CHAPTER II

HISTORICAL

1. Anthraquinones

1.1 Distribution of Anthraquinones

Long before anything was known of their chemistry, rhubarb, aloe, senna and cascara were recognized as firming a natural group of purgative drugs. Certain vegetable and animal dyestuffs such as madder and cocchineal were of great economic importance before the introduction of synthetic dyestuffs. Later the chemical similarity of these purgative drugs and dyestuffs became apparent. Substances of the anthraquinone type were the first to be recognized, both in the free state and as glycosides (Trease and Evan, 1976).

The anthraquinones are the largest group of natural quinones, nearly half of which have been found in higher plants and a similar number in fungi, especially in *Aspergillus* and *Penicillium* spp. and are found more frequently in lichens than in higher fungi (Thomson, 1971). There are the first 1,4-anthraquinones to be found in nature from the mycelium of *Aspergillus cristatus*. These are viocristin and isoviocristin (Thomson, 1985). However, studies on Australian toadstools belonging to the genus *Dermocybe* and its allies have led to the discovery of the novel anthraquinone

γ -lactone austrocorticin and noraustrocorticin (from an as yet unnamed species related to the European *Dermocybe cinnabarina*) and γ -lactone dermolactone and its 4-hydroxy derivative (from *Dermocybe sanguinea*, sensu Cleland). And a toadstool in New Zealand, *Dermocybe canaria*, has been found 4-aminophyscion which the first anthraquinone containing an amino group (Thomson, 1993).

In animals, a few occur in feather stars (Crinoidae) and in insects (Coccinae only) (Thomson, 1971). Carminic acid, the major constituent of cochineal, obtained from the dried bodies of female insects of species *Dactylopius coccus* that feed on the cactus *Nopalea coccinellifera* (Thomson, 1993). Some anthraquinones produced by enterobacters and nematodes. The symbiosis of the bacterium *Xenorhabdus luminescens* and the insectpathogen nematode *Heterorhabditis* spp. was found to produce 1, 3, 8 - trihydroxy -9, 10-anthraquinone and its methyl ether (Sztaricskai *et al*, 1992).

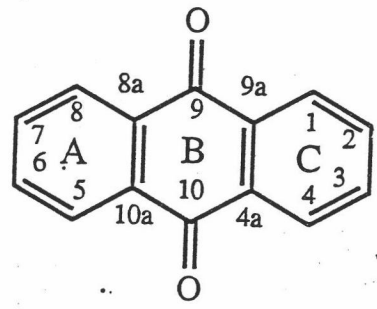
In higher plants, they are located chiefly in heartwood, bark and roots (often as glycosides), occasionally in stems, seeds and fruits the distribution of anthraquinones in higher plants are mostly in Rubiaceae, they account for half the total member (Thomson, 1971). Anthraquinones can be found among the following genera of the Rubiaceae:- *Morinda, Galium, Coprosma, Damnacanthus, Hymenodictyon, Hedyotis* (Gibbs, 1974) *Prismatomeris* (Lee, 1969) and *Coelospermum* (Thomson 1971). There was also a report of the anthraquinones in callus culture of *Cinchona ledgeriana* Maens (Rubiaceae) (Wijnsma *et at*, 1984). The other families also containing anthraquinone pigments

are Rhamnaceae [*Rhamnus* (Gibbs, 1974)], *Maesopsis* (Cumming, 1970), *Ventilago* (Cooke and Johnson, 1963), Polygonaceae [particularly *Rumex, Rheum* and *Polygonum* (Gibbs, 1974)], Leguminosae, subfamily Caesalpiniaceae [*Cassia* (Takido, 1958)], subfamily Papilionaceae [*Abrus* (Gibbs, 1974)], Bignoniaceae [*Tabebuia avellanedae* Lor.ex Griseb (Burnett and Thomson, 1967)], Verbenaceae [*Tectona grandis* Linn. (Ahluwalia and Seshadri, 1957)] and Scrophulariaceae [*Digitalis* species (Gibbs, 1974)]. Anthraquinones are also found in monocotyledons especially in Liliaceae and Xyridaceae. *Aloe* (Rheede, 1963), *Asphodeline, Asphodelus, Bulbine, Enemerus* (Van Rheede, 1964) and *Polygonatum* (Gibbs, 1974) represented the genera of the Liliaceae which contained anthraquinones, as well as Xyridaceae, *Xyris indica* Linn. containing chrysazin (Nijsiri Ruangrungsi, 1980) and *X.semifuscata* Baker containing chrysazin and 3-methoxy chrysazin (Fournier *et al.*, 1975). Some anthraquinones are found in Anacardiaceae, Apocynaceae, Asclepiadaceae, Caryophyllaceae, Compositae, Ericaceae, Euphorbiaceae, Lythraceae, Rhizophoraceae, Saxifragaceae (Gibbs, 1974) and Solanaceae (Knapp, 1972).

1.2 Chemistry of Anthraquinones

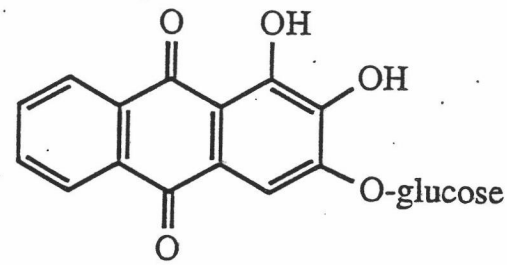
Most of anthraquinones are red yellow or orange yellow colouring matter. Anthraquinone is a tricyclic benzene ring structure having diketone at C-9 and C-10 position (9,10-anthracenedione).

The fundamental structure (1) is shown below with the ring numbering system.

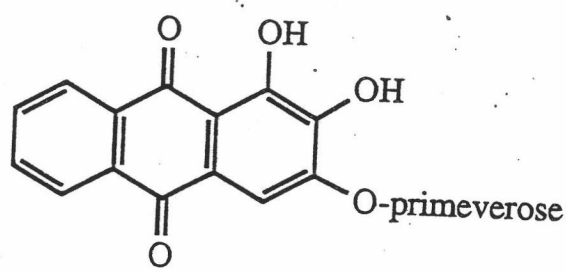


The fundamental structure (1)

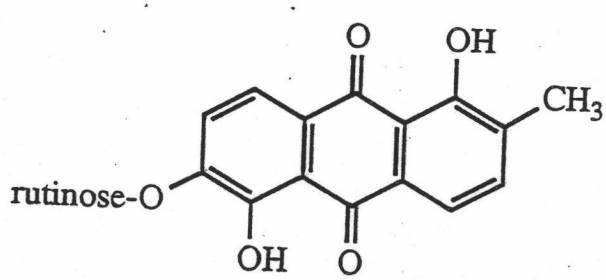
The anthraquinones actually existed in plants are apparently in several forms. They are often found as anthraquinone glycosides rather than hydroxylated anthraquinones or aglycones. Reports of the appearance of free anthraquinones must be regarded cautiously. Many anthraquinones occur as glycosides with the sugar residue linked through one of the phenolic hydroxyl group. The anthraquinone glycosides are classified in two types. One is O-glycoside, the sugar links at the phenolic oxygen. The other is C-glycoside with sugar attached through carbon-carbon bond (C-1 to C-8). Several different sugars are found in such glycosides. For example, alizarin occurs as a 3-glucoside (2) in *Rubia tinctorum* Linn.(madder) and as a 3-prime-veroside (3) in *Galium* species; and morindone occurs as a 6-rutinoside (4) in *Coprosma australis* Robinson (Robinson, 1967).



Alizarin-3-glucoside (2)



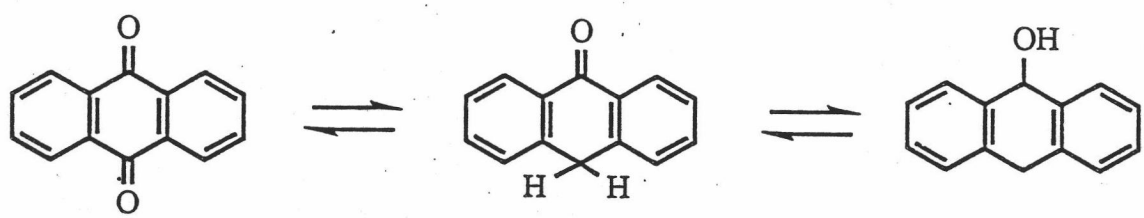
Alizarin-3-prime-veroside (3)



Morindone-6-rutinosidee (4)

Anthranols (5) and anthrones (6) are reduced anthraquinone derivatives occur either free or combined as glycosides. They are isomeric and one may be partially converted to the other in solution. The parent substance, anthrone, is a pale yellow, non-fluoresce substance which is insoluble in alkali, its isomer, anthranol, is brownish-yellow and forms a strongly fluoresce solution in alkali.

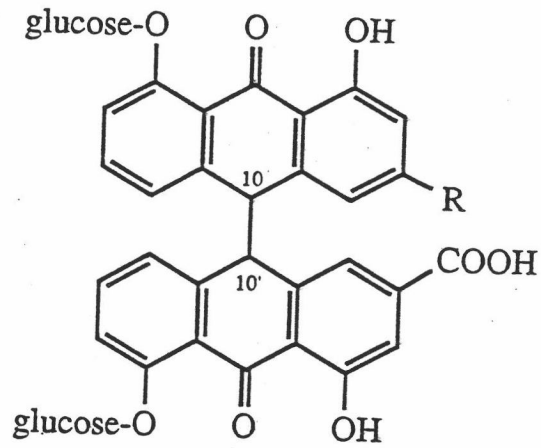
Anthranol derivative, such as are found in aloe, have similar properties and the strong green fluorescence which aloes gives in borax or other alkaline solution. It has long been used as a test for its identification. Anthranols and anthrones are the main constituents of chrysarobin, the mixture of substances prepared by benzene extraction from the material (araroba) found in the trunk cavities of the tree *Andira araroba*. If a little chrysarobin is treated on a white tile with a drop of fuming nitric acid, the anthranols are converted into anthraquinones. A drop of ammonia allowed to gradually mix with the acid liquid produces a violet colour. This modification of Borntrager's test has been used as a test for identify before the chemistry was known (Trease and Evans, 1976).



Anthraquinone Anthrone (6) Anthranols (5)

Dianthrones are compounds derived from two anthrone molecules, which may be identical or different. They are important aglycones in species of *Cassia*, *Rheum* and *Rhamnus*, in this group the sennosides are among the best-known example. The different between the aglycone of sennoside A and B (7) is one of optical activity and the same

relationship can be applied for the aglycone of sennoside C and D (7) (Trease and Evans, 1976).



	R	10-10′
Sennoside A	COOH	*trans*
Sennoside B	COOH	*meso*
Sennoside C	CH_2OH	*trans*
Sennoside D	CH_2OH	*meso*

Sennoside (7)

Anthraquinones can be deteced by the Borntrager's test in which an organic solution containing the test material is shaken with an aqeous base. In base, the anthraquinone can form phenolate-type ions which are coloured. The visible result of the test in which the basic layer goes a cherry red and the intensity of the colour can be used as a measure of the amount of anthraquinones in the material. Only free anthraquinones give a positive Borntrager's test and this fact can be used to distinguish between the O-and C-glycosides. The O-glycosides being hydrolyzed to free anthraquinones by reflux with diluted hydrochloric acid while the C-glycosides release the free anthraquinones only after oxidative cleavage. The colours given with alcoholic magnesium acetate solution are characteristic of different hydroxylation patterns (Robinson, 1967). Compounds containing, two hydroxyl groups in the *ortho* position e.g. alizarin, exhibit a violet colour, those with two in the *meta* position e.g. emodin, give and

orange-red or pink colour and those with two in para position e.g. guinizarin, produce a purple. These colour reactions are specific and stable.

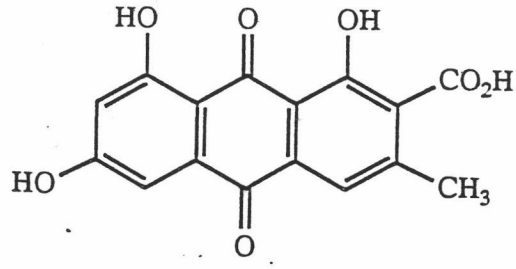
The most important groups of laxative drugs used todays is the group of the plant products derived from materials that contain anthracene derivatives. The active constituents are anthraquinone compounds containing phenolic group, either free, or methyl ethers, or as glycosides. The pharmacologically important compounds are free anthranols. Anthraquinones are active as cathartics only because they are reduced to anthranols by intestinal bacteria (Robinson, 1967).

