CHAPTER II

HISTORICAL

1. Chemical Constituents of Eupatorium [Tourn.] Linn.

The groups of compounds commonly found in the genus of Eupatorium are sesquiterpenoids, alkaloids, flavonoids, steroids and volatile oils.

List of the compounds found in various species of Eupatorium is shown in Table 1.

Table 1. Chemical Constituents of Eupatorium Spp.

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
Eupatorium	leave	coumarin	coumarin	8
acuminatum		umbelliferone	flavonoid	8
E. adenophorum L.	aerial part	isohexacosane	alkane	9
		n-hexacosanoic acid	organic acid	9
		β-amyrin	triterpene	9
		stigmasterol	sterol	9
		lupeol	triterpene alcohol	9
		taraxasterol	sterol	9,12
		salvigenin	flavonoid	9
		epifriedelinol	triterpene	9
	+	cadiene	sesquiterpenoid	10,11
	+	friedelin	triterpenoid	12
		stigmastadienone	sterol	12
		stigmasterol	sterol	12

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
E. album	+	kaurenic acid and its deriv.	miscellaneous	13,14
	+	eupatalbin	diterpenoid	15
		eupatoralbin	diterpenoid	15
E. altissimum	aerial part	germacranolide glycoside	glycosidic sesquiterpene lactone	16
	+	eupatorin	flavonoid	17
		5-hydroxy-3',4',6,7-tetramethoxyflavone	flavonoid	17
	aerial part	++	sesquiterpene lactone	18,19
E. amplum Benth	+	5-hydroxy-7-4'-dimethoxy-flavone	flavonoid	20

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
E. angustifolium (H.B.K.) Spreng	leave	5,4'-dihydroxy-7-methoxy-flavone (genkwanin)	flavonoid	21
		5-hydroxy-7,4'dimethoxy-flavone (7-Me acacetin)	flavonoid	21
E. anomalum	+	++	sesquiterpene lactone	22,80
E. apayana Vent.	leave	ayapanin	mixture of coumarin	23
	+	β-selinene	sesquiterpenoid	24
	+	ethereal oil	cruded oil	25
E. areolare	leave,	6-methoxykaempferol	flavonoid	26
var leiocarpum	head	patuletin	flavonoid	26
		eupatolitin	flavonoid	26
		quercitin	flavonoid	26

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
		penduletin	flavonoid	26
		ombuin	flavonoid	26
		eupalitin and its glycoside	flavonoid	26
E. azureum.	leave,	epifriedelinol	triterpene	27
	stem	taraxasterol acetate	sterol	27
E. cannabinum	root	euparin	sesquiterpene lactone	28
	leaves,	eupatolin	sesquiterpene lactone	29
		eupatoriopicrin	sesquiterpene lactone	29
	inflorescence	eupatoriopicrin	sesquiterpene lactone	29
	aerial part	echinatine	pyrrolizidine alkaloid	30
		supinine	pyrrolizidine alkaloid	30

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
	+	dammaradienyl acetate	sterol	31
		taraxasterol	sterol	31,32
		stigmasterol	sterol	31
	whole part	coumarin	coumarin	32
	root	euparin	sesquiterpene lactone	32,33
		eupatoriopicrin	sesquiterpene lactone	32,33
	aerial part	eucannabinolide	sesquiterpene lactone	33,34
	leaves	stigmasterol	sterol	36
		β-sitosterol	sterol	36
		campesterol	sterol	36
		taraxasterol	sterol	36
E. capillifolium	+	(2R,3R)-7-methoxy-3,5,4'-trihydroxyflavonone	flavonoid	37

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
		(2R,3R)-3,4'-dihydroxy-5,7-dimethoxyflavonone	flavonoid	37
	+	costic acid	miscellaneous	38
E. chinense.	aerial part	coumarin	coumarin	39
		palmitic acid	organic acid	39
		α-amyrin	triterpene	39
		epifriedelinol	triterpene	39
		friedelin	triterpene	39
		β-sitosterol	sterol	39
		α-amyrin acetate ester	triterpene	39
	+	peroxyeupahakonin A and B	sesquiterpene lactone	40
		eupahakonin A and B	sesquiterpenoid	40
		eupahakonenin A and B	sesquiterpenoid	40
		eupahakonesin	sesquiterpenoid	40

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
E. chinense var simmplicifolium (Makino) Kitam.	leaves	eupachifolin A,B,C,D, and E	sesquiterpene lactone	41
E. coelestinum.	aerial part	eupalestin	flavonoid	42
		5'-methoxynobiletin	flavonoid	42
		coumarin	coumarin	42
		nobiletin	flavonoid	42
		lucidin di-Me ether	flavanoid	42
		5,6,7,3',4'-pentamethoxy-flavone	flavonoid	42
E. cuneifolium.	+	eupacunin	sesquiterpene lactone	43
E. deltoideum	+	deltoidin A,B	sesquiterpene lactone	44

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
	root	bornyl p-coumarate	coumarin	45
E. fortunei	flower,	taraxsterol palmitate	sterol	46
	leaves	taraxasteryl acetate	sterol	46
		taraxasterol	sterol	46
		β-amyrin palmitate	triterpene	46
		β-amyrin acetate	triterpene	46
		octacosanol	miscellaneous	46
		stigmasterol	sterol	46
		β-sitosterol	sterol	46
		palmitic acid	miscellaneous	46
	+	eupafortunin	sesquiterpene lactone	47
E. formosanum	+	eupaformonin	sesquiterpene lactone	48
	+	eupatolide	sesquiterpene lactone	49

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
E. glabratum.	+	eupaglabrin	terpenoid	50
	+	eupaglabric acid	terpenoid	51
E. glechonophyllum.	thallus	10-acetoxy-8,9-epoxythymol-isobutyrate	thymol derivative	52
		8,9-dihydroxy-10-acetoxy-thymolisobutyrate	thymol derivative	52
		8,9,10-trihydroxythymol	thymol derivative	52
	leave	encecalin	chromene	53
		gleucolin	chromene	53
E. glutinossum	leaves	rutin	flavonoid	54
E. havanense.	+	sakuranetin	miscellaneous	55
		pulcherryl acetate	miscellaneous	55

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
	+	++	flavonoid	56
E. hebebotrya	+	++	diterpene acid	57
E. hyssopifolium	+	eupassopin	sesquiterpene lactone	58
		eupassopilin	sesquiterpene lactone	58
		eupassofilin	sesquiterpene lactone	58
		D(-)-3-hydroxyoctadecanoic acid ester	sesquiterpene lactone	58
E. inulaefolium.	+	5-6,3'-trihydroxy-7,4'-dimethoxyflavone	flavonoid	59,60
		pedalitin	flavonoid	59
	leaves, flower bud	jaceidin	flavonoid	61

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
E. japonicum	leaves	euponin	sesquiterpene lactone	62,63
	leaves	coumarin	coumarin	63
E. jhanii	aerial part	jhanol	diterpene oxide	64
		jhanyl acetate	diterpene oxide	64
		jhanidiol	diterpene oxide	64
		jhanidiol-18-monoacetate and diacetate	diterpene oxide	64
	+	jhanilactone	diterpene lactone	65
E. laevigatum Lam.	leaves	rutin	flavonoid	66
	flower	laevigatin	sesquiterpenoid furan	67
E. lancifolium	+	eupacunolin	sesquiterpene lactone	68
		eupacunin	sesquiterpene lactone	68

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
		desacetyleupacunin	sesquiterpene lactone	68
		++	sesquiterpene lactone	68
		coumarin	coumarin	68
E. leucolepis.	+	++	flavone	69
		nobiletin	flavone	69
		3',4'-methylenedioxy-5,6,7,8-tetramethoxyflavone	flavone	69
		3',4'-methylenedioxy-5,6,7,8,5'-pentamethoxyflavone	flavone	69
E. ligustinum.	+	ligustrin	sesquiterpene lactone	70
	+	eupalin	flavonol rhamnoside	71
		eupatolin	flavonol rhamnoside	71

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
E. lindleyanum DC.	+	eupalinin A,B,C, and D	sesquiterpene lactone	72
E. littorale	aerial part	rutin	flavonoid glycoside	73
E. macrocephalum.	aerial part	rhamnocitrin 3-glycoside	flavonoid	74
		7-methoxyaromadendrino 3-glycoside	flavonoid glycoside	74
E. micranthum	+	micrantoside	flavonoid glycoside	75
	aerial part	coumarin	coumarin	76
		docosanol	miscellaneous	76
		tetrecosanol	miscellaneous	76
	leave	7-methylaromadendrin	flavonoid	77
		rhamnocitrin	flavonoid	77

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
E. microphyllum L.	flower, leave	5-hydroxy-6,7,3'4'-tetra-methoxyflavone	flavonoid	78
		rutin	flavonoid	78
		quercetrin	flavonoid	78
		kaempferol	flavonoid	78
		jhanidiol 18-acetate	flavonoid	78
		β-amyrin 3-palmitate	triterpene	78
E. mikanioides.	+	2-hydroxy-8-(acyloxy)-trans-trans-1(10),4-germacra-dienolide	sesquiterpene lactone	78
		deacetyleupaserrin	sesquiterpene lactone	79
		eupatorin	flavonoid	79

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
E. mohrii.	+	++	sesquiterpene lactone	80
		eurecurvin	sesquiterpene lactone	80
E. odoratum	oil	eupatol	sesquiterpene alcohol	81
	oil	eupatene	sesquiterpenoid	82
	leave	isosakuranetin	flavonoid	83,84
		odoratin	chalcone	83,84
	whole plant	lupeol	triterpene alcohol	85
		β-amylin	triterpene alcohol	85
		salvigenin	flavone	83
	+	epoxylupeol	triterpene alcohol	86
	aerial part	isosakuranetin methyl ether	flavonoid	87
	+	isosakuranetin	flavonoid	88

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
		sakuranetin	flavonoid	88
		tamarixetin	flavonoid	88
E. pedale DC.	+	sakuranetin	flavonoid	89
		7-methoxyaromadendrin	flavonoid	89
		rhamnocitrin	flavonoid	89
E. perfloliatum	+	euperfolin	sesquiterpene lactone	90
		eufoliatin	sesquiterpene lactone	90
		euperfolitin	sesquiterpene lactone	90
		eufoliatorin	sesquiterpene lactone	90
E. peticlare	+	2-hydroxy-6-methoxy-benzoic acid	diterpenoid	91

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
E. quadrangulare.	aerial part	quadrangulin A	eudesmane sesquiterpene	92
E. recurvans	+	eurecurvin	sesquiterpene lactone	93
E. riparium Regal.	whole plant	taraxasteryl palmitate	sterol	94
		taraxasterol	sterol	94
		stigmasterol	sterol	94
E. rotundifoloum.	+	++	sesquiterpene lactone	95
		euparotin bromoacetate	sesquiterpene lactone	95
		eupachlorin	sesquiterpene lactone	95
		eupachlorin acetate	sesquiterpene lactone	95
E. rugosum. Lin	+	desmethylencecalin	chromene	96
		hydroxytremetone	cumaranone	96

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
E. sachalinense	+	peroxysachalinin	sesquiterpene lactone	97
		sachalinin	sesquiterpene lactone	97
		sachalin	sesquiterpene lactone	97
	leave	hiyodorilactone A,B and C	sesquiterpene lactone	98
	leave	hiyodorilactone D,E and F	sesquiterpene lactone	99
E. serotinum	+	++	sesquiterpene lactone	100
	+	euserotin	sesquiterpene lactone	101
E. semiserratum	+	eupaserrin	sesquiterpene lactone	102
		deacetyleupaserrin	sesquiterpene lactone	102
E. sessilifolium	+	eupasessifolide A and B	sesquiterpene lactone	103
		++	sesquiterpene lactone	103
E. sternbergianum	+	sternbin	flavonone	104

