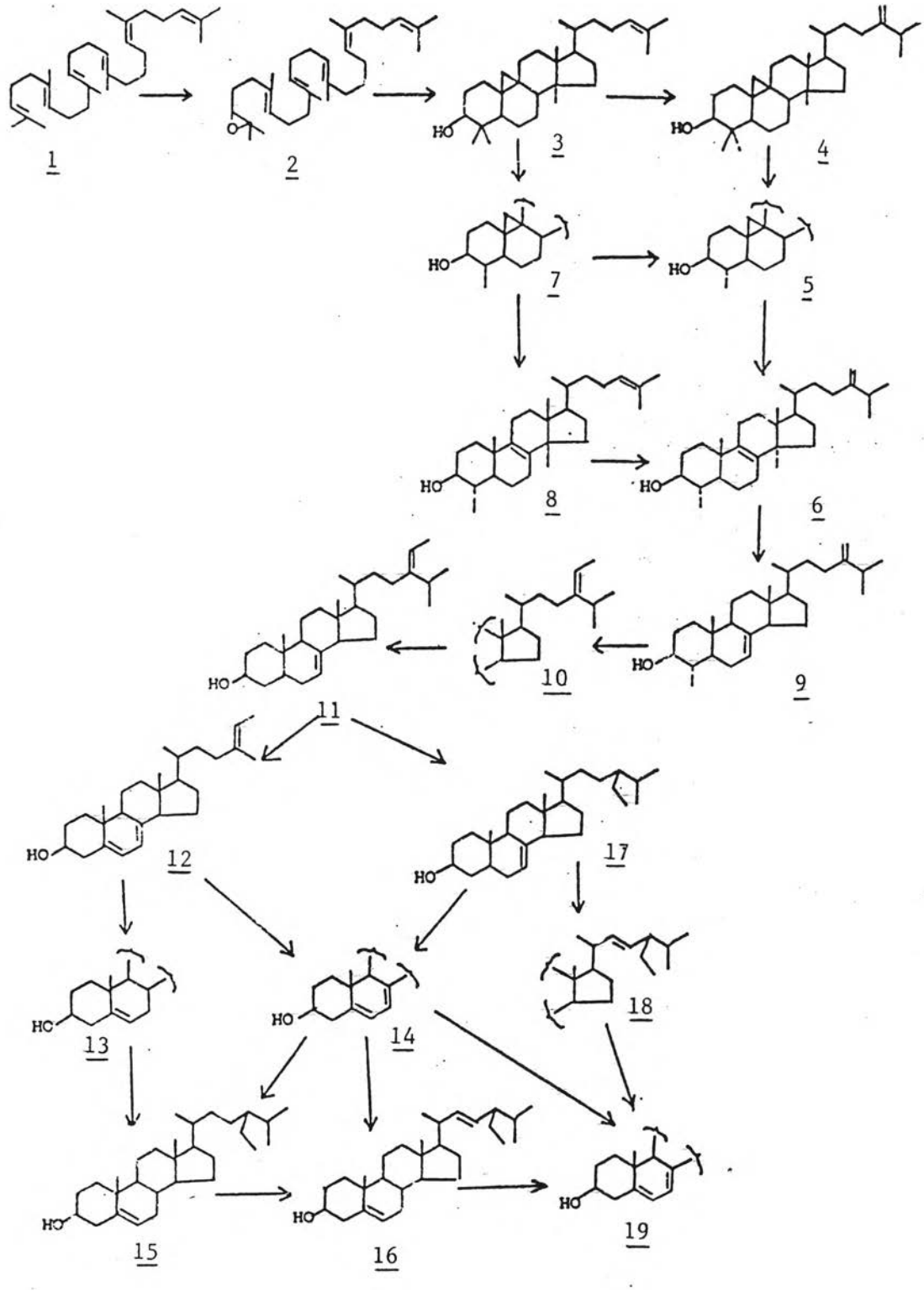


Figure 2.43 Biosynthetic pathway of plant sterols