1.3 Classification of Anthraquinones

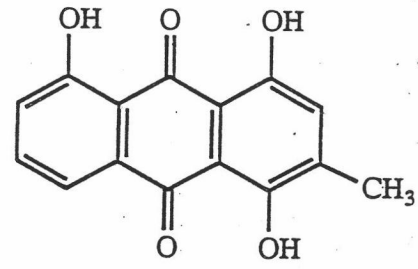
According to biosynthetic pathways, anthraquinones can be classified into two groups:

a) Anthraquinones with substitute in ring A and C

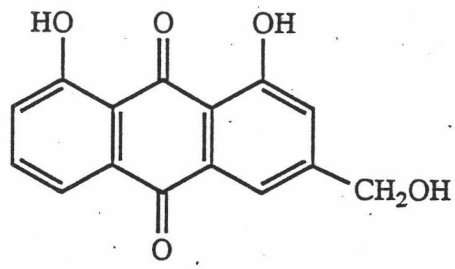
These anthraquinones are found in fungi and higher plants. In fungi they are endocrocin (8) and islandicin (9). Endocrocin was isolated from *Aspergillus amstelodami* and islandicin was isolated from *Penicillium islandicum* (Thomson, 1957). In higher plants, these anthraquinones distribute in Leguminosae (Subfamily Caesalpiniaceae and Papilionaceae), Polygonaceae, and Rhamnaceae e.g. aloe-emodin (10), chrysophanol (11), emodin (12). As the name



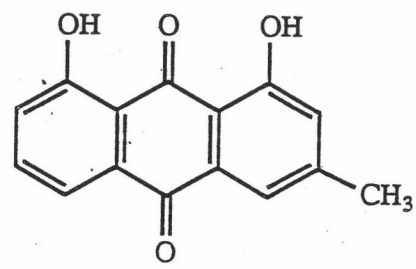
Endocrocin (8)



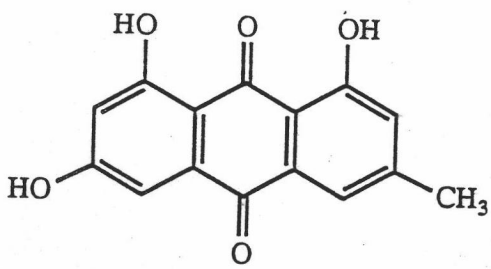
Islandicin (9)



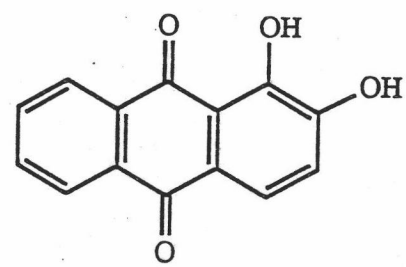
Aloe-emodin (10)



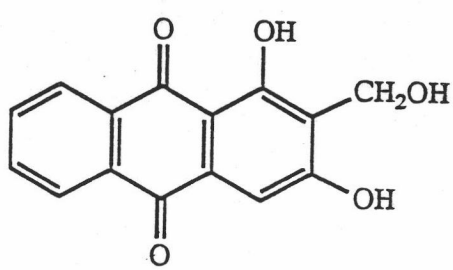
Chrysophanol (11)



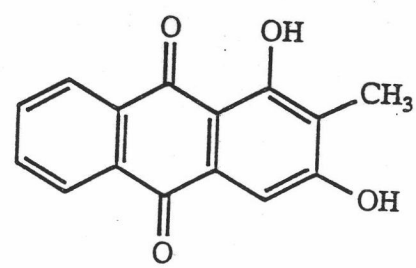
Emodin (12)



Alizarin (13)



Lucidin (14)



Rubiadin (15)

indicated, also occur in *Rhamnus* spp., *Rheum* spp. and *Rumex* spp. (Thomson, 1971).

b) Anthraquinones with substitute only in ring C

Members of these group are found mainly in Bignoniaceae, Rubiaceae, Scrophulariaceae and Verbenaceae e.g. alizarin (13), lucidin (14) and rubiadin (15) (Thomson, 1971 and Burnet and Thomson, 1964).

1.4 Biosynthesis of Anthraquinones

Like other secondary metabolites, anthraquinones are derived from a few key intermediates, principally acetate, shikimate and mevalonate, by a series of reactions which lead to formation of benzenoid compounds. Nearly all of anthraquinones are polyhydroxy (methoxy) derivatives with little variation in skeleton structure. Nevertheless they arise by at least two biosynthetic routes. Probably most anthraquinones arise by the acetate-malonate pathway, particularly those elaborated by fungi, but the extent to which shikimate is involved is still a matter for conjecture. It does, however, appear to be an important intermediate in the formation of many anthraquinones in higher plants (Thomson, 1971).

The two possible biogenetic pathways of the anthraquinone compounds are as follow:

1.4.1 Acetate-malonate pathway (Thomson. 1971)

Birch and his colleagues, and by Gatenbeck carried out labelling experiments with ^{14}C-acetate in order to establish the acetate derivation of emodin, islandicin and cynodontin. Additional confirmation was obtained using [^{14}C, ^{18}O] -acetate as precursor. Later investigations showed that aromatic polyketides were actually built up from a starter unit (usually acetate) and a chain of malonate units (formed by carboxylation of acetyl co-enzyme A). All these results were obtained using moulds in laboratory culture, and since all the fungal anthraquinones are structurally consistent with their formation by the acetate-malonate pathway. It seems reasonable to conclude that this is so. It is of interest too that the fungal component of the lichen *Xanthoria parietina* is known to be capable of elaborating anthraquinones in pure culture. As such typical fungal anthraquinones as emodin and chrysophanol are also found in higher plants it was tempting to assume that they are formed in the same way. Recent work by Leistner and Zenk has now shown that this is the case, the incorporation of labelled acetate into chrysophanol by both *Rumex alpinus* (Polygonaceae) and *Rhamnus frangula* (Rhamnaceae) being in complete agreement with polyketide biogenesis.

The majority of the anthraquinones which are assumed to be elaborated by the acetate-malonate pathway conform to emodin pattern and this is considered to arise by suitable folding and condensation of a polyketide chain derived from eight acetate units as shown in figure 3. Numerous variations of this basic structure exist resulting

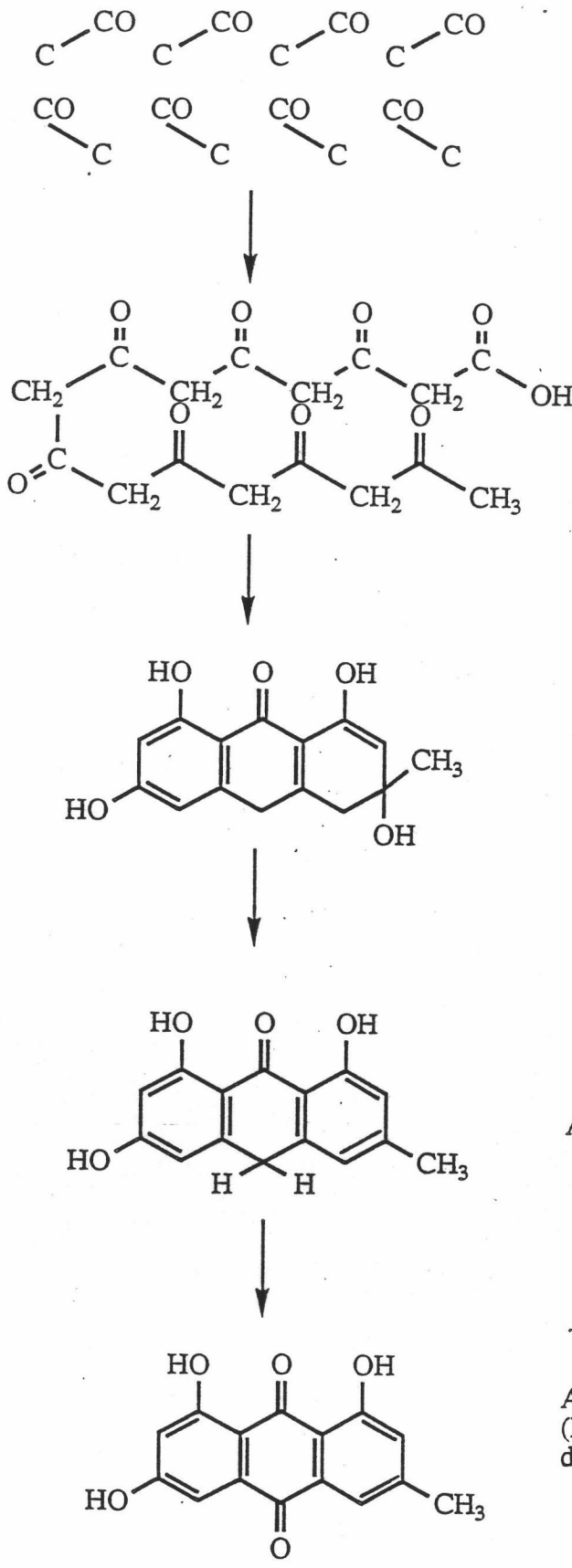
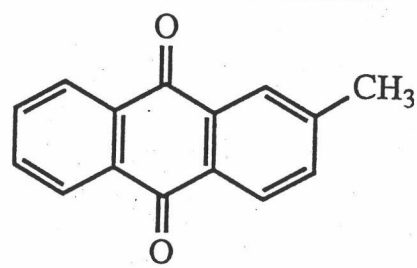


Figure 3 Acetate-malonate pathway of anthraquinones

from O-methylation, side chain oxidation, chlorination, dimerisation and the introduction or omission of nuclear hydroxy groups, while in endocrocin the terminal carboxyl group is retained.

1.4.2 Shikimate-mevalonate pathway (Thomson, 1971)

Antraquinones which have biosynthetic route via shikimate and mevalonate pathway are only found in higher plants. They are substituted in only one benzenoid ring and may be totally devoid of a carbon side chain or hydroxyl group e.g. alizarin (13), tectoquinone (16). The majority of these occur in the Rubiaceae (sub-family Rubioideae) and, to a lesser extent, in the Bignoniaceae and Verbenaceae, tectoquinone being present in all three.



Tectoquinone (16)

It seems likely that substituted ring C in this group of anthraquinone is derived from mevalonate. This was shown by feeding *Rubia tinctorum* Linn. plant with 2-^{14}C-mevalonate. Four radioactive pigments, rubiadin, pseudopurpurin, alizarin and purpurin were isolated. All of these compounds have only substitution

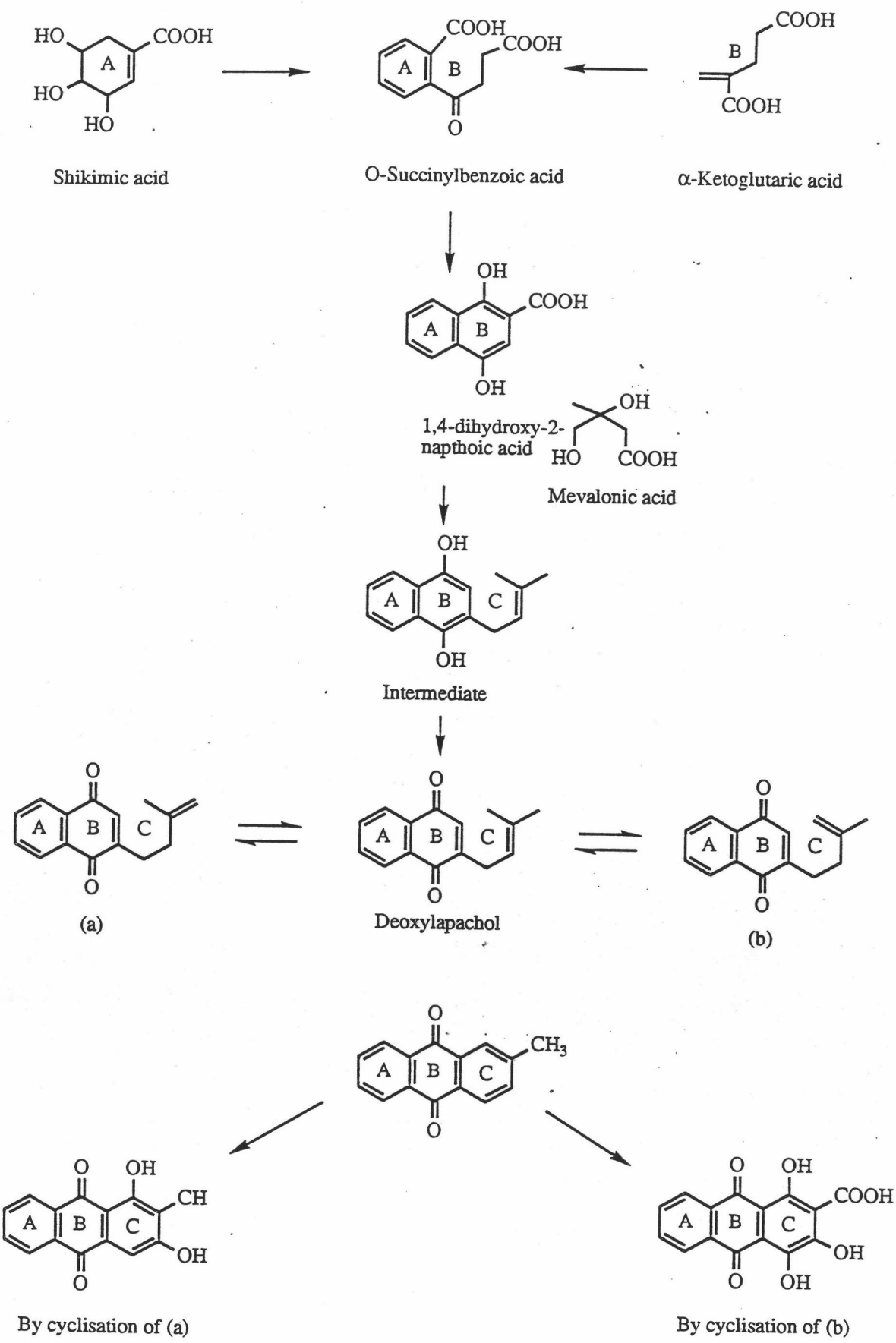


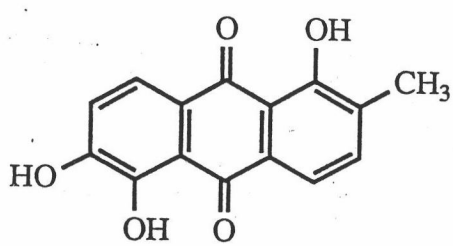
Figure 4 Shikimate-mevalonate pathway of anthraquinones

in ring C. Therefore it seems that ring C of the anthraquinones in Rubiaceae plants is formed as shown in **figure 4** and supposedly the same route is followed in Bignoniaceae and Verbenaceae.