Table 1. (Continue)

Botanical Origin	Plant Part	Chemical Substance	Category	Reference
E. tinifolium	+	tinifoline	thymol derivatives	105
		tinifoline diol	thymol derivatives	105
E. trapezoideum.	+	++	cadinenes	106
E. turbinatum.	aerial part	clerodane deriv.	diterpenoid	107
		abietane deriv.	diterpenoid	107
		friedolabdane	diterpenoid	107
E. urticaefolium	root	tremetol	miscellaneous	108
E. villosum Sw.	+	evillosin	diterpenoid lactone	109

+ = Unclassified part

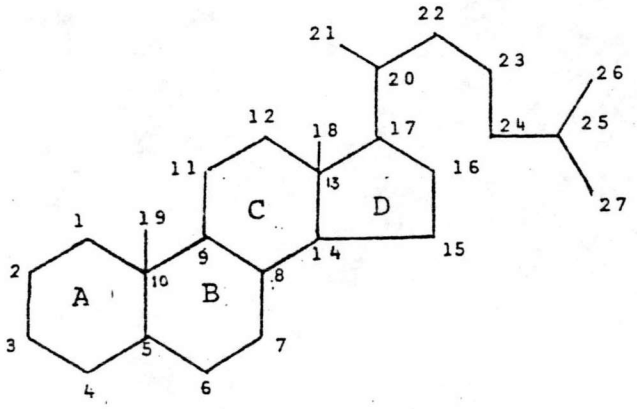
++ = Unclassified substance

2. STEROIDS

2.1 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 (110,111).

Steroid numbering system is as follows:-



2.2 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 exclusively in the free form (110,111).

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}) (111).

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 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 (111).

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} cholegenin 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 (111,112).

2.3 Classification of Steroids

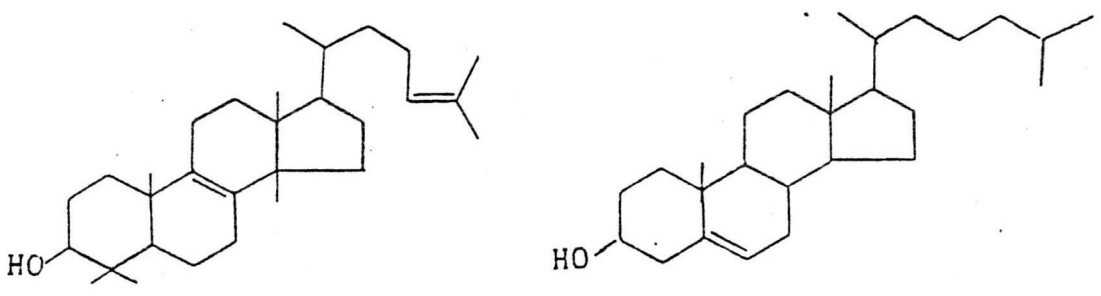
Steroids which are based on the cyclopentanoperhydrophenanthrene ring system, can be divided into at least five groups of compounds : sterols, steroid hormones, steroidal saponin, steroid alkaloids and cardiac

glycosides.

2.3.1 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 (113). 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 eigth-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 (114). The structures of lanosterol and a sterol are shown in Fig. 2.1.

The name STEROL is used for steroid alcohols. Since practically all plant steroids are alcohols with a hydroxyl group at C-3, they are all called sterols (114).



Lanosterol Cholesterol

Figure 2.1 Structures of lanosterol and cholesterol

Sterols can be classified into three groups, according to the number of carbon atoms in molecule.

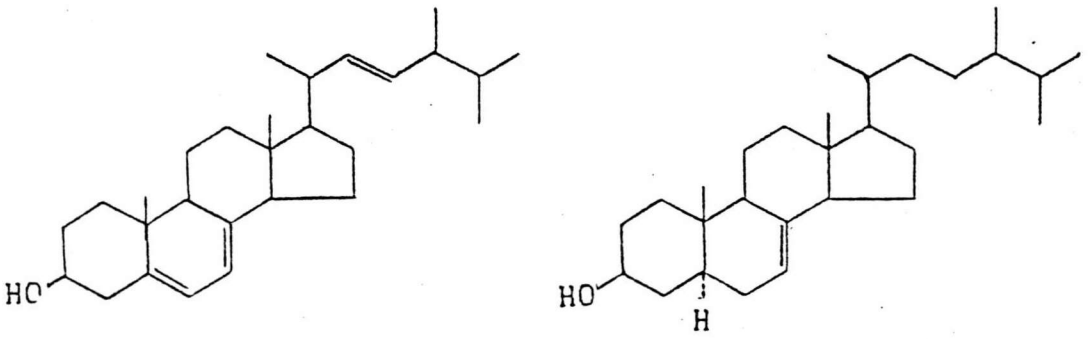
2.3.1.1 C_{27} Sterols

Cholesterol, C_{27} sterol, has been found to be widely distributed in 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 (111).

Cholesterol has an important function in plants. It serves as the starting material for biosynthesis of all other steroids (112,113).

2.3.1.2 C_{28} Sterols

The C_{28} sterols derived from the C_{27} sterols. The most important C_{28} sterol is ergosterol (Fig.2.2) which was first isolated from ergot (115,116) and also found in yeast and in most fungi. Recently it has also been found in higher plants (111). The structures of ergostrol and a C_{28} sterol are shown in Fig. 2.2.



Ergosterol Fungisterol

Figure 2.2 Some C_{28} sterols

2.3.1.3 C_{29} Sterols

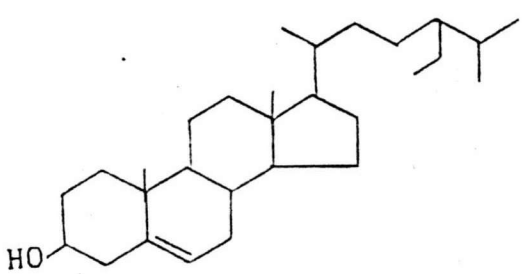
Sitosterol, stigmasterol and campesterol are the most widely distributed C_{29} sterols in higher plants which are called phytosterols. These commom sterols occur both free and as simple glycosides (111,115,117). The sterol most often isolated from plant is sitosterol but stigmasterol and campesterol are also quite common (118).

Sitosterol is generally called β-sitosterol. Since both α and γ-sitosterol have turned out to be mixture, the designation sitosterol is now equivocal (111).

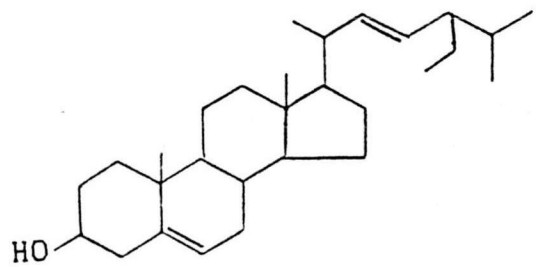
Stigmasterol was first isolated from the calabar bean (Physostigma venenosum Balf.). The commercial source is the soybean, but sugarcane wax also contained large amount of this sterol. Its abundance and the double bonds at carbon position 22 and 5 make stigmasterol to be an important

starting material for the synthesis of progesterol 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 differentiation (111,115).

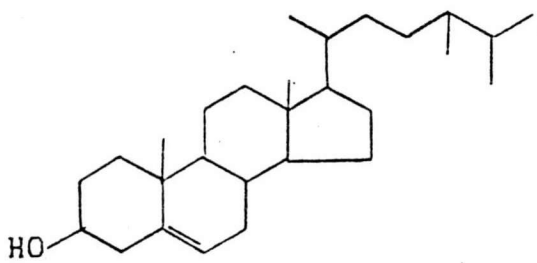
The structures of phytosterols are shown in Fig. 2.3.



Sitosterol



Stigmasterol



Campesterol

Figure 2.3 Some plant sterols (Phytosterols)

2.3.2 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 in the skeleton.

2.3.2.1 C_{27} to C_{29} Steroid Hormones

This group is the most insect-molting hormones. Since the insect-molting hormones were first isolated from insects and called ecdysone (Fig. 2.4), these hormone from plants are sometimes called phytoecdysones.

Their common features are the 14 α-hydroxy group and the Δ^7-6-keto group. Other hydroxy groups may be attached to various positions (111).

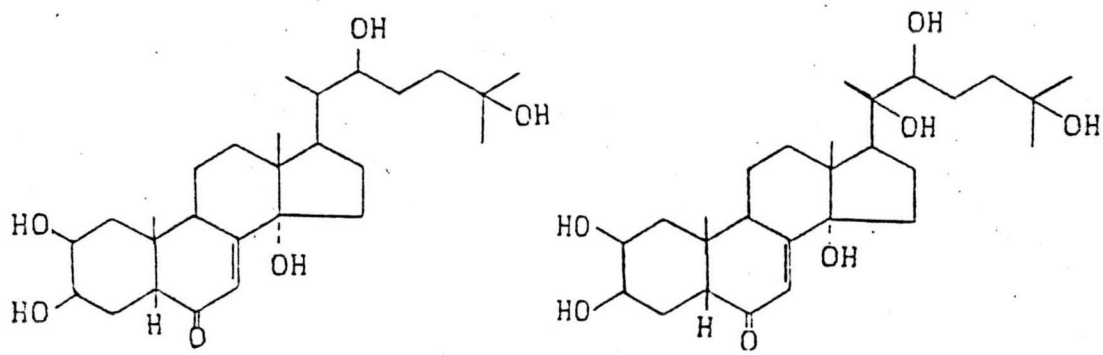


Figure 2.4 Some insect-molting hormones

2.3.2.2 C_{18} to C_{21} Steroid Hormones

The degradation of cholesterol to pregnenolone and its conversion to progesterone (Fig. 2.5) which is the key reaction in the biosynthesis of steroid hormones in animal is also observed in higher plants (112,119). 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. 2.6) (119). Tomatoes carry out the same degradation of tomatidine to allopregnenolone (Fig. 2.6) as the basic step in the partial synthesis of steroid hormones (112). Microorganisms are also known to be capable of converting sapogenins to C_{21} and C_{19} steroids (119).

In animal, the C_{19} and C_{18} steroids are formed from C_{21} by successive degradation steps (Fig. 2.5). Fig. 2.7 shows C_{19} steroids identified in plants (119).