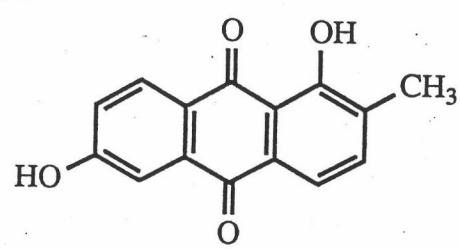
Labelled precursors, carboxyl-^{14}C-D-shikimic acid, in *Rubia tinctorum* Linn. root, led to labelled alizarin. The distribution of **radioactivity** in the alizarin molecule was determined by degradation of the alizarin dimethylether which yielded benzoic acid and veratric acid. The result of this degradation showed that the carboxyl group of shikimic acid is exclusively incorporated into C atom of alizarin. It is proposed that glutamic acid is deaminated to α -ketoglutaric acid and combined with shikimic acid to form naphthoquinones. It is assumed that shikimic acid is transformed to chorismic acid prior to incorporation into quinones. Chorismate and α -ketoglutarate are supposed to combine to give O-succinylbenzoic acid which cyclises to give a naphthalene of unknown structure. After ^{14}C-2-glutamic acid feeding, it showed that C-2 of glutamic acid gives rise specially to C-10 of naphthalene or alizarin anthraquinone. This naphthalene could be 1,4-dihydroxy-2-naphthoic acid which is linked to γ , γ -dimethylallyl pyrophosphate derived in turn from mevalonic acid, in the meta position to C-9 of alizarin. The latter observation emerges from the fact that activity from C-5 mevalonic acid is specially incorporated into C-4 of alizarin so suggesting that ring C-1 to C-4 are derived from mevalonic acid by way of γ , γ -dimethylallyl pyrophosphate. Decarboxylation and ring C closure would lead to anthraquinone alizarin.

Leistner (Leistner, 1973) has shown the biosynthesis of alizarin in *Rubia tinctorum* Linn. by using tracer technique. Specific incorporation of labels from carboxyl-^{14}C-D shikimic acid, 2-^{14}C-D glutamic acid and 5-^{14}C-DL mevalonic acid suggests that these compounds provide the skeleton of alizarin. Experimental data indicate that α -ketoglutaric acid or derivative therefore combines with shikimic acid, chorismic acid, or phrephemic acid to give O-succinylbenzoic acid which is then transformed to a nonsymmetrical 1-4 naphthoquinone intermediate, and γ , γ - dimethylallyl pyrophosphate, is then attached. Ring closure and further modification lead to alizarin.

Morindone (17) and soranjidiol (18) anthraquinones of *Morinda citrifolia* Linn. are hydroxylated in both ring A and ring C.



Morindone (17)



Soranjidiol (18)

Experiments carried out by Leistner showed that anthraquinone morindone is derived from shikimic acid via O-succinylbenzoic acid as the same biosynthetic pathway as alizarin. The hydroxyl groups attached to ring A are introduced at the latter stage of biosynthesis

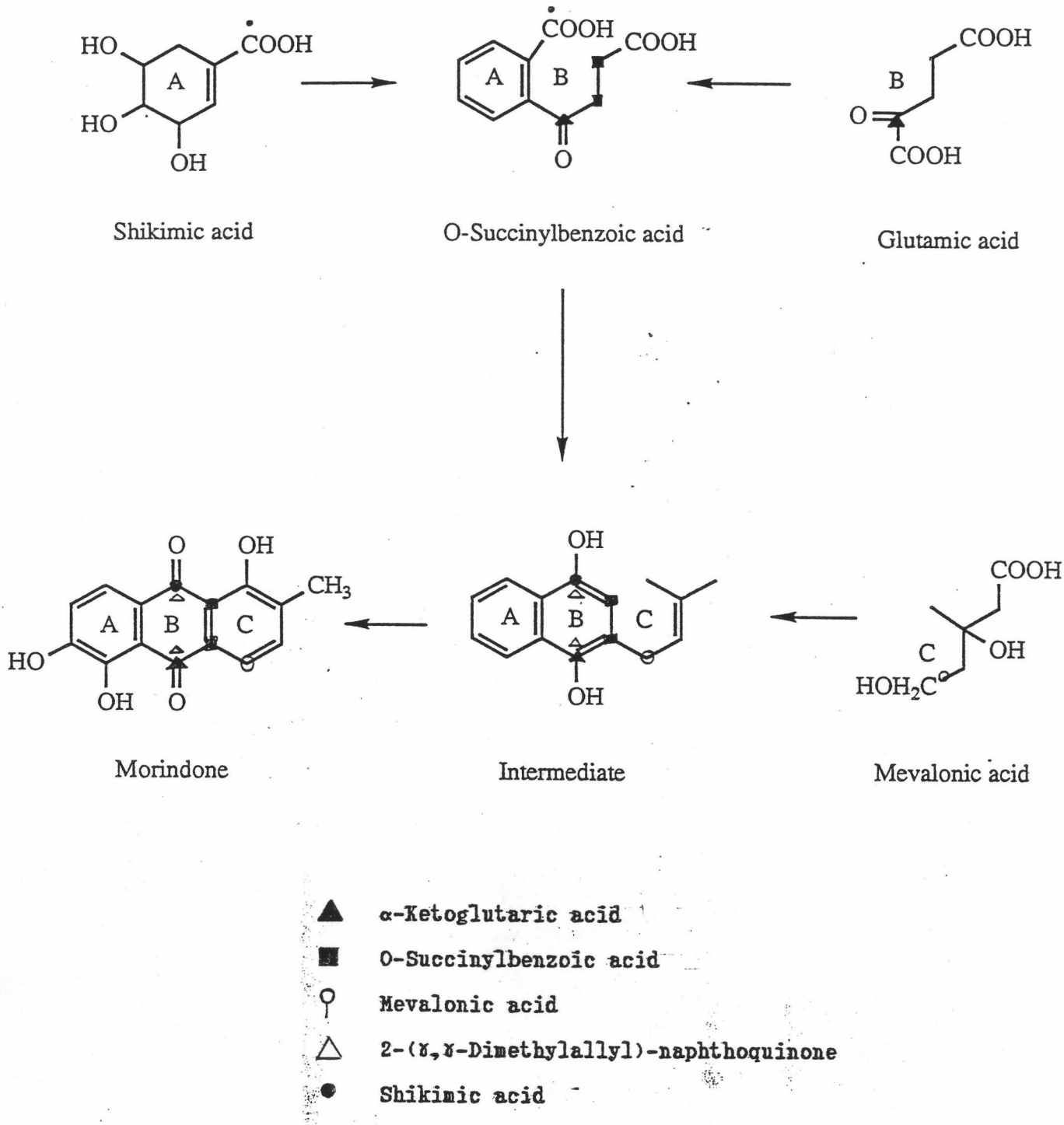


Figure 5 Migration of radioativity from different precursors to morindone

and are not derived from hydroxyl groups of shikimic acid as shown in figure 5 (Leistner, 1973).

2. Steroids

2.1 Distribution of Steroids

Steroids can be divided into at least 5 groups of compounds: sterols, steroid hormones, steroid saponins, steroid alkaloids and cardiac glycosides (Gilman *et al*, 1957). In nature, the sterols are widely distributed, both free and combined as esters or glycosides. Ester of the sterols with fatty acids are common to both animal and plant life, but the glycosides occur only in plants. On the basis of occurrence, the sterols are divided into zoosterol (animal sterol), phytosterol (plant sterol), mycosterol (sterol of yeast and fungi) (Gilman *et al*, 1957) and marine sterol (sponges) (Shoppee, 1964).

The number of steroid hormones found in nature are quite large so. One is the insect-molting hormone group. Compounds of this type were first isolated from insects, but the variety and amount of insect-molting hormones in plants are far greater than insects. The molting hormones interact directly with the chromosome of insects to release genetic information. This result is in metamorphosis. Ecdysterone, Makisterone A, Makisterone C and cyasterone are representatives of this group. Furthermore, the steroid hormone, estrone, has now found in the seed of palm, pomegranate and apple trees and in the pollen of the date palm. (Heftmann and Mosettig, 1960).

The steroidal saponins are rather widely distributed among higher plants than animals or fungi. They occur in *Yucca, Trillum* and *Smilax* (Liliaceae), in *Agave* (Amaryllidaceae), in *Dioscorea* (Dioscoreaceae), in *Digitalis* (Scrophulariaceae) and in *Solanum, Lycopersicon* and *Cestrum* (Solanaceae) (Miller, 1973).

Steroid alkaloids occur in numerous *Solanum* species and in certain *Veratrum* species. Solanine, from the potato *S.tuberosum*, was discovered with five minor glycosides, β -and γ -solanine and α -, β -, and γ -chaconine, whist solacauline has been isolated from the leaves of *S.acaule*. A chemically closely related glycoside demissine occurs in *S.demissum*. Isorubijervasine, from *V. album* (European hellebore) and *V. viride* (American hellebore), is accompanied by the rubijervine. Tomatine occurs in wild *S. lycopersicum* varieties and in some cultivated tomato plants. Solasonine occurs in the Dead Sea Apple, *S. sodomaeum*, in *S.aviculare*, and in a variety of New Zealand plants, whilst the related glycosides solamargine and solasodamine from *S.marginatum* have been described. An isomer solalauricine occurs in *S.auriculatum*, whilst a closely related glucoside solangustine occurs in *S.augustifolium* (Shoppee, 1957).

The chief sources of the cardiac glycosides are the members of the plants orders Apocynaceae and Scrophulariaceae. Of the latter order, certain genera of *Digitalis* (foxglove) furnish most of the drugs of therapeutic value.

2.2 Chemistry of Steroids

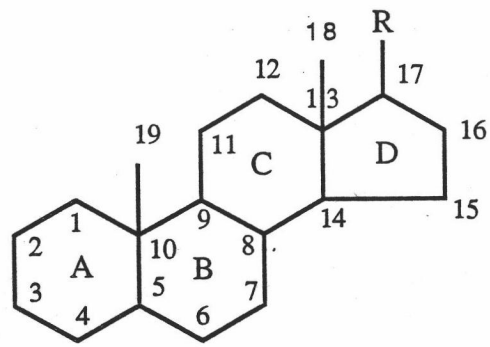
Steroids (Gk.,Stereos=solid) are solid alcohols that are widely distributed in the animal and plant kingdoms, the major sterol being cholesterol, a C_{27} compound. The basic skeleton consists of 17 carbon atoms arranged in the form of perhydrocyclopentanophenanthrene (19) and numbering of the carbons and lettering of the rings are shown. (Nakanishi *et al*, 1974).

Steroids belong to a large group of compound known as terpenoids or isoprenoids (Miller, 1973). Terpenes are formed by the polymerization of isoprene units (20) and steroids are triterpenes or triterpenoids.

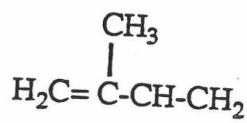
Steroids are widely varied in structure and encompass compounds of vital importance to life, such as cholesterol, the bile acid, vitamin D, sex hormone, corticoid hormone, cardiac aglycone and antibiotic (Nakanishi, 1974).

Because nucleus of the steroids are seven asymmetric centres (C-5, C-8, C-9, C-10, C-13, C-14, C-17) so giving a total of 128 possible stereoisomerides. Most of the steroids are hydroxylated at C-3, while some also have hydroxyl groupings in other positions of the ring system or the side chain. In cholesterol, the hydroxyl group on C-3 is on the same side as the methyl group on C-10, i.e., both project towards the front of the molecule (β -orientation). When the hydroxyl group of C-3 lies on the side of the ring opposite to that of

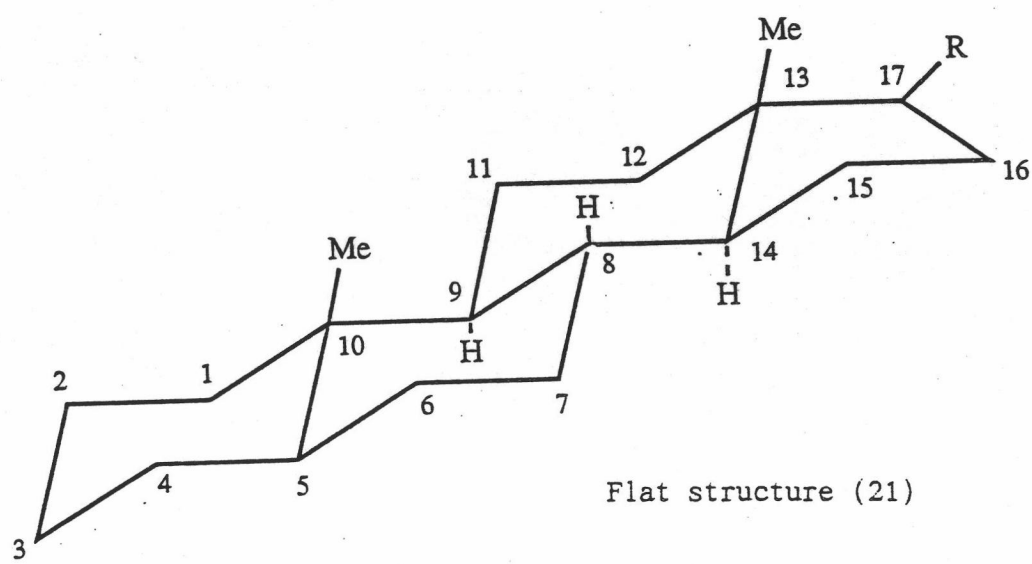
the methyl group at C-10, the configuration at C-3 is α . The stereochemistry of the side chain, the angular methyl groups, and the B/C and C/D *trans* ring fusions of bile acids in the same as that of sterol. The only difference is at C-5 since the bile acid are A/B-*cis* (5 β) compound, while the sterol are A/B-*trans* (5 α) compound (Ikan, 1969). So the stereochemistry of the steroids in a series of-*trans* relationships between substituents at adjacent ring junctions along the backbone of the molecule C-5-10-9-8-14-13 which give an almost flat structure (21) (Gilman *et al*, 1957).