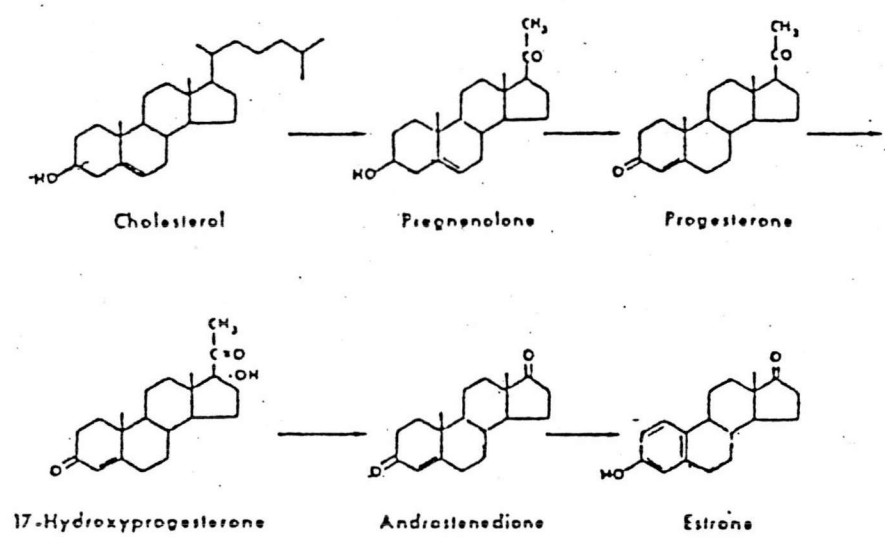


Figure 2.5 Biogenesis of esterone

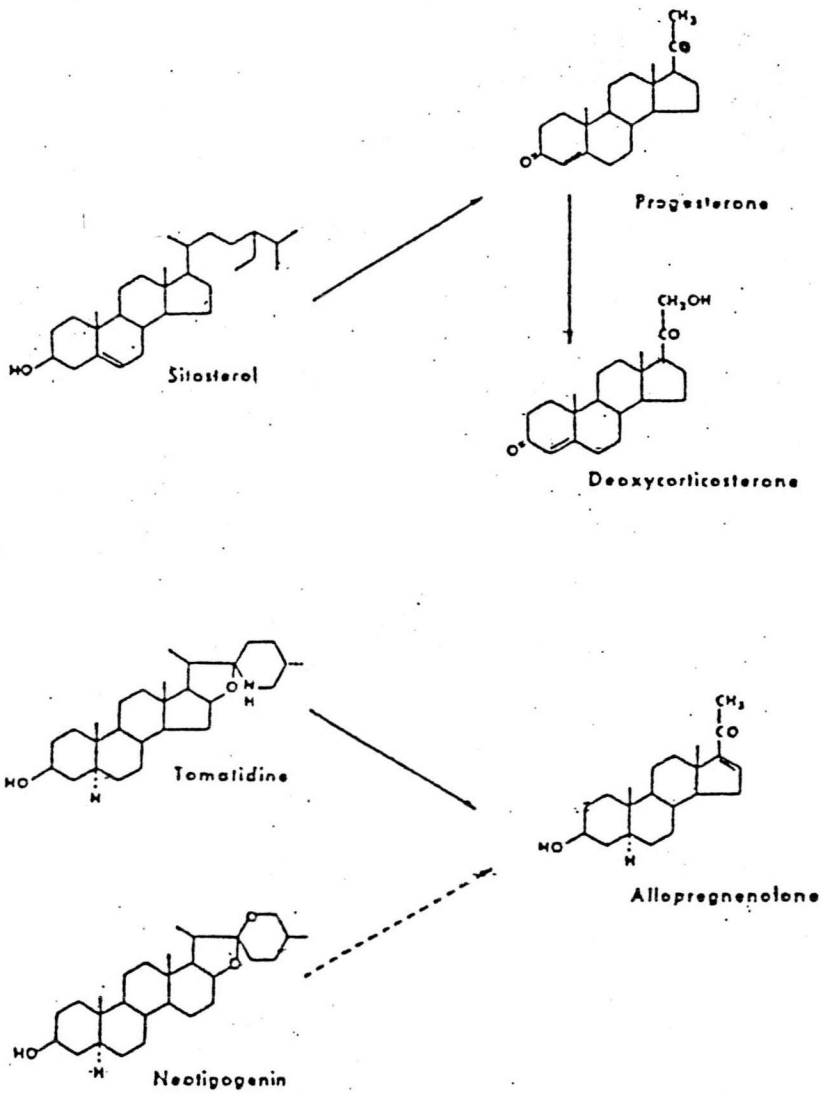
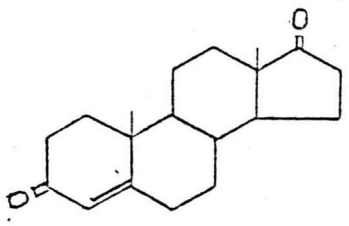
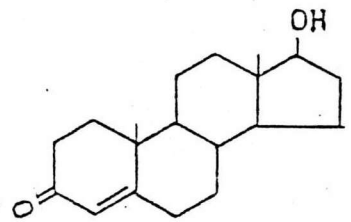


Figure 2.6 Biogenesis of C_{21} steroids



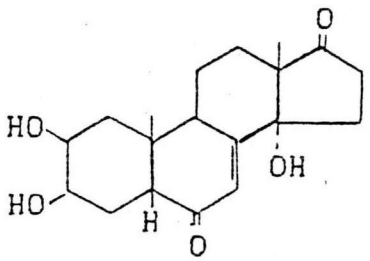
Androst-4-ene-3-,17-dione

(Pinus sylvestris)



Testosterone

(Pinus sylvestris)

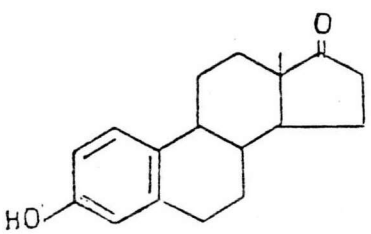


Rubrosterone

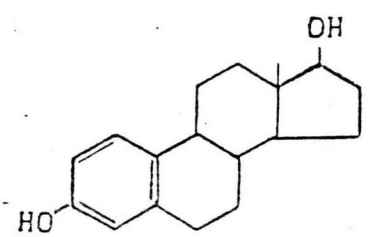
(Achyranthes rubrofusca)

Figure 2.7 Some C_{19} steroids in higher plant

Steroidal estrogens have been identified in plants, such as estrone, a C_{18} steroid hormone (Fig. 2.8), occurs in palm seeds, pollen of the date palm and pomegranate seeds.



Estrone



Estradiol

Figure 2.8 Some C_{18} estrogens in higher plant

2.3.3 Steroidal Saponins

The saponins are generally glycosides which have two common characteristics : (a) they foam in aqueous solution; and (b) they cause haemolysis. The dilute solutions of them are quite toxic to fish.

There are two types of saponins, steroidal saponins and triterpenoid saponins. Steroidal saponins are glycosides of a particular steroid structure described as having a spiroketal side chain (Fig. 2.9). 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 saponins occur in the form of their glycosides, the saponin. Glycosylation is generally at C-3 (111,112).

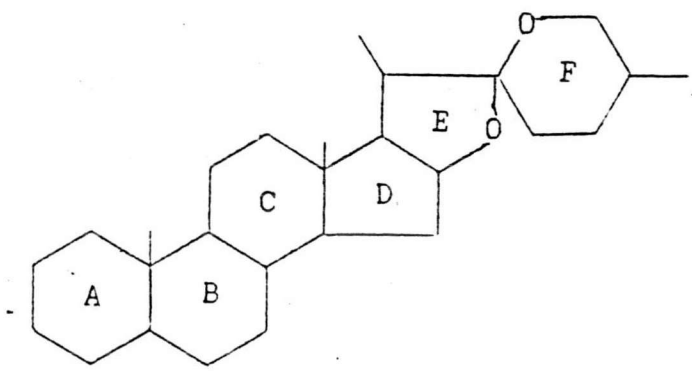
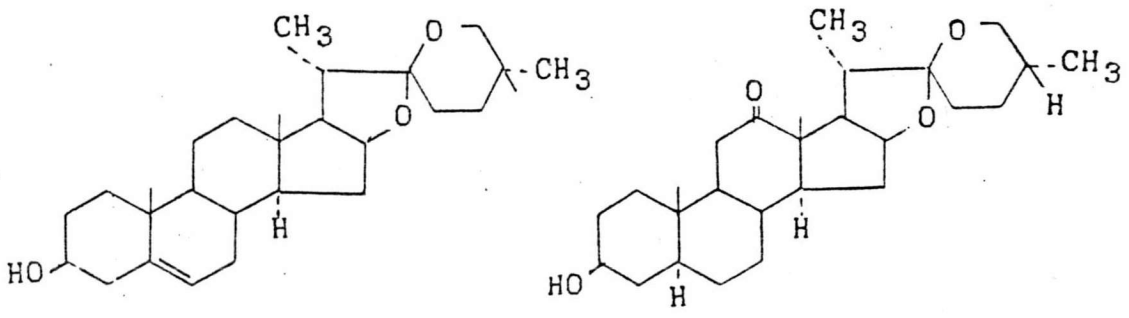


Figure 2.9 Structure of spiroketal steroid nucleus.

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 to be the most useful as starting materials for chemical conversion to medicinal hormone substances are diosgenins, hecogenin, botogenin and their stereoisomers (Fig. 2.10). They are most common in the families Liliaceae, Agavaceae, Amaryllidaceae and Dioscoriaceae. The discovery and isolation of these plant saponins, accompanied by advances in the chemical technique have greatly increased the availability and decrease the cost of the steroidal hormone substances used in medicines (120,121).



Diosgenin Hecogenin

Figure 2.10 Steroid sapogenins

2.3.4 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 (122).

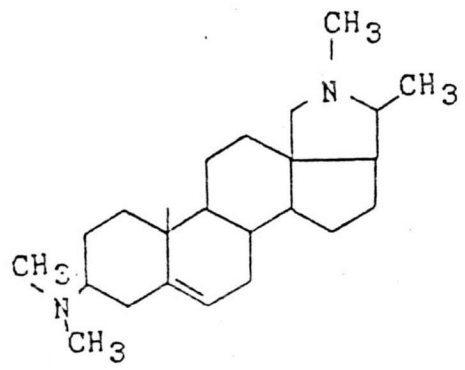
According to Sato (122), steroidal alkaloids are divided into two general types:- C_{21} alkaloids and C_{27} alkaloids.

2.3.4.1 C_{21} Alkaloids

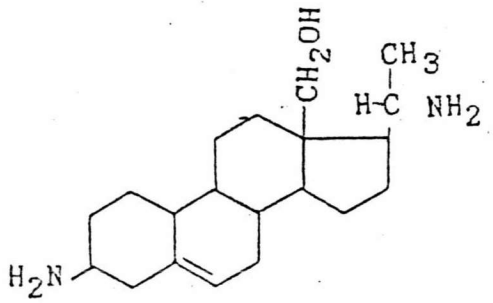
Alkaloids of this group are the pregnane derivatives. A great number of C_{21} alkaloids have recently been isolated from Apocynaceae and Buxaceae. The Apocynaceae alkaloids, found mainly in Holarrhena and Funtumia species, are undoubtedly produced from pregnenolone by amination at either C-3 or C-20, 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. 2.11) are synthesized by direct amination of labeled pregnenolone (111).

The pyrrolidine ring in conessine and other alkaloids of that type is probably derived from a precursor in which C-16 is oxygenated, such as holarrhimine (Fig. 2.11). Conessine, the most abundant C_{21} alkamine, is a

desirable starting meterial for the synthesis of certain hormones, such as aldosterone (111).



Conessine

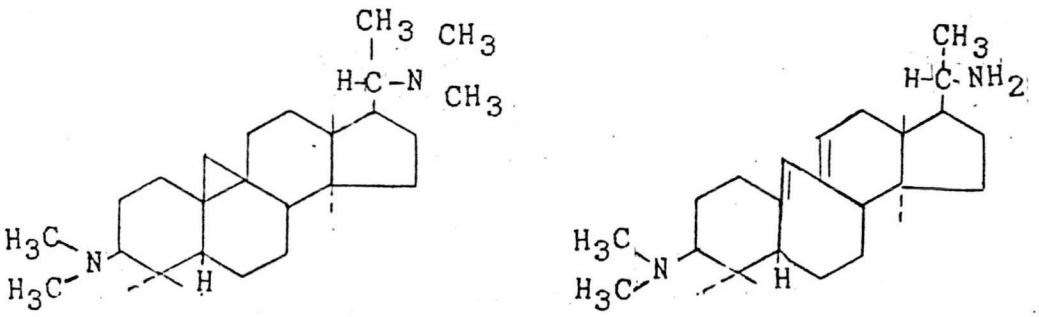


Holarrhimine

Figure 2.11 Structures of some Holarrhena alkaloids (the C_{21} alkaloids)

The alkaloids of the Buxaceae, in addition to the type of alkamines described above, are biogenetically related to cycloartenol and other steroid precursors. Cycloprotobuxine A, (Fig. 2.12) found in various Buxus species, may be regraded as a prototype. Analogs with primary and secondary amino groups at C-3 and C-20 and with a hydroxy 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, there is an additional cycloproprane ring,

especially one of the buxamines (Fig. 2.12) in which the ring B is enlarged (111,118).



Cycloprotobuxine A

Buxamine G

Figure 2.12 Structures of some Buxus alkaloids (the C_{21} alkaloids)

2.3.4.2 C_{27} Alkaloids

Many of these alkaloids, such as solasodine (Fig. 2.13), 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 sugar as the analogs saponins. However, the distribution of C_{27} alkaloids is restricted to the genera Veratrum and Fritillaria (Liliaceae); Solanum, Lycopersicon and Cestrum (Solanaceae) (111).

Alkaloids in Solanum species, conjugated with sugar

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. 2.13) (123). Solàsodine 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. It has some fungistatic and bacteriostatic effect but it is perhaps of greater interest as a potential raw material for steroid hormone synthesis (111,115).

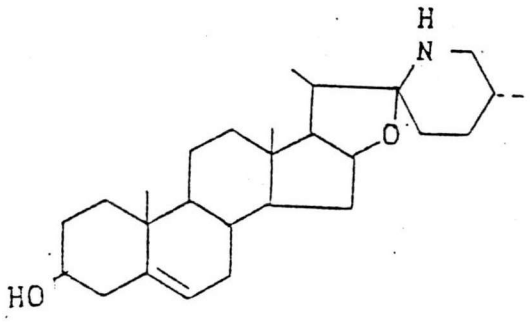
The second group of Solanum alkaloids has no cyclic oxygen atom but it has a condensed ring system and tertiary nitrogen such as solanidine (Fig. 2.13) (123).

Both groups of solanum alkaloids are known to be synthesized by plants from cholesterol (111).

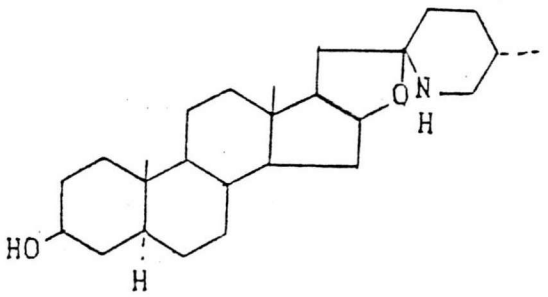
The alkaloids which occur in Veratrum species are not steroids because they contain a five-membered C ring and a six-membered D ring. Veratramine and jervine are representatives of Veratrum alkaloids (Fig. 2.14). Fritillaria alkaloids have structure similar to Veratrum alkaloids such as sipieimine which have been used as the Chinese medicine for a long time (115,123).

All C_{27} alkaloids are toxic to animals, and some of them are also toxic to fungi. The Veratrum alkaloids and

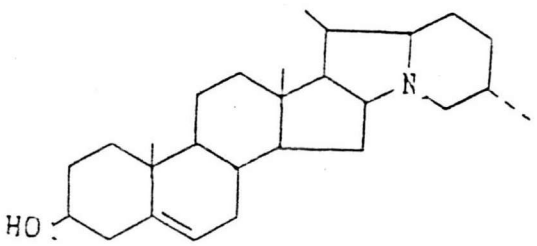
their derivatives are used in medicine as hypotensive agents (111).



Solasodine

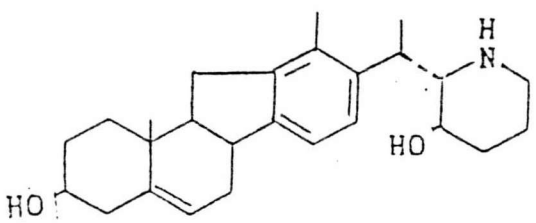


Tomatidine

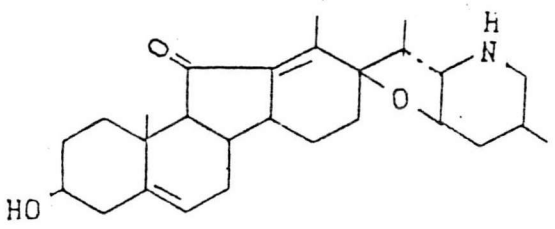


Solanidine

Figure 2.13 Stuctures of some Solanum alkaloids



Veratramine



Jervine

Figure 2.14 Structures of some Veratrum alkaloids

2.3.5 Cardiac Glycosides

The principle structures of cardiac glycosides can be divided into three parts, 1) steroidal nucleus 2) unsaturated lactone ring and 3) sugar portion.

1) Steroid nucleus

The steroidal structures of cardiac glycosides generally have saturated tetracyclic carbon skeleton and an unsaturated lactone ring, the second part of the cardiac glycoside structures, attached to C-17 of the carbon skeleton. The structure of steroidal skeleton and unsaturated lactone ring are refered as aglycone (124).

In the aglycones of these steroidal cardiac 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 cardiac 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 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. However the

naturally occuring steroidal glycosides are known to have configuration different from these, for instance with 3- α-hydroxyl group, or with an α-configuration at C-5 or with the A/B ring fusion has the <u>trans</u> configuration and C/D ring fusion has the <u>cis</u> configuration.

2) Unsaturated lactone ring

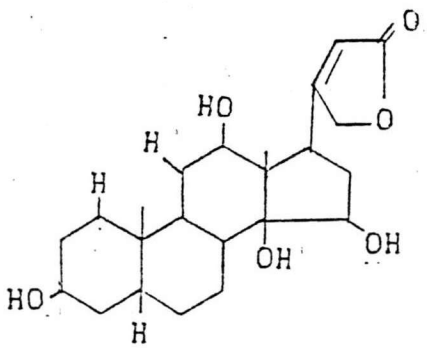
The unsaturated lactone ring is usually attached to C-17 of the carbon skeleton. On basis of the lactone ring structure, these aglycones may be grouped into two groups (124):-

a) The cardenolides (aglycone with 23 carbons), the 5-membered lactone ring attached to C-17 is a butenolide (4-carbons) which is also known as a $\Delta^{\alpha\beta}$-γ-lactone. The structure of this aglycone group are shown in Fig. 2.15.

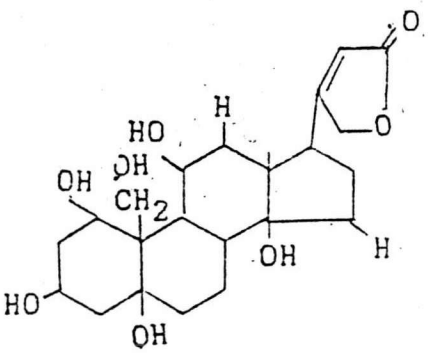
b) The bufadienolides or scilladienolides (aglycone with 24 carbons), the 6-membered lactone ring attached to C-17 is a pentadienolide (5 carbons, with two double-bonds) which is also called a pentenolide, or otherwise known as a $\Delta^{\alpha\beta,\gamma\delta}$-δ-lactone. The structure of this aglycone are shown in Fig. 2.16.

3) Sugar portion

The different cardiac glycosides may have one, two, three, or four monosaccharide units in the sugar portion of molecule.

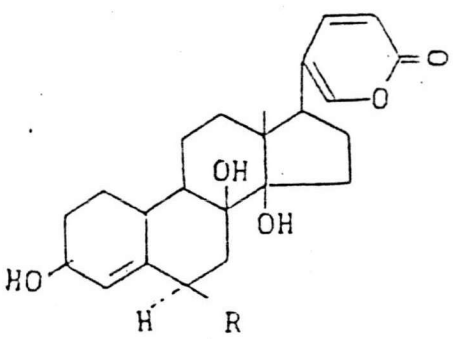


Diginatigenin



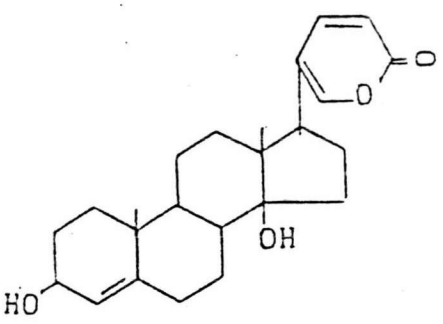
Ouabagenin

Figure 2.15 Structures of some aglycones of cardenolide group



Scillirubrosidin R = H

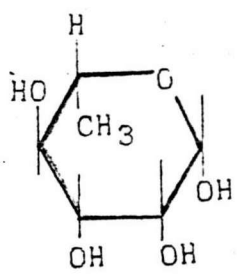
Scillirosidin R = -O-CO-CH_3



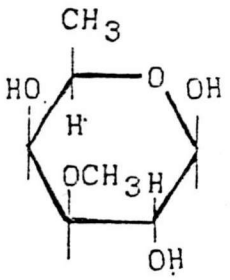
Scillarenin

Figure 2.16 Structures of aglycones of scilladienolide group

There are many kinds of sugar, found in structure of cardiac glycosides, such as glucose, rhamnose, and deoxysugars (rhamnose, digitoxose, digitalose etc) which are sometimes refered to as the "rare sugar" (124), Fig. 2.17.



Rhamnose



Digitalose

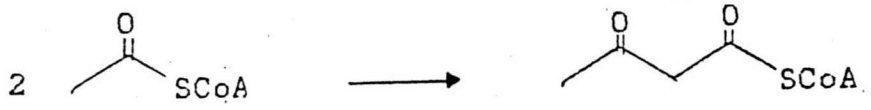
Figure 2.17 Some sugars of cardiac glycosides

2.4 Biosynthesis of Steriods

Steroids are a group of tetracyclic triterpenoids which is a group of isoprenoid compounds. All isoprenoid compounds originate from isopentenyl pyrophosphate which is synthesized from acetyl CoA in the same manner by both plants and animals as follows:-

2.4.1 Conversion of Acetyl CoA to Acetoacetyl CoA

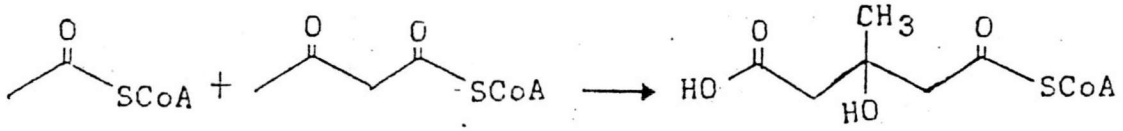
Acetoacetyl CoA thiolase catalyzes the condensation of two molecules of acetyl CoA to form acetoacetyl CoA (125).



Acetyl CoA Acetoacetyl CoA

2.4.2 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 (126).