Perhydrocyclopentanophenanthrene (19)



Isoprene unit (20)



Flat structure (21)

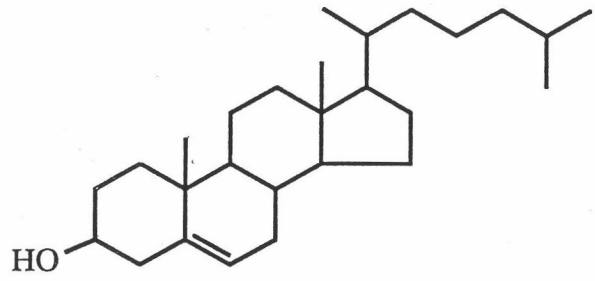
2.3 Classification of Steroids

2.3.1 Sterols

The sterols are 3-monohydroxysteroids of the C_{27}, C_{28} and C_{29} series, all the naturally occurring compounds have a 3 β -hydroxyl group, and nearly all have one or more double bonds. The commonest position is at 5; next come 22 and 7. Ring A/B, B/C and C/D are *trans* fuseds. The hydrocarbon skeleton has a few angular methyl group at C-10 and C-13 generally and 8 to 10 carbon chain at C-17. The hydroxyl, the two methyl group and the C-17 side chain all have the β -orientation (Bell and Charlwood, 1980).

C_{27} Sterols

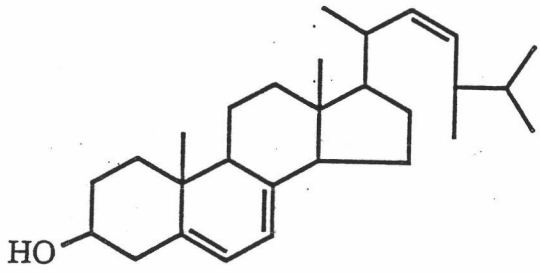
Cholesterol (22) is one of the most abundant compounds. It is present in vertebrates and invertebrates and also found in plants (Ikan, 1969). So far, cholesterol has been found in the red algae, in blue-green algae, in bacteria, and in the pollens of many plants (Goodwin and Mercer 1983); the date palm, cotton wool, sunflower and mustard, in the spore of the fern *Polystichum filix-mas*, in the seeds of many plants; soybean, peanut, oat, apple, avogado and oil palm ; in the bark of pine tree and *Erythrina suberosa* Roxb. and in the roots of the cactus *Wilcoxia viperina* Britton & Rose (Miller, 1973) and in oysters and clams (Gilman *et al*, 1957). Cholesterol is the key intermediate from which animals make all the other steroids; such as vitamin D_3, bile acids, progesterones, adrenocortical hormones, androgenic and estrogenic hormones (Miller, 1973).



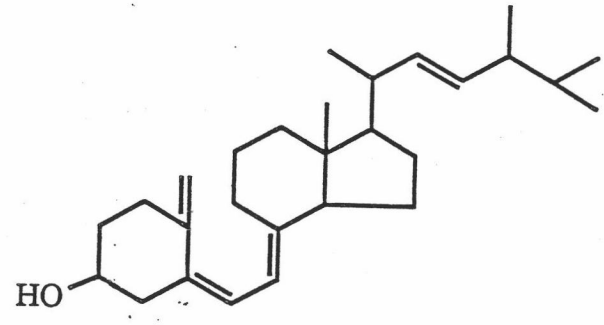
Cholesterol (22)

$\underline{C_{28}}$ Sterols

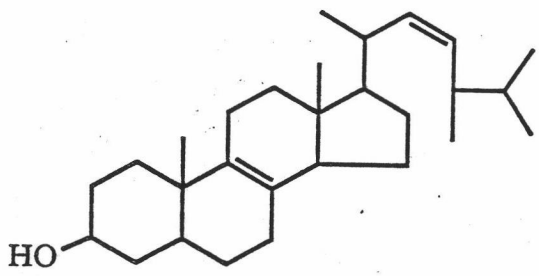
The C_{28} sterols derive from the C_{27} sterols and from either the carboxyl or methyl carbon of acetate for C-28 (Heftmann and Mosettig, 1960). The most important C_{28} sterol is ergosterol (23), the principal mycosterol, which first isolated from ergot (Weete, 1973) but also occurs in yeast and in fungi, but more recently it was also been discovered in higher plants. The ergosterol attracted little attention until 1926-1927, when it was discovered that irradiation with ultra-violet light converts it into a vitamin D (24). Subsequent work has shown that ergosterol is the principal yeast sterol. The content in yeast varies considerably in the different species and is influenced greatly by the nature of the substrate on which the yeast is culture (Gilman *et al*, 1957). Ascosterol (25) and fecosterol (26) rank among the minor yeast sterols (Ikan, 1969).



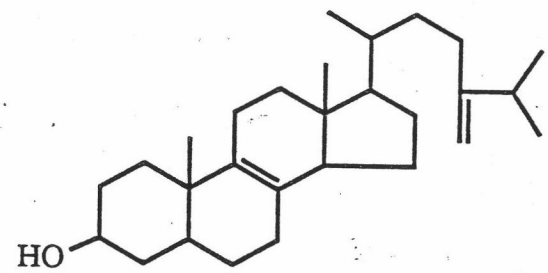
Ergosterol (23)



Vitamin D (24)



Ascosterol (25)



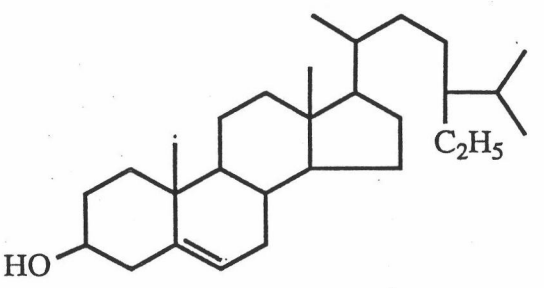
Fecosterol (26)

$\underline{C_{29}\text{ sterol}}$

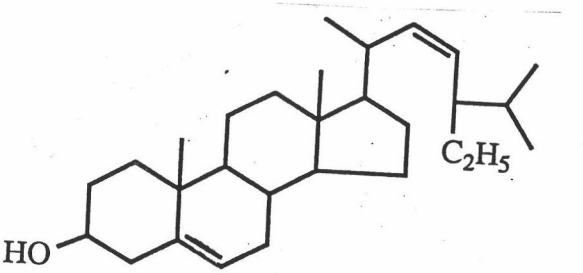
The important C_{29} sterol in higher plants are sitosterol (27), stigmasterol (28), spinasterol (29) and **brassicasterol** (30) which called phytosterols (Ikan, 1969). These common sterols occur both free and as simple glycoside.

The five sitosterols, α 1-, α 2-, α 3-, β -, and γ -sitosterols, $C_{29}\ H_{50}\ O$ are the most widely distributed plant sterols; they occur as mixtures with 5 α -stigmastanol [5 α :

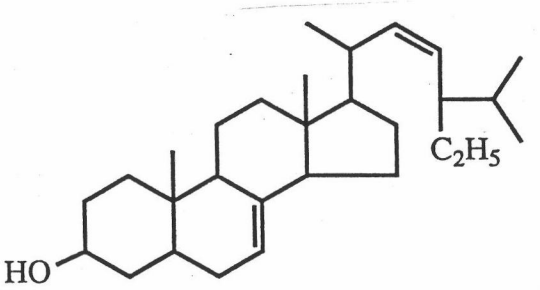
6-dihydro- β -sitosterol] and are unusually difficult to purify and identify (Shoppee, 1964). α 1-Sitosterol probably has the structure 3 (β)-hydroxyl-24-ethyl- $\triangle^{5,8(14)}$-cholestadiene. The structure of α 2- and α 3-sitosterol are doubly unsaturated and are precipitated by digitonin. β -Sitosterol, $C_{29}H_{50}O$ is the principle sterol of cottonseed oil and calycanthus oil; it has been obtained from soya-bean oil, wheat-germ oil, corn oil, rye-germ oil, cinchona wax, and crepe rubber. γ -Sitosterol apparently differs from β -sitosterol merely in the spatial configuration of the C_{17} side chain (Gilman *et al*, 1957).



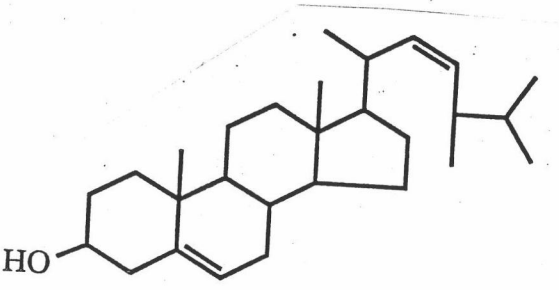
Sitosterol (27)



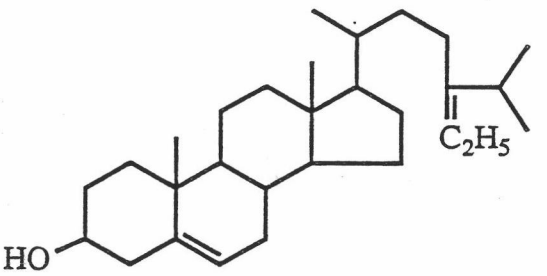
Stigmasterol (28)



Spinasterol (29)



Brassicasterol (30)



Fucosterol (31)

Although stigmasterol ($C_{29}H_{48}O$) is widely distributed in plants both as the free sterol and as glycosides, only soybean and the seeds of *Physostigma venenosum*, the calabar bean, contain enough of the sterol to be used as sources. From soybean oil, stigmasterol is conveniently separated as its sparingly soluble acetate tetrabromide from the accompanying sitosterols. Sugarcane wax also contains substantial amounts of this sterol. Its abundance and the double bonds at C-22 and C-5 make stigmasterol to be an important starting material for the synthesis of progesterone and other steroid hormones. 5-Dihydrostigmasterol has been isolated from a slime mold, *Dictyostelium discoideum* (Heftmann and Mosettig, 1960).

From spinach, and also from alfalfa,the characteristic sterol, α -spinasterol, has been isolated. In spinach, this sterol is accompanied by the allied compounds, β and γ -spinasterol. The isomeric spinasterol (C_{29} H_{48} O), β , γ , and δ spinasterol all give 5 α -stigmast-8(14)-enol by hydrogenation under isomerizing

conditions; they are probably $\triangle^7$-compound differing in the position of the side-chain double bond (Shoppee, 1957).

Miscellaneous Plant Sterols

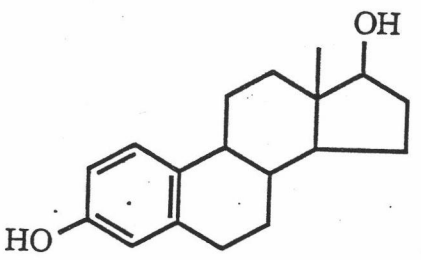
In addition to the above plant sterols, there are a number of others which have been investigated. Noteworthy among these are brassicasterol from rapeseed oil and fucosterol (31) from bladder wrack. Brassicasterol has the structure 3 (β)-hydroxyl-24-methyl-$\triangle^{5,22}$-cholestadiene and is exceptional in that it has a methyl group at C-24 rather than the ethyl group usually found at this position in plant sterols. Fucosterol contains two double bonds and, on catalytic hydrogenation, is reduced to stigmastanol. One of the double bonds of fucosterol is probably at C-5, but the position of the other is uncertain.

2.3.2 Steroid hormones

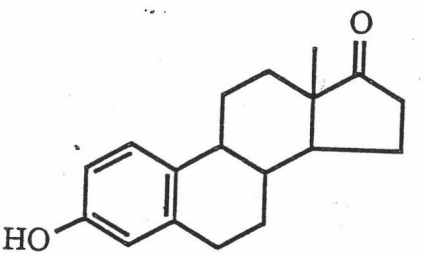
Hormones are substances secreted by specific glands, which exert control over various body process. The steroid sex hormones are divided as follow: female hormones, such as the estrogens; estradiol (32), estrone (33), and estriol (34), progestational hormones, such as progesterone (35); and androgens-testosterone and androsterone-which have male hormone activity.

The naturally occuring estrogens differ from other steroid hormones in the presence of an aromatic ring A, the absence of

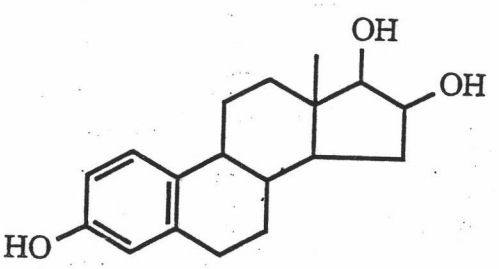
a methyl groups at C-10, and the presence of a keto-or hydroxyl-group at position 17 and sometimes at position 16.