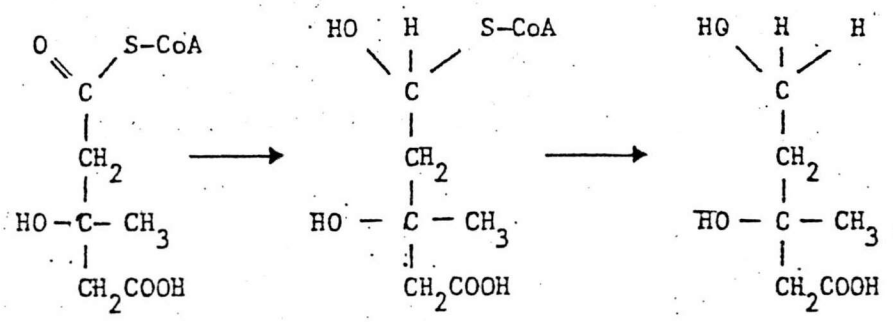


Acetyl CoA Acetoacetyl CoA HMG CoA

2.4.3 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 (127).

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



HMG CoA

Mevalonic acid

2.4.4 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 (128,129).

The overall reaction for the conversion of acetyl CoA to isopentenyl pyrophosphate are shown in Fig. 2.18 (129).

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 (128).

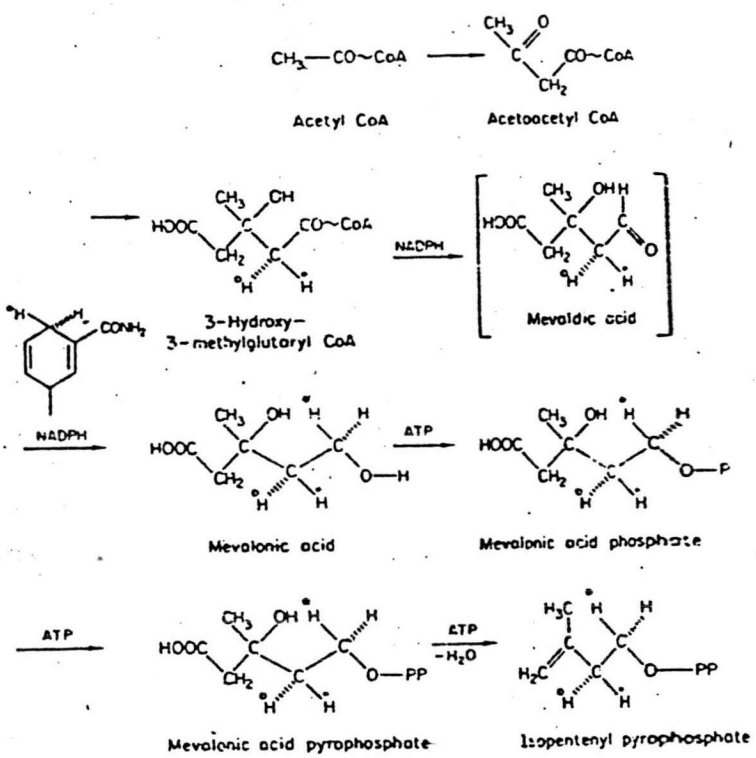
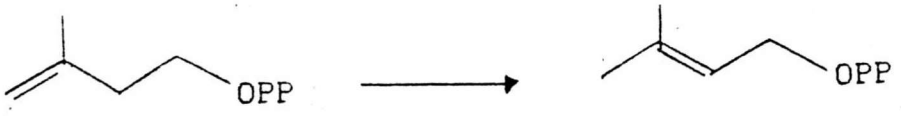


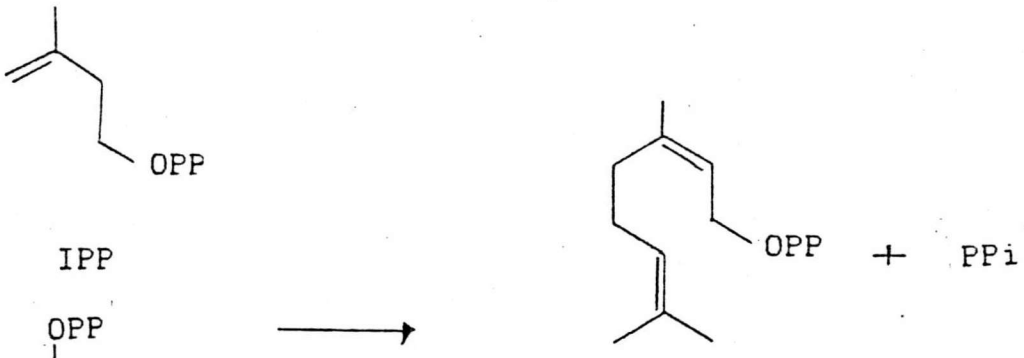
Figure 2.18 Biosynthesis of isopentenyl pyrophosphate from acetyl CoA

2.4.5 Isomerization of Isopentenyl Pyrophosphate (IPP) to Dimethylallyl Pyrophosphate (DPP) (130).



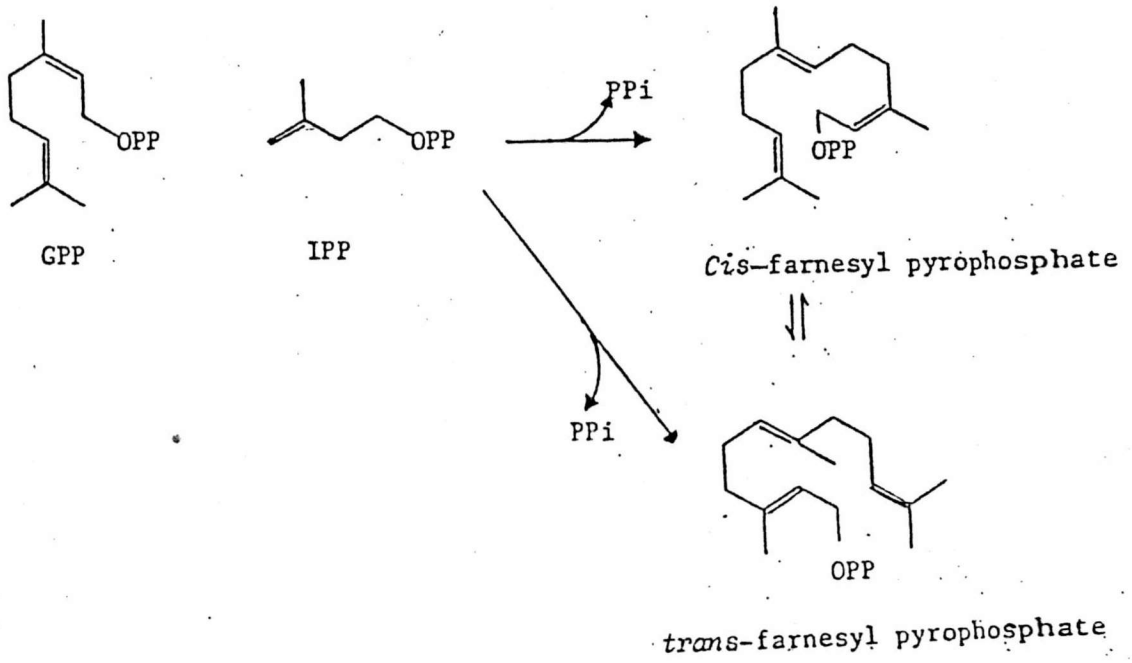
IPP DPP

2.4.6 Formation of Geranyl Pyrophosphate (GPP) from Isopentenyl Pyrophosphate (IPP) and Dimethylallyl Pyrophosphate (DPP) (130).



Geranyl Pyrophosphate (GPP)

2.4.7 Formation of Farnesyl Pyrophosphate from Geranyl Pyrophosphate (GPP) and Isopentenyl Pyrophosphate (IPP) (130).



2.4.8 Formation of Squalene from farnesyl pyrophosphate

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. 2.19 (129).

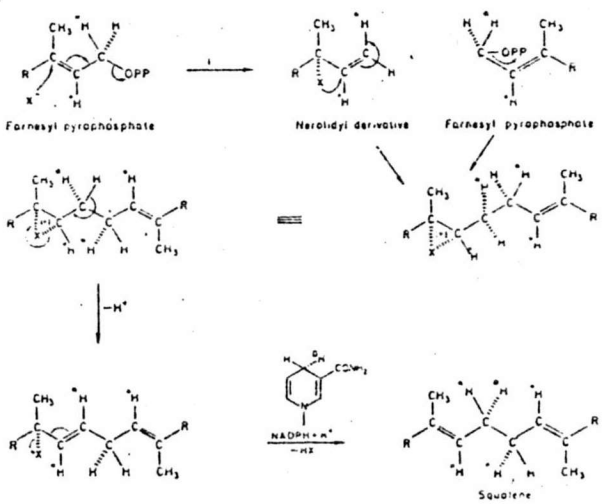


Figure 2.19 Formation of squalene from farnesyl pyrophosphate

2.4.9 Formation of cyclic triterpene ring system from squalene, Fig. 2.20 (115,118,129)

The cyclisation of squalene 1 to form the cyclopentanophenanthrene 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 hydroxy 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.

2.4.10 Conversion of first cyclic intermediate (cycloartenol) to the sterol products (115,118,129).

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

cycloartenol 4, a 4,4-dimethyl sterol. Demethylation at C-4 is probably the next step, producing cyclocucalenol 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 cycloartenol → 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 rearrangememt 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)-triene-3 -ol 12 → stigmasta-5,7-diene-3 β -ol 14 → sitosterol 15 or stigmasterol 16. This sequence would not involve avenasterol.

Another pathway for the biosynthesis of major higher plant sterol is first reduction of $\Delta^{24(28)}$ of Δ^{7} - avenasterol to form stigmasta-7-en-3 -ol 17. This reaction must be through a $\Delta^{24(25)}$ intermediate since the C-25 hydrogen atom is lost. Next, stigmasta-7-en-3 -ol goes through the $\Delta^{7(8)}$ - $\Delta^{5,7}$ - $\Delta^{5(6)}$ 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 on species and environmental conditions.