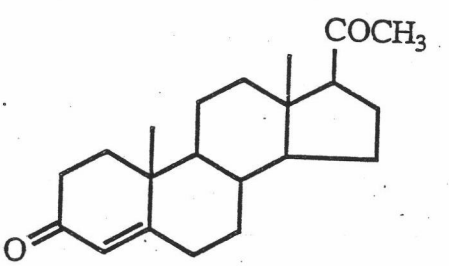
Estradiol (32)



Estrone (33)

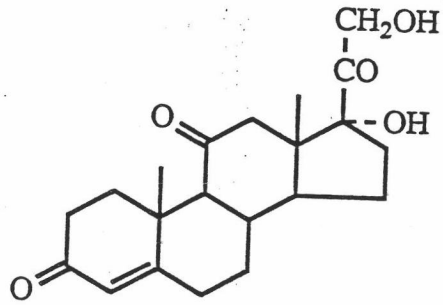


Estriol (34)

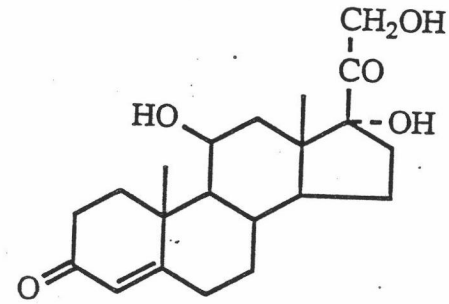


Progesterone (35)

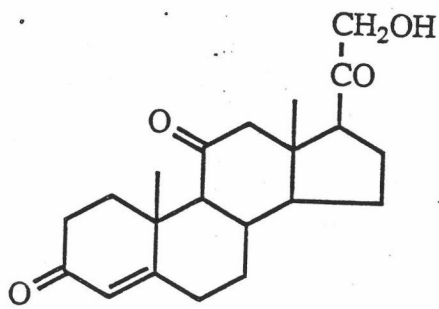
The adrenocortical hormones have 21 carbon atoms, ketonic groups at C-3 and C-20, a double bond at the 4(5)-position, and a hydroxyl group at C-21. These compounds may be divided into the highly active glucocorticoids, e.g., cortisone (36) and cortisol (37) and highly active mineralocorticoids, e.g., 11-dehydrocorticosterone (38) and aldosterone (39).



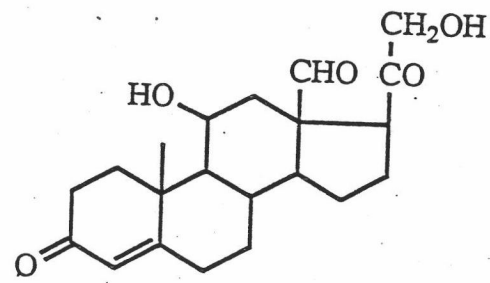
Cortisone (36)



Cortisol (37)

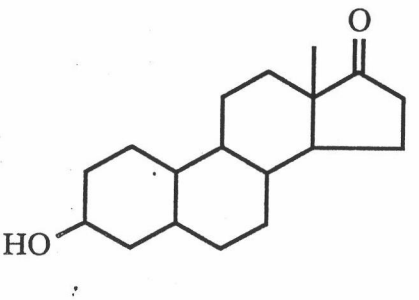


11-Dehydrocorticosterone (38)

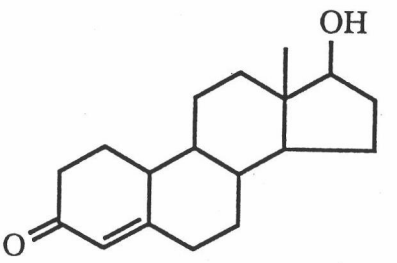


Aldosterone (39)

The naturally occurring androgens such as androsterone (40) has 19 carbon atoms. The principal androgenic steroid is the male sex hormone testosterone (41).



Androsterone (40)

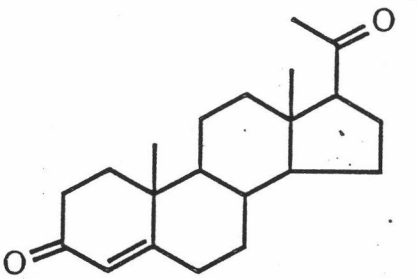
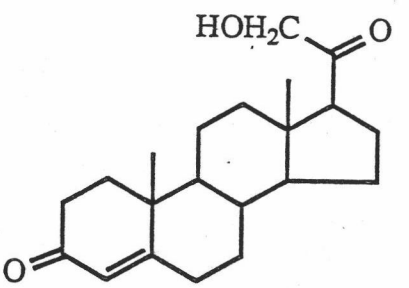
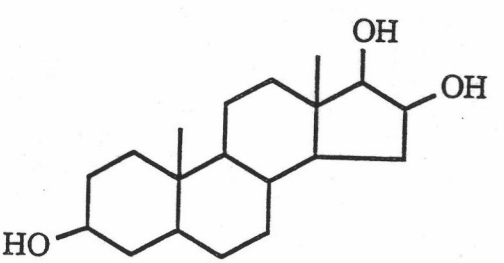


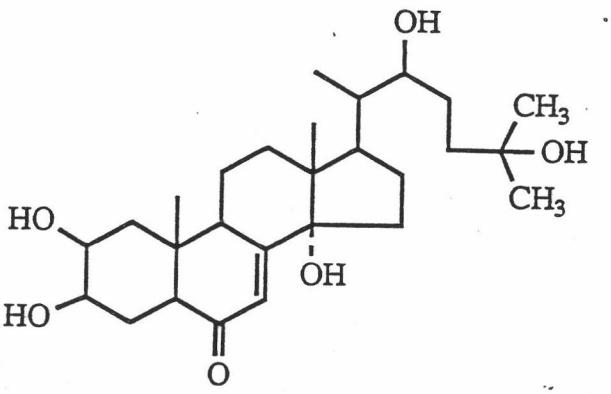
Testosterone (41)

A number of steroids, which are known to be hormones in animals, are somewhat widespread in plants but their biological activity, if any, in plants has yet to be clearly demonstrated. The animal hormones are derived from cholesterol after partial or complete removal of the side chain and this is probably how they arise in plants. The more common of these compounds that are produced by plants are outlined in table 1 (Goodwin and Mercer, 1983).

Ecdysteroids are extremely polar substances which have only recently been discovered in plants. They have the same basic structure as the insect molting hormones and are not widely distributed. Typical examples are ecdysone (42) isolated from bracken (*Pteridium*) rhizomes and ponasterone A, which is 20R-hydroxyecdysone, from the conifer *Podocarpus* (Goodwin and Mercer, 1983).

Table 1 Occurrence of some animal steroid hormones in plants

Name (animal source)	structure	Plant source
Progesterone (corpus luteum of the ovary placenta)		*Holarrhena floribunda*
Deoxycorticosterone (adrenal cortex)		*Digitalis lanata*
Androstanetriol (testes)		*Haptopapus heterophyllus*



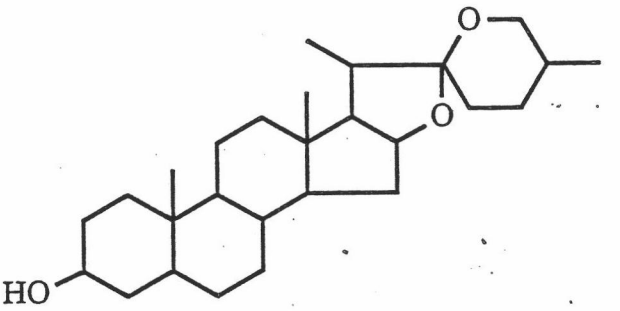
Ecdysone (42)

2.3.3 Steroid saponins

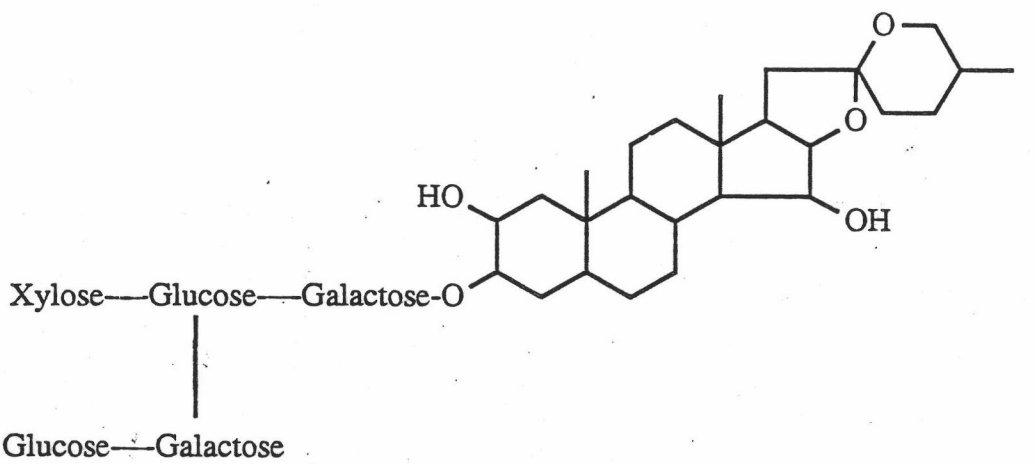
The saponins were originally named because of their soap-like characteristics. They are powerful surface active agents which cause foaming when shaken with water, these solution are haemolytic when inject into blood stream of the animals and therefore highly toxic intravenously. In very dilute solution, they are quite toxic to fish and plants containing them have been used as fish poison for hundreds of years. As glycosides, they are hydrolysed by acids or enzymes to give an aglycone (sapogenin) and various sugars. According to the structure of aglycone, two kinds of saponin are recognized, the steroidal and triterpenoid types.

Steroidal saponins are C_{27} sterol in which the side chain has undergone metabolic changes to produce a spiroketal. Such a compound is tigogenin (43) from *Digitalis lanata*. Sapogenins occur naturally as the saponins, which are 3-O-glycosides of the parent

steroid. A well known example is digitonin (44) from *Digitalis purpurea* (Goodwin and Mercer, 1983).



Tigogenin (43)



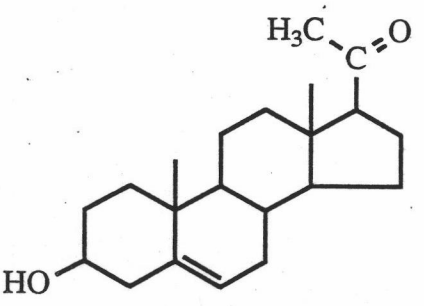
Digitonin (44)

2.3.4 Steroid alkaloids

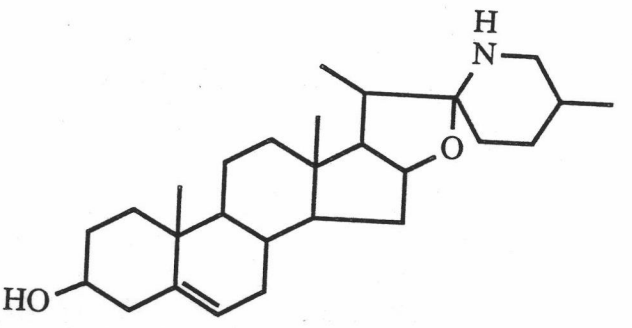
Two main groups of steroid alkaloids exist, the C_{27} alkaloids, which appear to be formed directly from cholesterol, and the C_{27} alkaloids presumably produced from pregnenolone (45).

Many of the C_{27} alkaloids are nitrogen analogues of the C_{27} sapogenins; solasodine (46) is a typical example. Other groups, as exemplified by rubijervine (12 α - hydroxysolanidine) (47), contain nitrogen as part of the six-membered condensed ring system of the molecule. Rubijervine is obtained from *Veratrum* roots, which also produce polyhydroxy triterpenoid alkaloids such as germine (48) which occurs naturally as various esters with acids such as α -methylbutyric acid and acetic acid.

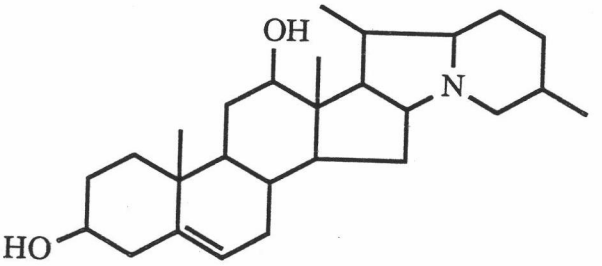
A number of C_{21} alkaloids have been recently isolated from the Apocynaceae and Buxaceae. For example, holaphyllamine (49) has been isolated from *Holarrhena* (Apocynaceae) (Goodwin and Mercer, 1983).