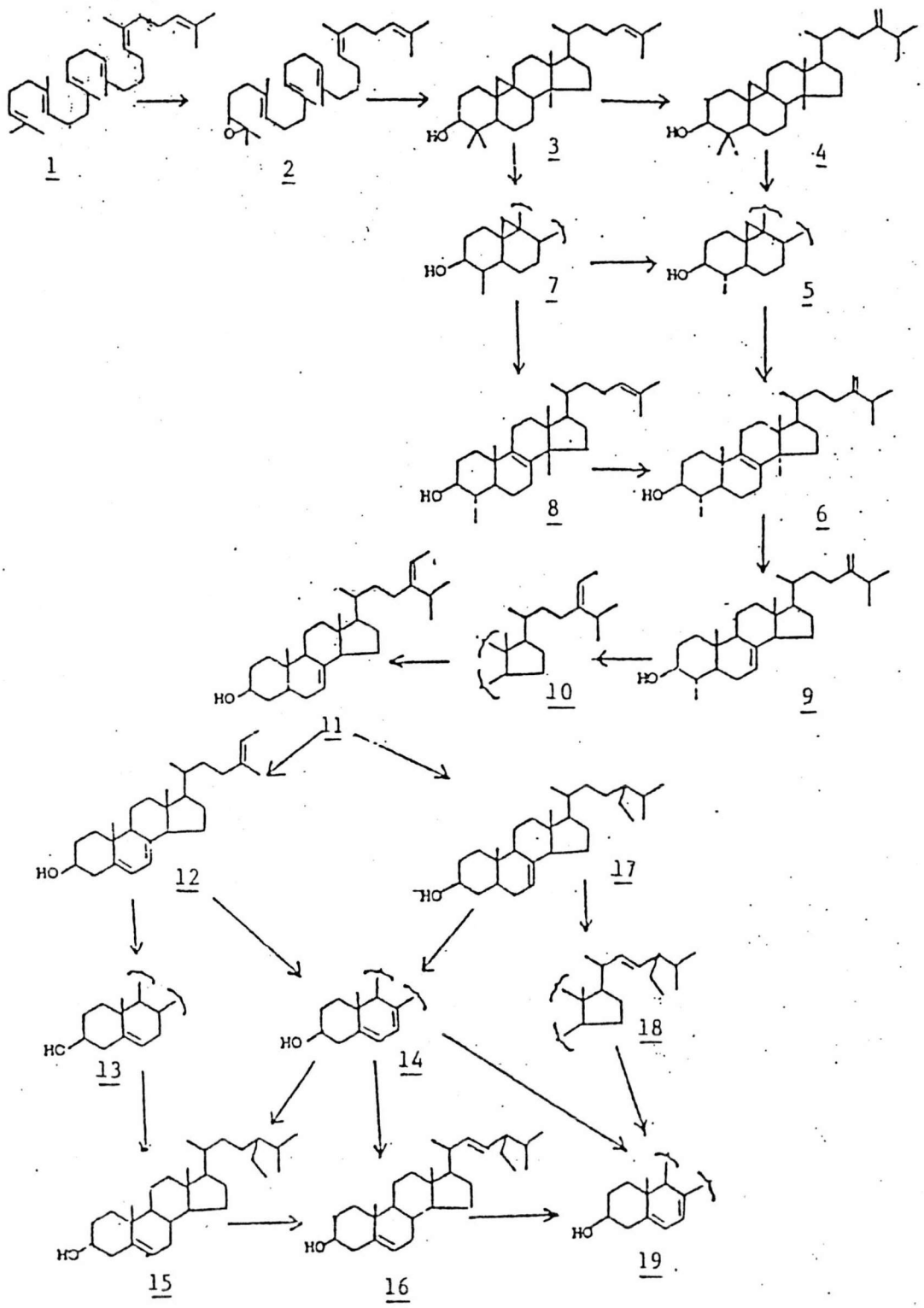


Figure 2.20 Biosynthetic pathway of plant sterols

3. Sesquiterpenoids

Up till now, over 1,200 sesquiterpenoids(131) have been isolated and identified from many species of higher plants and lower plants, chiefly from species of Compositae (132). Sesquiterpenoids of the species of the genus Eupatorium (Compositae) are also listed in table 1.

Three reasons can be given for an increase in the interest in this group of natural products. First, sesquiterpenoids, especially sesquiterpene lactones, have been used successfully as markers in biochemical systematic (chemotaxonomy) studies, mainly in Compositae. Second, a number of compounds have recently been found to have their various biological activities such as anti-tumor, cytotoxic, antimicrobial and phytotoxic activities and finally, the development of methods and appartus of isolation, purification and structure elucidation make research more easily (132, 133, 134).

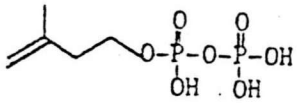
3.1 Chemistry of Sesquiterpenoids

Sesquiterpenoids are a class of terpenoids. This group of compounds possess many functional groups, such as alkanes, alcohols, lactones, etc. (134).

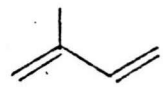
Terpenoids refer to the group of natural products which are derived biosynthetically from the 5-carbon compound, isopentenyl pyrophosphate which is also known as "activated isoprene". Isoprenoid compounds are also refered

to as terpene or terpenoids (110,135).

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



Isopentenyl Pyrophosphate



Isoprene

3.2 Classification of Sesquiterpenoids

Classification of sesquiterpenoids is based on their carbocyclic skeleton in which they are divided into subgroups according to their functional class : nonlactonic and lactonic. The lactonic functional names end with "-olide".(133,134).

The sesquiterpenoids appear to be composed of three isoprene units linked by head-to-tail condensation. Their carbon skeletons are varied greatly owing to subsequent secondary C-C bond formation and molecular rearrangement during their biogenetic course.

Table 2. Natural products formed from isoprene units.

Class	Number of Isoprene Units	Occurrence
Hemiterpene (C_5)	1	emitted by the leaves of different species of higher plants
Monoterpenes (C_{10})	2	Constituents of volatile oils, iridoid substance
Sesquiterpenes (C_{15})	3	Constituents of volatile oils, resins
Diterpenes (C_{20})	4	Constituents of volatile oils and resin, 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

3.2.1 Germacrane Class

So far about 80 compounds of this class have been isolated. Although these compounds include hydrocarbons, alcohols, epoxides, ketones (nonlactonic), γ-lactones (germacranolides) and furanolides, they are thermally unstable, owing to the fact that they have an unsaturated ten-membered ring containing two endo-cyclic double bonds or epoxides at the 1(10)- and 4-positions as a commom structure (Fig. 2.21). Especially, the sesqui-hydrocarbons of this class contain an additional double bond in the interior or exterior of the ring and are sensitive to heating.

3.2.1.1 Nonlactonic Compounds

Among ten members of nonlactonic compounds of this class, shiromool and shiromodiol, mono- and diacetate (Fig. 2.22) have been found to be insect antifeedant (136).

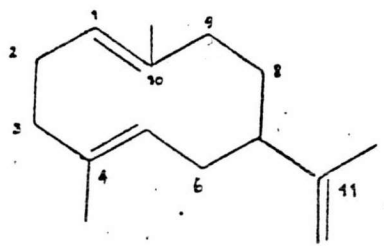
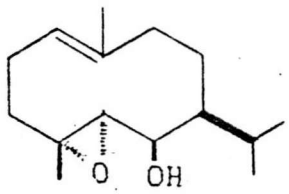
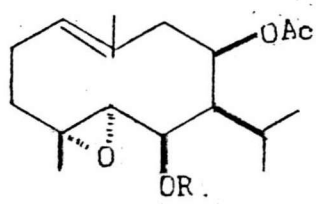


Figure 2.21 Common Structure of Germacrane Class

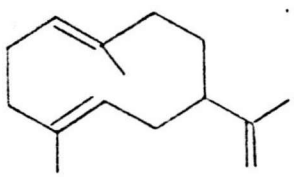


Shiromool

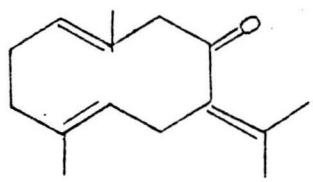


Shiromodiol monoacetate

Shiromodiol diacetate



Germacrane A



Germacrone

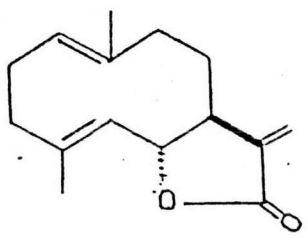
Figure 2.22 Some nonlactonic sesquiterpenoids of the germacrane class

3.2.1.2 Germacranolides

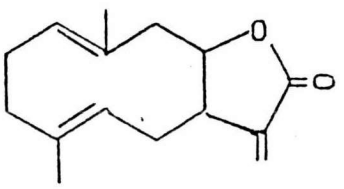
This group of germacrane class includes monolactone and dilactone, and all their members have one lactone functional group at C-12 and C-15. The germacranolides, accordingly, are classified into two subgroups ,i.e. C-6 closing and C-8 closing types depending upon direction of the lactone formation involving the C-12 functional group (Fig. 2.23). As far as the stereochemistry is concerned, both closing types uniformly have a stereostructure of C-12 lactone function attaching to C-6 or C-8 in trans-fashion;

H-7 always takes α-orientation and H-6 or H-8, β-pyrethrosin is the only exception with a <u>cis</u>-fused lactone (Fig. 2.23).

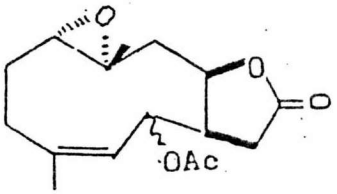
In the dilactones of C-6 closing type, the additional lactone ring is oriented between C-14 and C-2 accompanied by α-oriented H-2, and in those of the C-8 closing type, between C-15 and C-6 with β-oriented H-6 (Fig. 2.23).



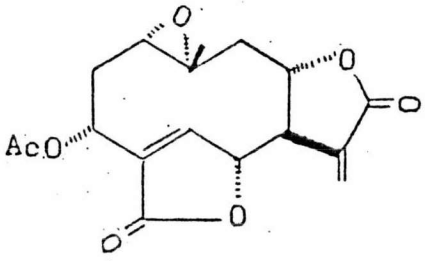
Costunolide



Inunolide



Pyrethrosin

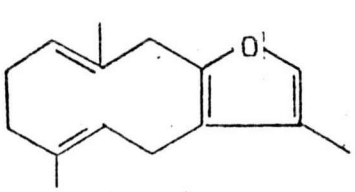


Scandenolide

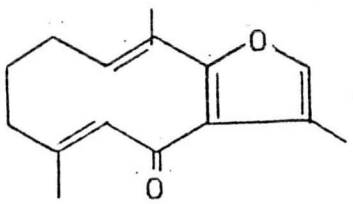
<u>Figure 2.23</u> Some germacranolides

3.2.1.3 Germafuranolides

About 20 members of this group, which have the isopropenyl side chain of the germacrane carbon skeleton as a part of a furan ring, have been mainly detected from plants of the Lauraceae family. In all the members the oxide bond of the furan ring is oriented between the C-8 and C-12 position; the alternative 6,12-oxide form has never been found. Among them, also, several members have an additional lactone ring between C-15 and C-6 with the above-mentioned furan ring. Thus, sericenic acid isolated from leaves of Neolitsea sericea is thought to be an intermediate compound showing biogenetic transformation from the simple germafuranolide to the lactonized germafuranolide. Besides, some pairs of geometric isomers such as furanodienone and isofuranodienone, neosericenine etc (Fig. 2.24), have been isolated regarding the cyclodeca-1,5-diene system of the molecule. The pairs were respectively characterized by NOE examination to comprise one isomer having two endo-cyclic double bonds in trans-trans fashion and a second isomer containing two trans-cis oriented double bonds.



Furanodiene



Furanodienone

Figure 2.24 Some Germafuranolides

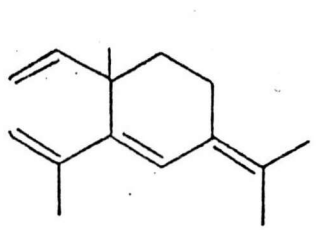
3.2.2 Elemane Class

This class also contains γ-lactone and furanolides together with hydrocarbons, alcohols and carbonyl compounds like the germacrane class.

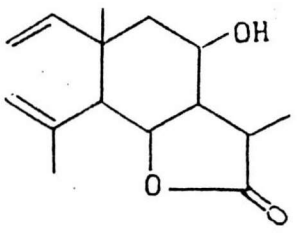
As to the stereochemistry at the C-5, C-6 and C-10 centers, it can be said, in general, that two isopropenyl groups on C-5 and C-7 have β- and a vinyl group on C-10 has α-configuration in the absolute configuration of the members of this class.

Thermal instability of germacrene hydrocarbons has been mentioned in the foregoing section. A large number of germacrane sesquiterpenoids containing a cyclodeca-1,5-diene system such as germacrene A, Germacrone, etc. easily undergo thermal cleavage of the ten-membered ring at the allylic position for the two endo-double bonds to give elemane type compounds. Accordingly, it is to be questioned whether compounds of this elemane class collected here are true natural products or not.