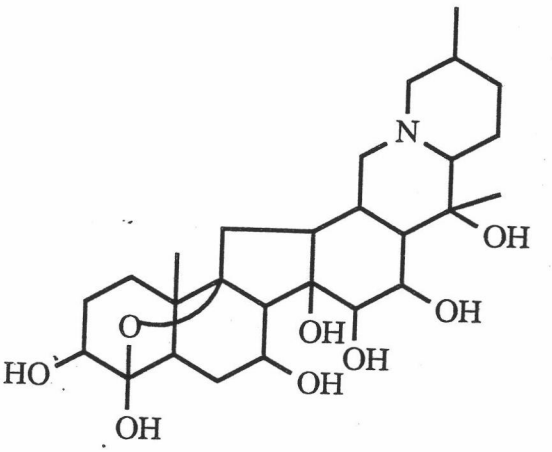
Pregnenolone (45)



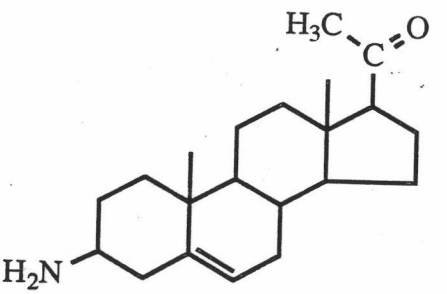
Solasodine (46)



Rubijervine (47)



Germine (48)

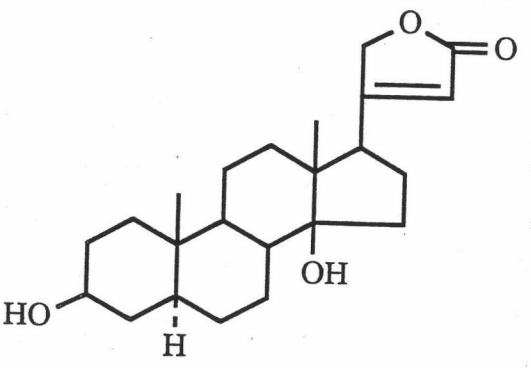


Holaphyllamine (49)

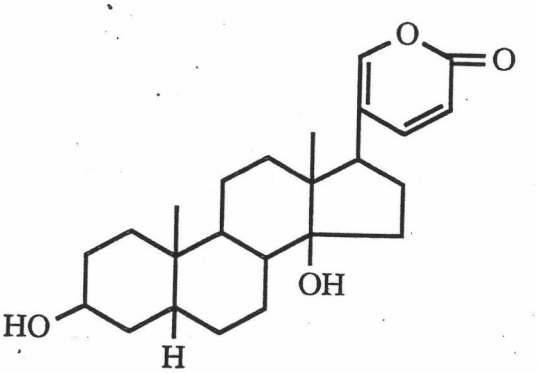
2.3.5 Cardiac glycosides

Certain steroids exert a specific and powerful effect on the cardiac muscle and are called cardiac-active (cardiotonic) principles. Many cardiac glycosides have been isolated from plants in tropical regions and have been employed by natives of Africa and

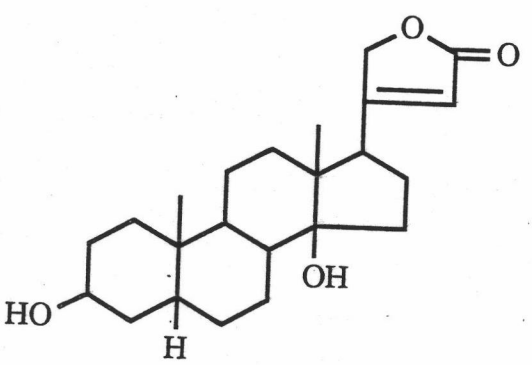
South America for the preparation of arrow poisons. Some occur in the secretions of poisonons toads. The aglycones are of two types : cardenolides (50) and bufadienolides (51). The cardenolides are C_{23} steroids having as side chain an α—, β -unsaturated five membered lactone ring and a C_{14} hydroxy group. Aglycone in this type was isolated from *Digitalis* and *Strophanthus* such as digitoxigenin (52), gitoxigenin (53) and strophanthidin (54) (Sim, 1968 and Miller, 1973).



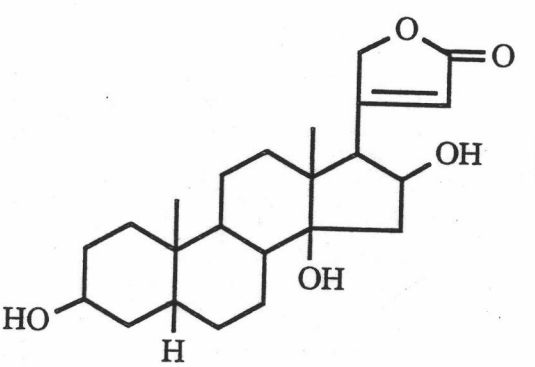
Cardenolides (50)



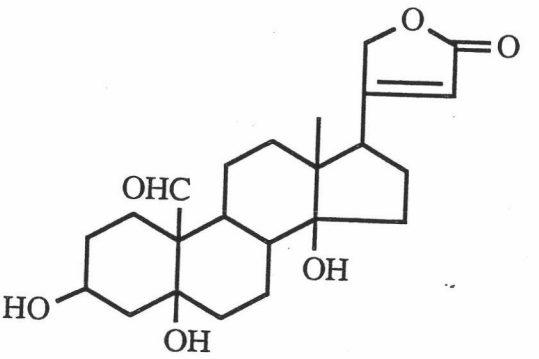
Bufadienolides (51)



Digitoxigenin (52)

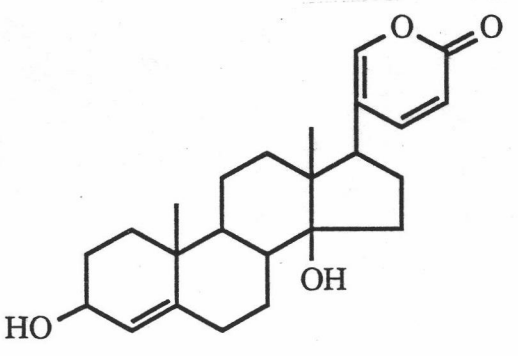


Gitoxigenin (53)

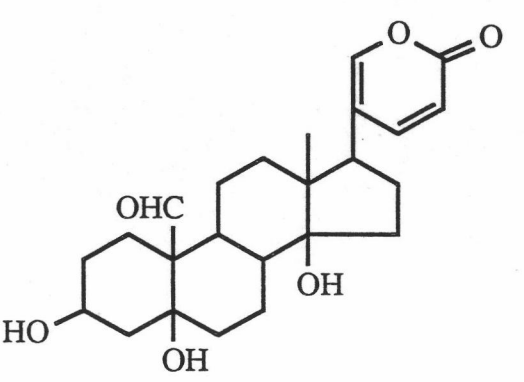


Strophanthidin (54)

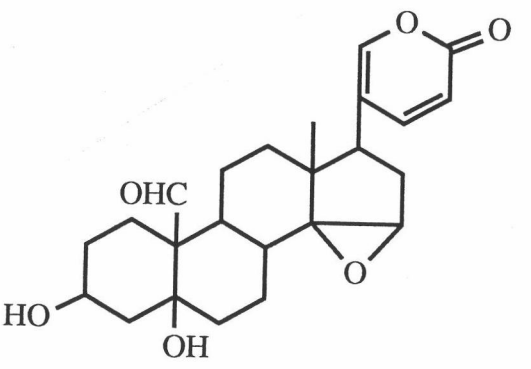
The bufadienolides are C_{24} steroids having as side chain a doubly unsaturated, six-membered lactone ring and a 14 β -hydroxy group or its modification. Bufadienolide type has been found in plant only in the buttercup family (Ranunculaceae) and lily family (Liliaceae). Scillarenin (55) is the cardiac genin found in the bulb of *Bowiea* and *Scilla* in Liliaceae, hellebrigenin (56) is a genin from the rhizomes of the Christmas rose (Goodwin and Mercer, 1983) and Bufotalidin (57) from toad venom (Sim, 1968).



Scillarenin (55)



Hellebrigenin (56)



Bufotalidin (57)

2.4 Biosynthesis of plant sterols (Shoppee, 1957)

The formation of plant sterols pathways occur through there principal stages :

a) Squalene biosynthesis

Mevalonic acid is formed by activation of acetate (58) to acetyl-coenzyme A (59), conversion of this to acetoacetyl-coenzyme A (60), and condensation of these compound to give the monocoenzyme A ester of 3-hydroxy-3-methylglutaric acid (61). Mevalonic acid (62) is converted by way of the 5-phosphate into the 5-pyrophosphate (63), which by dehydration and decarboxylation gives isopentenyl pyrophosphate (64). Then it is reversibly isomerized to the extent of 93 % to dimethylallyl pyrophosphate (65). The next important intermediate is formed by electrophilic attack of a molecule of dimethylallyl pyrophosphate on the terminal methylene groups of a

molecule isopentenyl pyrophosphate with simultaneous elimination of the allylic pyrophosphate ion to afford a carbonium ion, which expels a proton to give geranyl pyrophosphate (66), this by similar electrophilic attack on the terminal methylene group of a second molecule of isopentenyl pyrophosphate then yields farnesyl pyrophosphate (67). Farnesyl pyrophosphate is isomerized to nerolidyl pyrophosphate; electrophilic attack of the former on the terminal methylene group of the later results in "tail-to-tail" union to give a dehydrosqualene, reduced to squalene (68).

b) Squalene cyclization

The cyclization of squalene to form the cyclopentanophenanthrene ring system is squalene-2, 3-oxide (69) which is also the intermediate during cyclization. Cyclization is initiated by cation OH^+ attack at the squalene position which gives rise to C-3 of the sterol molecule. The epoxidase, which converts squalene to the 2,3-oxide, is microsomal in nature and requires NADPH and molecular oxygen, and addition of the sterol inhibitor, tri (2-di-ethylaminoethyl) phosphate, results in an accumulation of squalene-2, 3-oxide. Formation of the tetracyclic steroid ring system is through molecular rearrangement, a migration of two hydrogen atoms and two 1,2-methyl shifts from C-8 to C-14 and from C-14 to C-13. The 3 β -hydroxyl is derived from atmospheric oxygen and not from water. The conversion of squalene-2, 3-oxide to cycloartenol (70) requires the cyclase enzyme. It is generally accepted that cycloartenol is the first cyclic product in plants.

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

To form the major phytosterols from cycloartenol, an alkylation at C-24 is probably the first step and this occurs through transmethylation involving S-adenosyl methionine which is the product in this step is 24-methylene cycloartanol (71), a 4,4-dimethyl sterol. Demethylation at C-4 is probably the next step, producing cycloeucalenol (72) the first 4-methyl sterol. The next step, the 9 β ,19 β -cyclopropane ring can be opened most efficiently to form obtusifoliol (73) and 31-norlanosterol (75) by C-14 demethylation to occur, a $\Delta^{8(9)}$ bond. The most generally accepted pathway is through 24-methylene cycloartanol ——> cyclocucalene ——> obtusifoliol but the sequence cycloartenol ——> 31-norcycloartenol (74) ——> 31-norlanosterol (75), obtusifoliol (73) has also been indicated. From obtusifoliol to 24-methylene lophenol (76) occurs through molecular rearrengement by migration of double bond. The formation of 24-ethylidene lophenol (77) occurs by the second alkylation of C-28. Methionine is again the methyl donor for the second alkylation. During this process, a cationic site at C-24 of the steroid molecule is created which is stabilized through the loss of a hydrogen atom from C-28. The removal of the second C-4 methyl group from 24-ethylidene lophenol (77) is also through oxidative decarboxylation and this product is Δ^{7}-avenasterol (78). The conversion of Δ^{7}-avenasterol 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 form