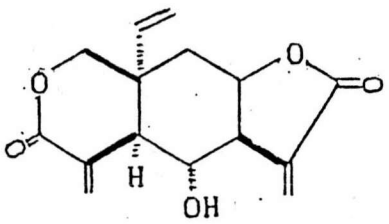
Recently, some new types of sesquiterpenoids with the elemane carbon skeleton such as vernomenin, occidenol, etc. have been isolated (Fig. 2.25).



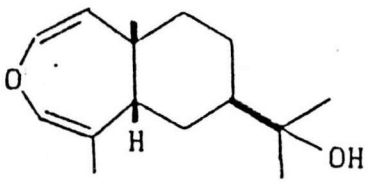
α-Elemane



Temisin



Vernomenin



Occidenol

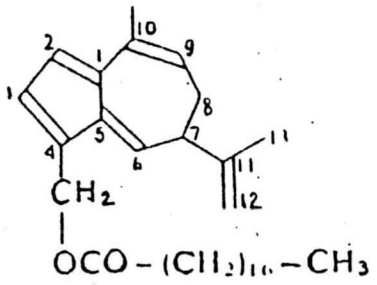
Figure 2.25 Some sesquiterpenoids of the elemane class

3.2.3 Guaiane Class

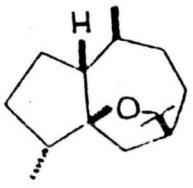
3.2.3.1 Nonlactonic Compounds

This class further includes three hemiketals which have a 5,8-epoxy-8-ol system and some others of two types, 5,11-oxide and 10,11-oxide together with a large number of γ-lactones (guaianolides) to be described in the following section. The 5,11-oxides isolated from zedoary such as liguloxide, liguloxidol and guaioxide have a *cis*-fused bicyclo[5.3.0]decane system accompanied by an α-oriented H-5, while the 10,11-oxides isolated from japanese valerian root such as kessane, α-kessyl alcohol, kessanol and

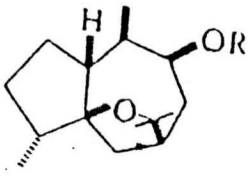
kessoglycol diacetate possess a <u>trans</u>-fused bicyclo[5.3.0]decane system accompanied by the same α-oriented H-5; in both types of ether, the oxide ring are in β-orientation.



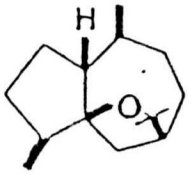
14-Hydroxyguaia-1,3,5,9,11-pentenyl stearate



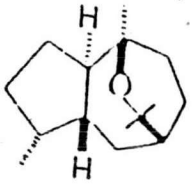
Liguloxide



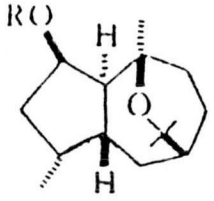
Liguloxidol



Guaioxide



Kessane



α-Kessyl alcohol

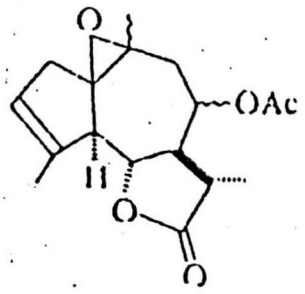
<u>Figure 2.26</u> Some nonlactonic sesquiterpenoids of the guaiane class

3.2.3.2 Guaianolides

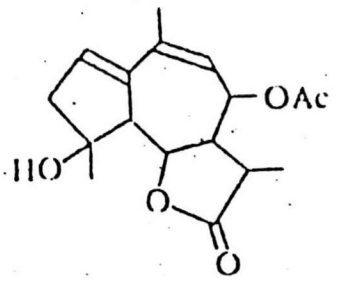
During chemotaxonomic investigations on the Compositae, over 100 compounds of sesquiterpene lactones which comprise two main classes, the guaianolides and the ambrosanolides (pseudoguaianolides), have been isolated. Since all the members of both groups have a lactone function on C-12, they are again classified into two subgroups of C-6 closing and C-8 closing types according to the direction of lactone formation. As far as the stereostructure has been established, all the guaianolides of the C-6 linkage type have the configurations of H-5(α), H-6(β), and H-7(β); the C-8 closing guaianolides predominantly possess H-5(α), H-7(β), and H-8(α). Three chlorine-containing guaianolides, eupachlorin, its acetate and eupachloroxin, which show tumor inhibitory activity have been isolated from the alcoholic extract of Eupatorium rotundifolium, and the stereostructures were confirmed by means of X-ray analysis. These compounds are new type of sesquiterpenoids.

3.2.4 Ambrosane Class

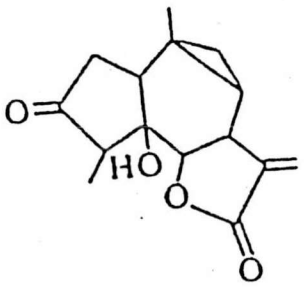
Formation of the ambrosane skeleton is explained by the biogenetic 1,2-shift of a methyl group in the guaiane skeleton from C-4 to C-5. Up till now a large number of the members of this class have been isolated and all the compounds are characterized as γ-lactone (ambrosanolides) with the exception of damsic acid which is the anly sesquiterpene acid having the ambrosane carbon skeleton and



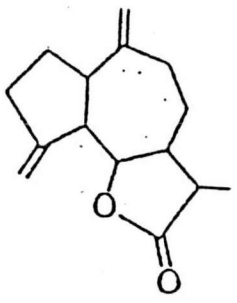
Globicin



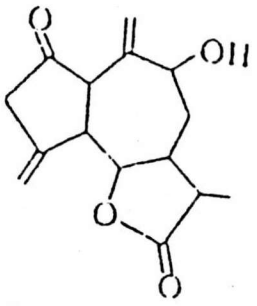
Sieverin



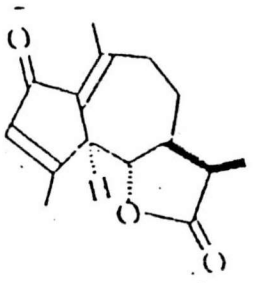
Ivaxillarin



Mokko Lactone



Grosshemin



Achillin

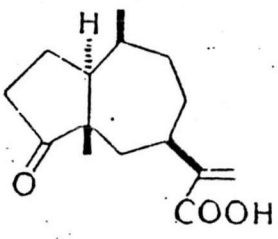
Figure 2.27 Some lactonic sesquiterpenoids of the guaiane class

was isolated from Ambrosia ambrosioides.

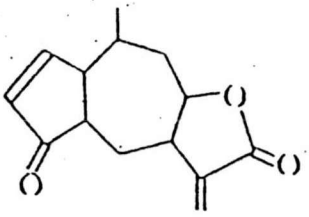
The ambrosanolides, which include both C-6 and C-8 closing types, have a trans-fused bicyclo[5.3.0]decane system along with a β-oriented C-15 methyl group. With respect to the stereochemistry of the lactone ring, the C-6

closing ambrosanolides all have cis-fusion (α-oriented H-6), but such common figure has not been recognized in the C-8 closing type.

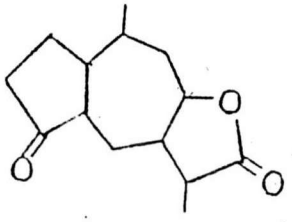
Mexicanin E and dihydromexicanin E are nor-sesquiterpene lactone without a C-15 methyl group and mexicanin D is an abnormal guaianolide having a methyl group at the C-2 position instead of C-15.



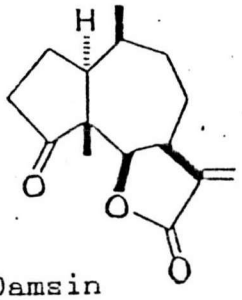
Damsic acid



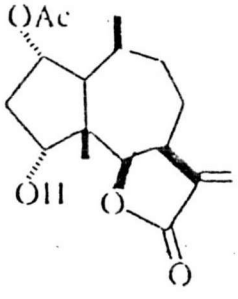
Mexicanin E



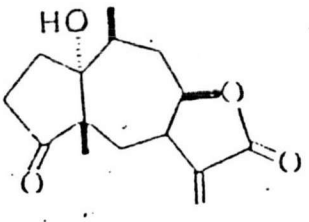
Dihydromexicanin



Damsin



Salsolin



Peruvin

Figure 2.28 Some sesquiterpenoids of the ambrosane class

3.2.5 Miscellaneous classes of sesquiterpenoids

Furthermore, there are several new sesquiterpenoid skeleton compounds, which are recently discovered, for instances, eremophilane, chrymorane, etc.(136).

3.3 Distribution of Sesquiterpenoids

Sesquiterpenoids have been isolated from many species of higher plants and also from algae and other non vascular plants. Several novel sesquiterpenoids have been discovered from many species of the liverworts (Hepaticae), placed phylogenetically between the vascular plants and algae(134).

Sesquiterpenoids possess several functional group; hydrocarbons, alcohols, epoxides, ketones, and lactone(136). They have been found that the data of distribution of sesquiterpene lactone are very useful for chemotaxonomy especially for the Compositae (132).

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 Umbelliferae, Magnoliaceae, Lauraceae, Winteraceae, Illiciaceae, Aristolochiaceae, Menispermaceae, Cortiariaceae, Acanthaceae, Burserae, Hepaticae, and Amaranthaceae (132,137).

3.4 Biosynthesis of Sesquiterpenoids

Biosynthesis of sesquiterpenoids involving modification and/or cyclisation of pyrophosphate ester of trans, trans-farnesol, cis, trans-farnesol or nerolidol (133,138,139).

Biosynthetic pathways of sesquiterpenoids involve similar early steps as the biosynthesis of steroids to obtain farnesyl pyrophosphate. Further reaction steps, then take place to obtain the various class of sesquiterpenoids (140).

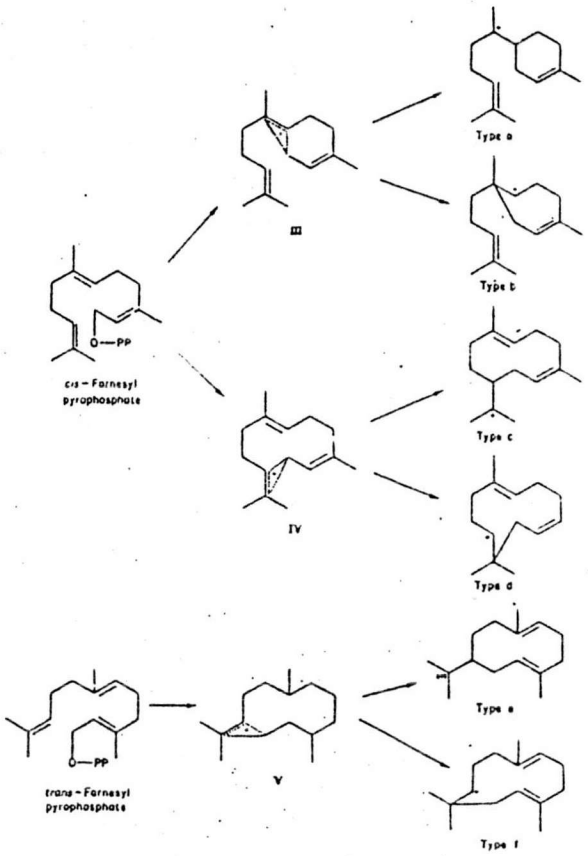


Figure 2.29 Possible ways for the formation of different types of cyclic sesquiterpenoids from farnesyl pyrophosphate.

The formation of cyclic sesquiterpenoids has been investigated to only a small extent. The ring system most frequently found may be directly formed after elimination of the pyrophosphate group by the unstable cation III, IV and V shown in Fig. 2.29. The positively charge intermidiate products of the type a-f may stabilize by elimination of a proton or reaction with another compound Fig. 2.30 (110).

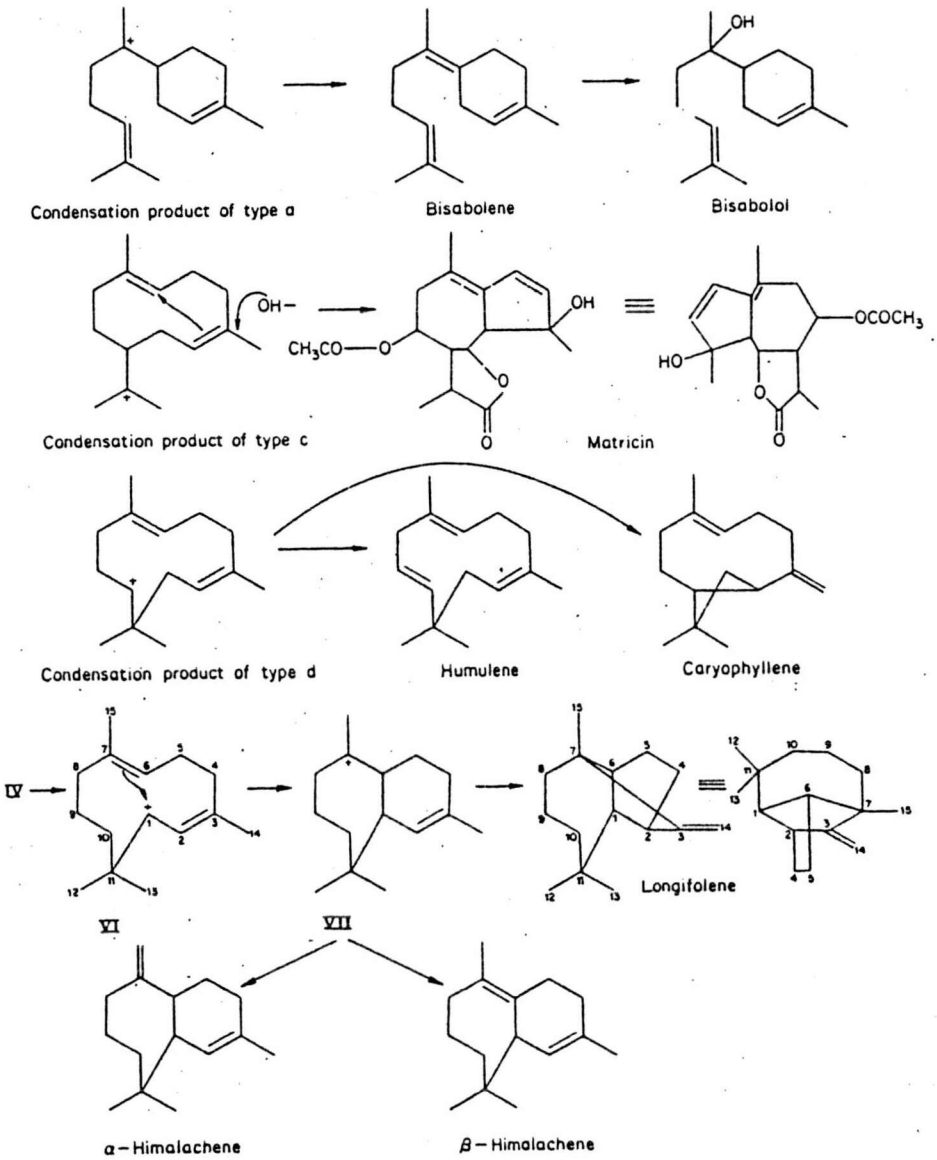


Figure 2.30 Possible ways for the formation of some sesquiterpenoids

3.5 Biological activities of sesquiterpenoids

Sesquiterpenoids are a group of natural products which exhibit various interesting biological activities and some of them are on developing for clinical uses (140).

Some important biological activities and clinical uses of sesquiterpenoids are listed in Table 3.

1. Antiphlogistic and Spasmolytic Agents

For a long time, antiinflammatory sesquiterpenoids, such as guaiazulene and chamazulene (Fig. 2.31), have been known. Azulenes practically do not exist naturally. They are formed as artifacts from sesquiterpenic precursors such as matricin, achillin or artabsin during steam distillation, by the equally unstable chamazulene-carboxylic acid.

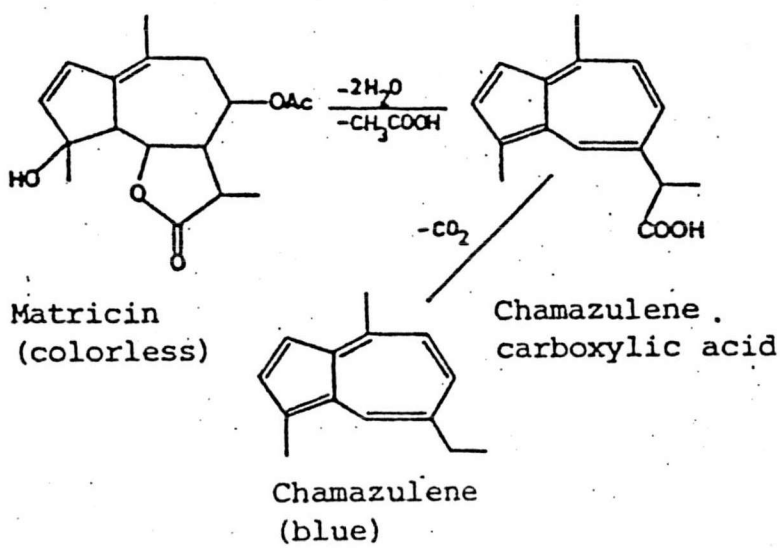
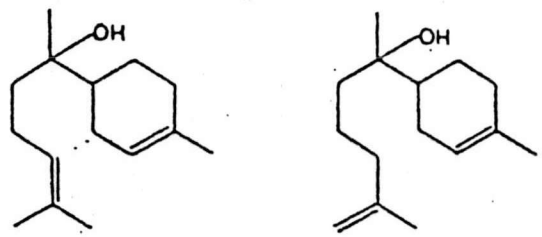


Figure 2.31 Formation of chamazulene from matricin

Table 3 The important bioactivity and clinical use of some sesquiterpenoids

Activity	Biological activity	clinical use
Analeptic	+	+
Analgesic	+	
Anthelmintic	+	+
Antiarrthythmic	+	
Antibiotic	+	+
Antiseptic	+	
Antiepileptic	+	
Antiinflammatory	+	
Antitumor	+	
Choleretic	+	
Hypotensive	+	
Irritant	+	
Juvenile hormone	+	
Organoleptic	+	
Pheromone	+	
Phytohormone	+	
Sedative	+	
Spasmolytic	+	

Furthermore, (-)-α-bisabolol (Fig. 2.32) showed a clear antiinflammatory action against the carrageenene edema of the rat foot and against the cotton pellet granuloma of the rat. The antiphlogistic effect is clearly less toxic.



Isopropylidene form

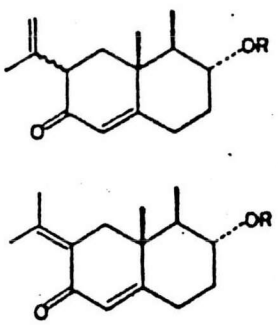
Isopropenyl form

Figure 2.32 α-Bisabolol

The usual naturally occuring levorotatory form of bisabolol (e.g. in chamomile oil) has more powerful antiphlogistic and spasmolytic activities than the dextrorotatory form or the racemate. The biochemical studies showed that (-)-α- bisabolol has a primary antipeptic action depending on dosage, which is not caused by an alteration of the pH-value. The proteolytic activity of pepsin is reduced by 50% through addition of bisabolol in the ratio 1/0.5.

Although spasmolytic activity is found rather widely among the sesquiterpenoids, only the petasins shall be mentioned here. Petasin and isopetasin are beside S-petasin and S-isopetasin (Fig. 2.33) the spasmolytic agents of the

leaves and roots of Petasites hybridus. The petasins belong to the group of eremophilane sesquiterpenoids. They are esters of the C_{15} alcohol petasol or isopetasol, with angelic or β-methylthioacrylic acid, respectively. Petasin is 14 times more active than papaverine. The esters of isopetasol are less active.



Petasin R = Angelic acid

S-Petasin R = β-Methylthioacrylic acid

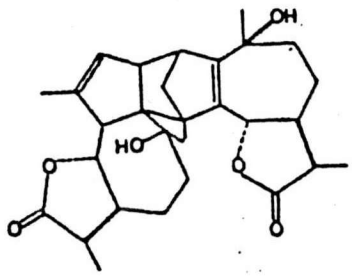
Iso-Petasin

Iso-S-Petasin

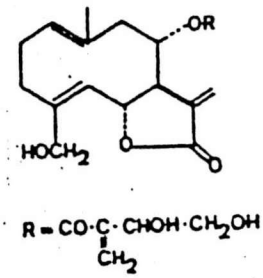
Figure 2.33 The petasins and isopetasins

2. Bitter Substances

Bitterness is typical for a great number of sesquiterpenoids, being particularly marked in the majority of lactones present in the family of Compositae. But only Artemisia absinthium and Cnicus benedictus are important in pharmacy and in food industry. The bitter taste of Artemisia is due to the guaianolide lactone absinthin and relates compounds (Fig. 2.34). Cnicin, the bitter principle of C. benedictus, is an ester of 3,4-dihydroxy-1-butene-2 carboxylic acid with a sesquiterpene lactone of the germacrane type.



Absinthin



Cnicin

Figure 2.34 Important sesquiterpene lactones with bitter taste

3. Antitumor Activity

The available of modern, refined methods for testing anticarcinogenic agents has encouraged the systematic search for cancerostatic agents among natural products. Many of the sesquiterpenoids in the Compositae family, chiefly lactones from the germacranolide, guaianolide, pseudoguaianolide and elemanolide class are especially active. Table 4 shows some sesquiterpene lactones that have been isolated and shown to have antitumor activity.

More recent studies of several cytotoxic sesquiterpene lactones and related compounds confirmed the requirement for an unsaturated lactone ring (Fig. 2.35) for cytotoxicity. As a rule, activity of germacranolides was higher than that of guaianolides (131).

Table 4. Some sesquiterpene lactones with antitumor activity

Substance	Class	Plant
Elephantin	Germacranolide	*Elephantopus elatus*
Elephantopin	"	"
Eupatoriopicrin	"	*Eupatorium cannabinum*
Eupacunin	"	*Eupatorium cuneifolium*
Eupacunoxin	"	"
Eupatocunin	"	"
Eupatocunoxin	"	"
Eupacunolin	"	"
Eupatolide	"	*Eupatorium formosanum*
Eupaformonin	"	"
Cnicin	"	*Cnicus benedictus*
Euparotin	Guaianolide	*Eupatorium rotundifolium*
Euparotin acetate	"	"
Eupachlorin	"	"
Eupachlorin acetate	"	"
Eupatoroxin	"	"

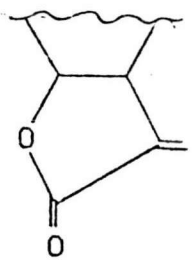


Figure 2.35 Structure of unsaturated lactone ring