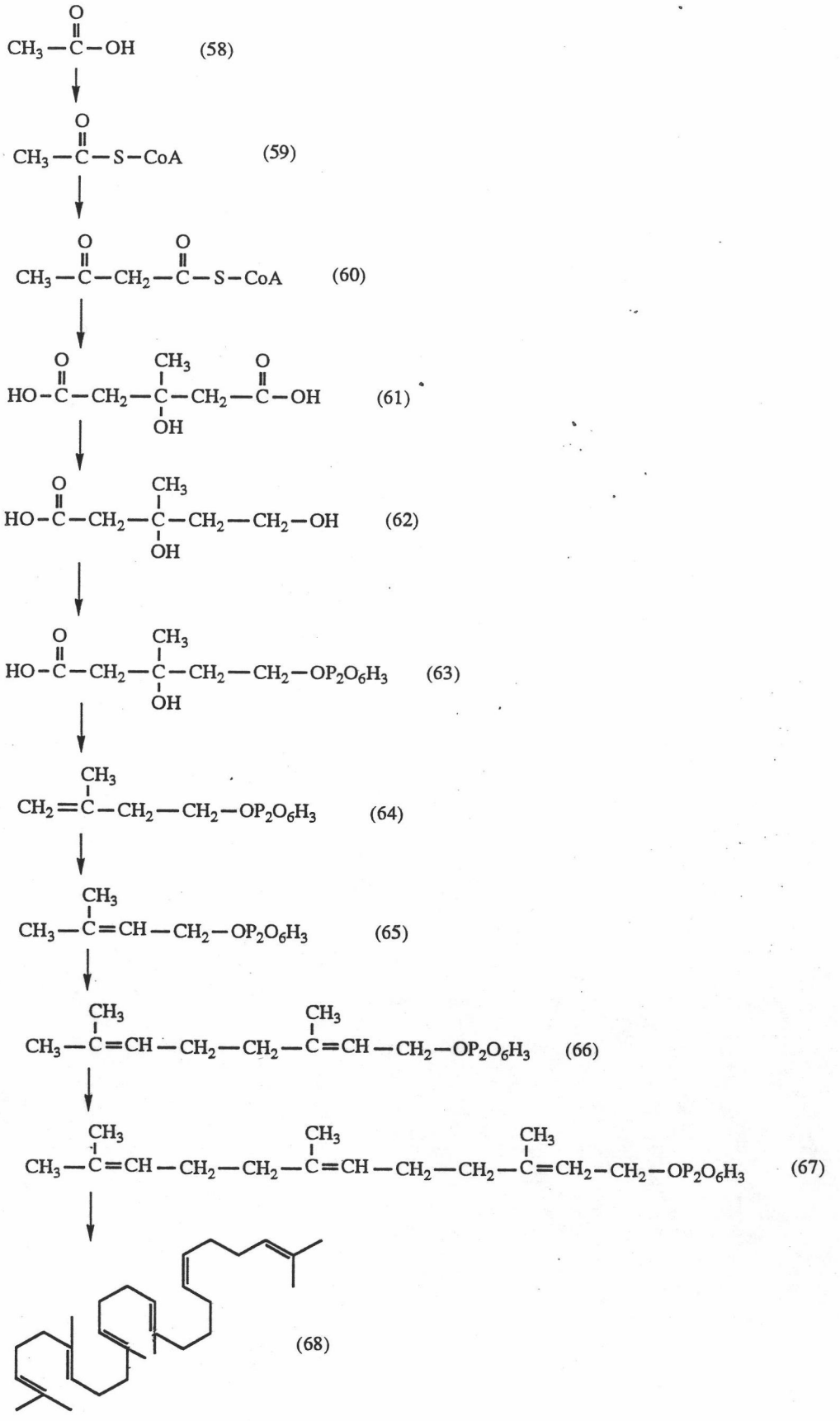


Figure 6 Squalene biosynthesis

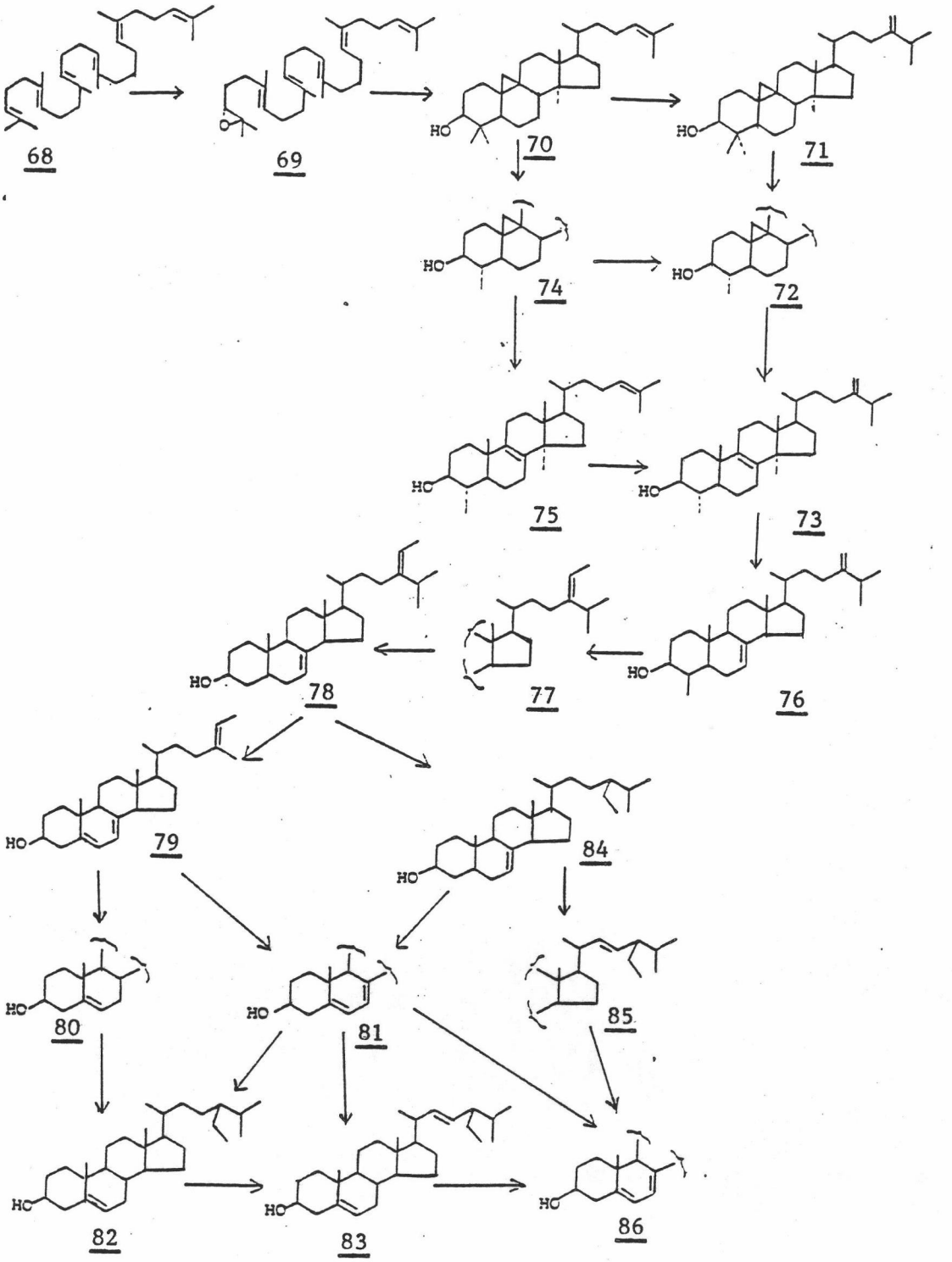


Figure 7 Biosynthetic pathway of plant sterol

avenasterol (80). Formation of sitosterol from avenasterol required hydrogenation of $\Delta^{24(28)}$ and reduction of the 24-ethylidene. Formation of stigmasterol is assumed to occur through sitosterol by the enzyme 22,23-dehydrogenase. Formation of sitosterol and stigmasterol may also be Δ^{7}-avenasterol (78) ——> stigmasterol-5, 7, $\Delta^{24(28)}$-trien-3 β -ol (79)—> stigmasta-5, 7-diene-3 β -ol (81) —> sitosterol (82) or stigmasterol (83). 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-3 β -ol (84). This reaction must be through a $\Delta^{24(25)}$ intermediate since, the C-25 hydrogen atom is lost. Next, stigmata-7-en-3 β -ol goes through the $\Delta^{7(8)}$- $\Delta^{5,7}$ ——> $\Delta^{5(6)}$ rearrangement to form sitosterol. For stigmasterol formation can be through spinasterol (85) ——> 7-dehydrostigmasterol (86).

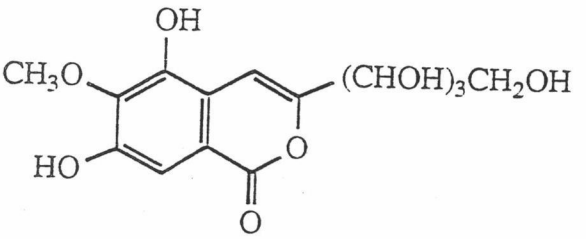
It is quite possible that all of the discussed pathways operate in plants, and depending upon species and environmental conditions.

3. Isocoumarins

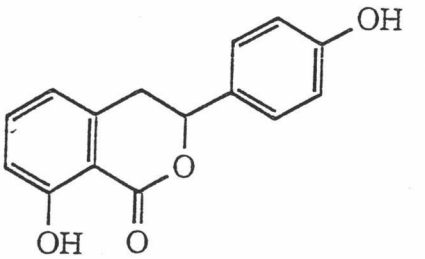
3.1 Distribution of Isocoumarins

Isocoumarins are found in higher plants especially Saxifragaceae and Hydrangeaceae (Murray *et al*, 1982). Bergenin (87) has

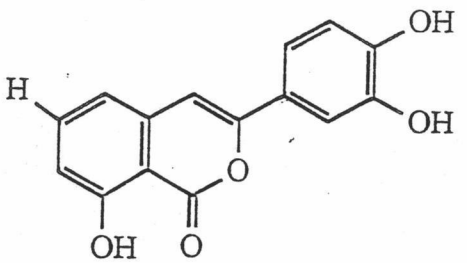
been found from the root stems of various members of the Saxifragaceae (Elderfield, 1951) and usually found hydrangenol (88) from Hydrangeaceae (Swain, 1963). In addition, isocoumarin, thunbergenol A (89) and B (90) were isolated from Hydrangea Dulcis Folium, the fermented and dried leaves of *Hydrangea macrophylla* Seringe var. *thunbergii* Makino which is used as an oral refrigerant and a sweetener (Yoshikawa *et al*, 1992, vol 40 No.11). And thunberginols C (91), D (92), and E (93), dihydroisocoumarins, and thunberginol G 3′-O-glucoside (94) and hydrangenol 4′-O-glucoside (95), dihydroisocoumarin glycosides also were isolated from Hydrangeae Dulcis Folium (Yoshikawa *et al*, 1992, vol 40 No.12). *Coriandrum sativum* L. is widely distributed and its aerial parts and mature fruit are commonly used as a spice and crude drug in Southeast Asia to China. Studying of the phenolic components of this umbelliferous plants, two new isocoumarin coriandrone A (96) and B (97) were isolated, together with two known isocoumarins, coriandrin (98) and dihydrocoriandrin (99) (Baba *et al*, 1991). Feralolide (100), a new dihydroisocoumarin, was isolated from a dried exudate of *Aloe ferox* Miller (Liliaceae) (Cape aloe) and occasionally, of its hybrids with *A. africana* Miller or *A. spicata* Baker (Speranza *et al*, 1993). There was also a report of polygonolide (101) has been isolated from the root of *Polygonum hydropiper*. The isocoumarin ester (102), a degraded gallic acid dimer, is produced in the leaves of *Flueggea microcarpa* (Thomson, 1993).



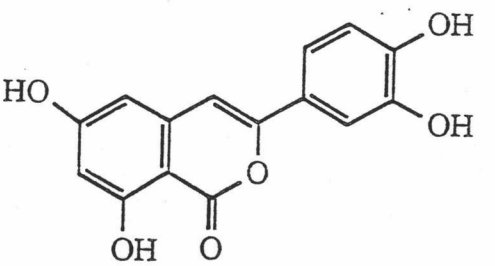
Bergenin (87)



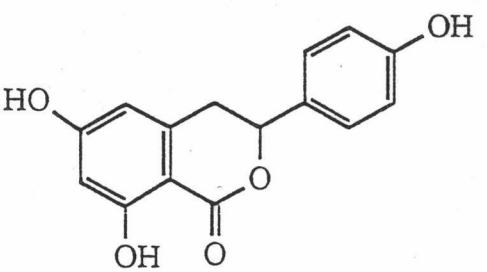
Hydrangenol (88)



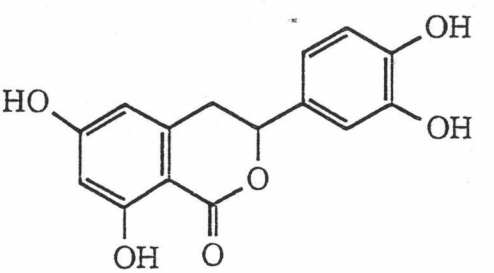
Thunberginol A (89)



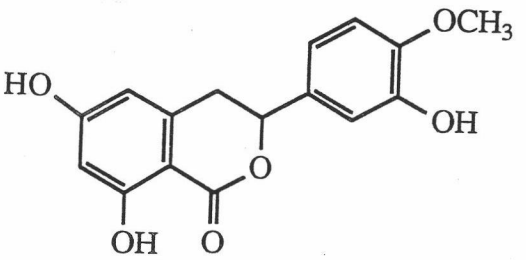
Thunberginol B (90)



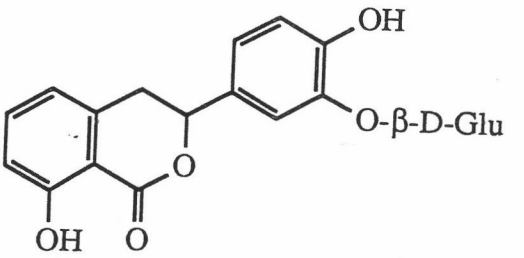
Thunberginol C (91)



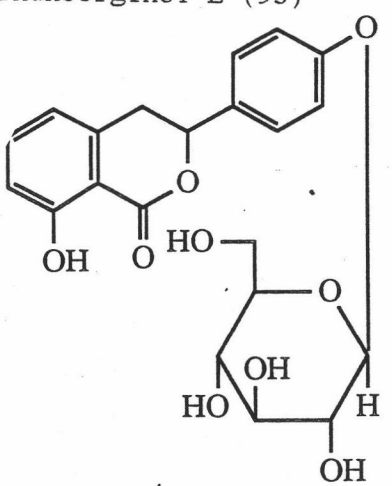
Thunberginol D (92)



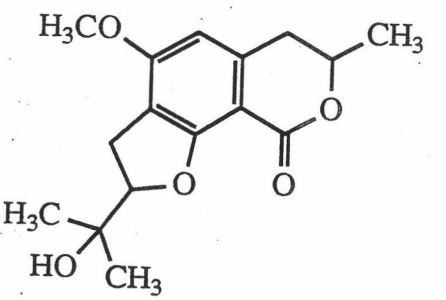
Thunberginol E (93)



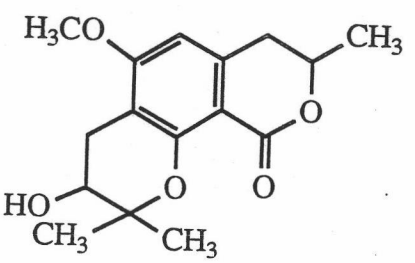
Thunberginol G-3′-O-glucoside (94)



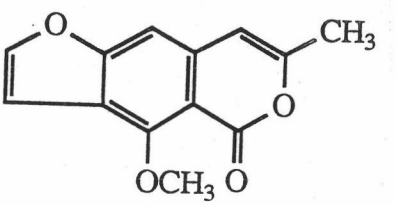
Hydrangenol 4′-O-glucoside (95)



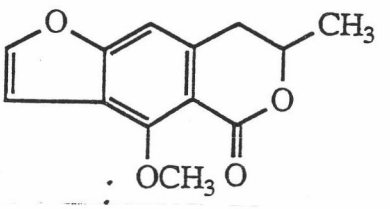
Coriandrone A (96)



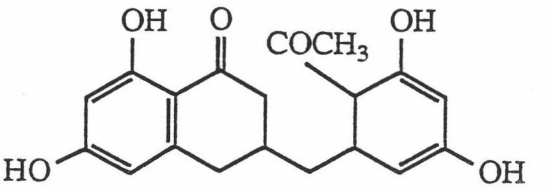
Coriandrone B (97)



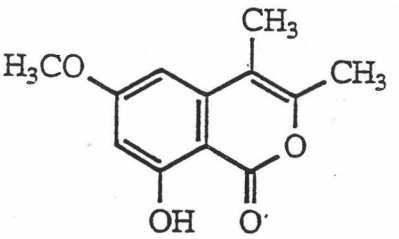
Coriandrin (98)



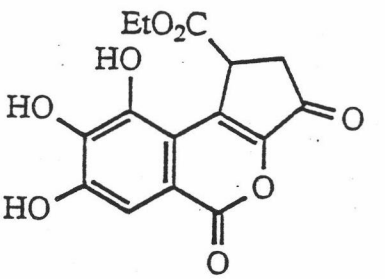
Dihydrocoriandrin (99)



Feralolide (100)



Polygonolide (101)

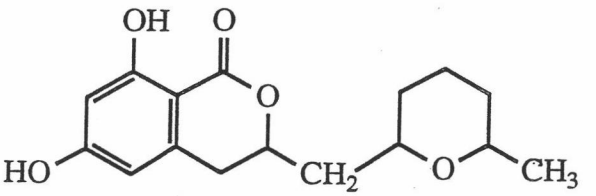


Gallic acid (102)

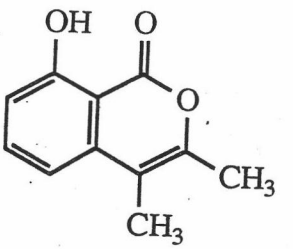
In addition, isocoumarin compounds could found in fungi. Example, cladosporin (103) was a new antifungal metabolite produced in good yield in the mycelium of the *Cladosporium cladosporioides* (Scott, Van Walbeek and Wilhelmina, 1971). And 3, 4-dimethyl-8-hydroxy isocoumarin (104) was isolated from *Leinzites thermophila* (Le Blance and Babineau, 1972). Hallock *et al* (1988) show that a phytotoxic metabolite has been isolated from cultures of pathogenic fungus *Drechslera siccans* and characterized as 6,8-dihydroxy-3-(2 -hydroxypropyl) isocoumarin (de-O-methyldiaporthin)

(105).For *Aspergillus* spp.,there was a report of isocoumarin compounds asperentin (106) and 5 -hydroxy asperentin (107) in surface culture of *Aspergillus flavus* (Grove and Frederick, 1972). Some isocoumarin, mellein (108), 4-hydroxy mellein (109) and ochratoxin A (110) were isolated from synthetic media of *Aspergillus ochraccus* (Moore *et al*, 1972). The other fungi that can produce mellein is *Aspergillus mellus* (Narasimhan and Bhide, 1971). Mellein has been found in many sources in nature, including fungi and insects. It has been shown to posses an impressive array of biological activities. For example, it is one of the constituents of the mandibular secretion of Carpenter ants and the defensive secretion of termites. It occurs in the male hair pencils of the oriental fruit moth and has been shown to have pheromonal properties in the castes of *Camponotus pennsylvanicus*. Some report showed that mellein presence in the Australian ponerine ant *Rhytidoponera chalybaea* Emery (Formicidae) (Sun and Toia, 1993).

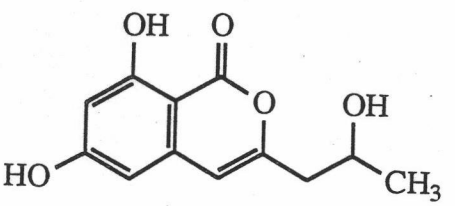
In mushroom, *Gloeophyllum striatum*,can produce oosponal (111), It decreases the blood pressure of hypertensive mice and increases capillary permeability in rabbits (Umezawa *et al*, 1972). Futhermore, oosponal was isolated from fungus *Lenzites trabea* (Mir, Ahmad and Hamid, 1972).



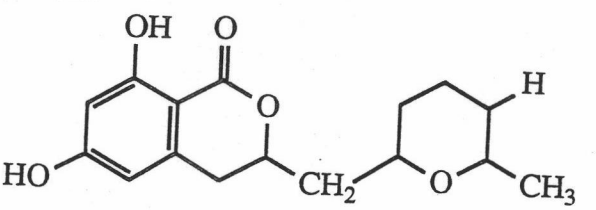
Cladosporin (103)



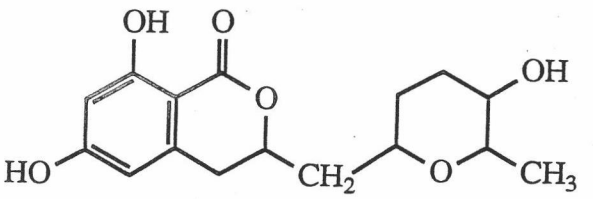
3,4-Dimethyl-8-hydroxy isocoumarin (104)



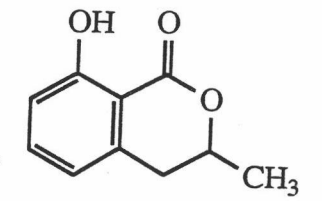
De-O-methyldiaporthin (105)



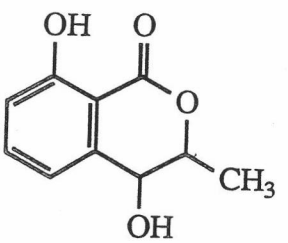
Asperentin (106)



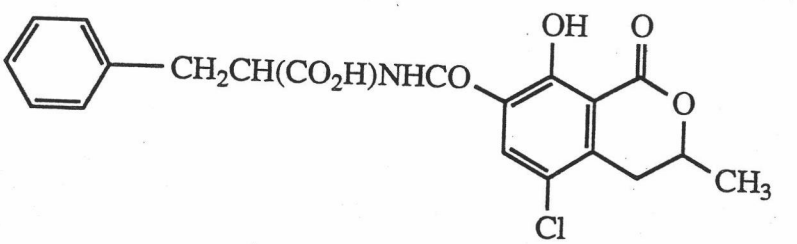
5 -Hydroxy asperentin (107)



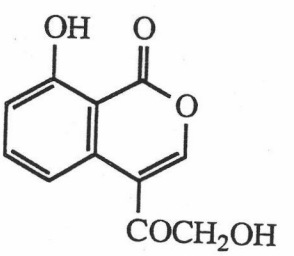
Mellein (108)



4-Hydroxy mellein (109)



Ochratoxin-A (110)

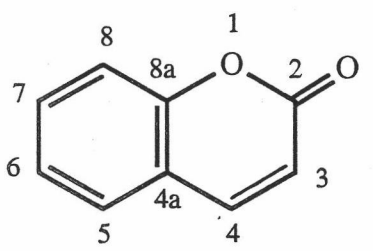


Oosponol (111)

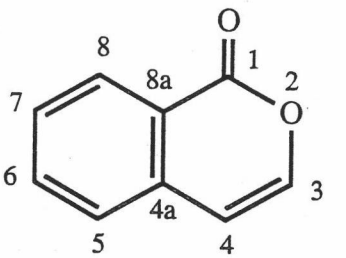
3.2 Chemistry of Isocoumarins

Isocoumarin or 1H-2-benzopyran-1-one is an unsaturated lactone which differs from coumarin (112) in that the olefin linkage is not conjugated with the carbonyl groups. It may be regarded as a vinyl ester (Elderfield, 1951).

The numbering in practise with isocoumarin (113) is illustrated in the following formula.



Coumarin (112)



Isocoumarin (113)

3.3 Biosynthesis of Isocoumarins

The biosynthesis of isocoumarins are derived via polyketides pathway which formed from acetate and malonate units (Herbert,1981). For example, the biosynthesis of mellein (114) and 2, 4-dihydroxy acetophenone (115) which occur in the Australian poneine ant *Rhytidoponera chalybaea* Emery were studied by feeding the ants with an aqueous solution of sodium [1,2 - $^{13}C_2$] acetate. The isolated mellein and acetophenone were examined by ^{13}C-NMR spectroscopy and

^{13}C-^{13}C couplings from the intact acetate units were detected. The results established that they arise via the polyketide pathway (Sun and Toia, 1993). In considering the biosynthesis of 2,4 - di hydroxyacetophenone, while it can be predicted that it will arise from the acetate/malonate pathway, there are two possible foldings of the polyketide chain (Figure 8) which could yield this product. The other way indicated that it derived from a tetraketide precursor with the folding.

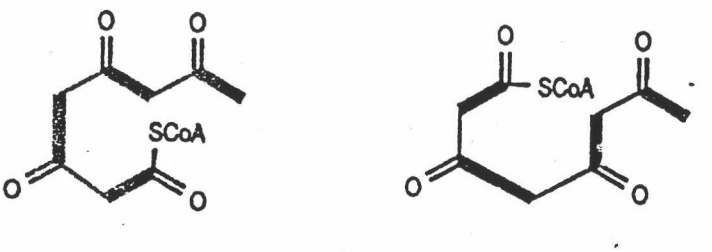


Figure 8 Alternative possible foldings for cyclization of the C - 8 polyketide relative to the position of the [1,2 - $^{13}C_2$] acetate units.

Two possible ways are suggested for the mode of action on a polyketide synthetase for aromatization (Figure 9, p.59). One of the routes is (i) the reduction of the carbonyl group at C - 5 and then dehydration to form an unfunctionalized double bond. The other route is (ii) the reduction of carbonyl group at C - 5 along with enolization at C - 3. Both way form a *cis* double bond so that three contiguous C-C bonds are held in a *syn* arrangement and the appropriate reactive sites (C - 1 and C - 6) are brought together for cyclization (Sun and Toia, 1993).

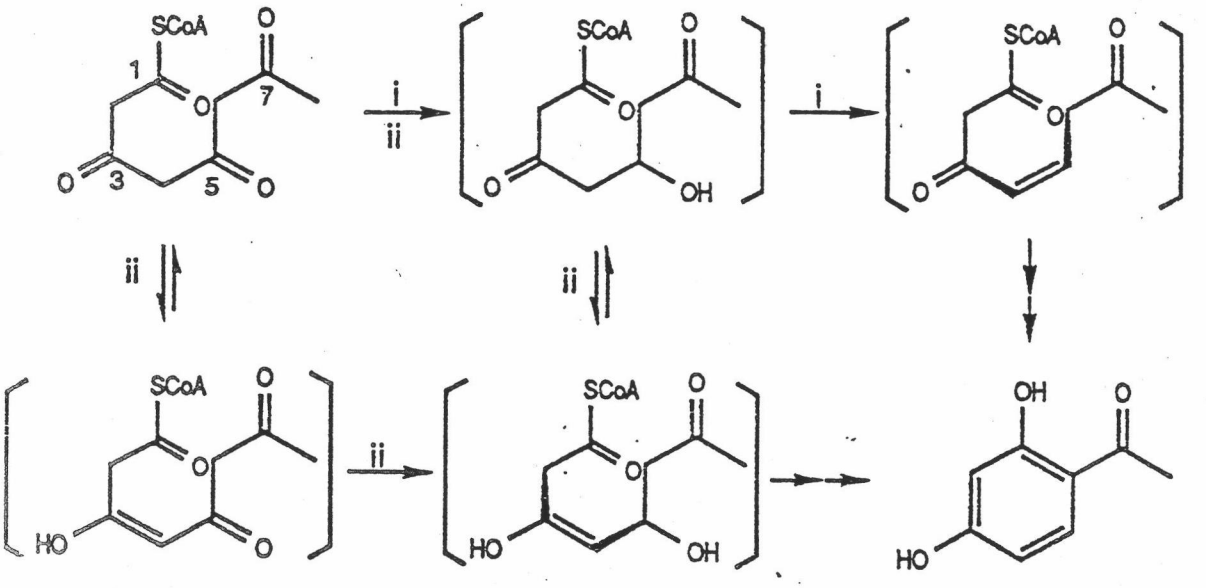


Figure 9 Possible cyclization sequence of the C-8 polyketide leading to 2, 4 -dihydroxyacetophenone as defined by the observed connectivity of the [1, 2 -$^{13}C_2$] acetate unit.

Mellein was determined for its doubly labelled ^{13}C NMR spectrum, and the observed ^{13}C-^{13}C couplings. It confirmed that mellein is formed by the anticipated folding of a pentaketide chain (Figure 10) (Holker and Simpson, 1981).

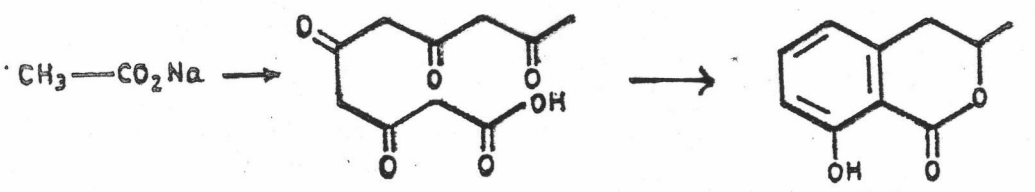


Figure 10 Incorporation of [1,2 - $^{13}C_2$] acetate into mellein