CHAPTER II

1. Chemical compounds isolated from species of *Cassia*

1.1 Alkaloids.

Alkaloid	Botanical source	Plant part	Reference
Cassine	*Cassia carnaval* Speg.	leaf	70
	C. excelsa Shrad.	leaf,twig	15,70
	C. jahnii Britton et Rose.	leaf	16a
	C. spectabilis DC.	leaf,stem, seed	16b,16c
Iso-6-cassine	*C. spectabilis* DC.	leaf,seed	16c,16d
Dihydrocassine (Carnavaline)	*C. carnaval* Speg.	leaf	70
	C. jahnii Britton et Rose.	leaf	16a
Iso-6-carnavaline	*C. spectabilis* DC.	seed	16c
Casselsine	*C. excelsa* Shrad.	leaf,twig	70
Cassilysine	*C. excelsa* Shrad.	leaf,bark	17,61
Cassilysidine	*C. excelsa* Shrad.	bark	17,61
Cassinicine	*C. spectabilis* DC.	leaf,stem, seed	16b,16c
Chaksine	*C. absus* Linn.	seed	18,61

Alkaloid	Botanical source	Plant part	Reference
Isochaksine	*Cassia absus* Linn.	seed	18
Prosopinone	*C. carnaval* Speg.	leaf	19
Siamin	*C. siamea* Lamk.	pod	20
Spectaline	*C. spectabilis* DC.	leaf,seed	16c,16d
Spectalinine	*C. spectabilis* DC.	seed	16c
N-methyl morpholine alkaloid	*C. occidentalis* Linn.	seed	21
N-methyl-β-phenethylamine alkaloid	*C. marylandica* Linn.	leaf	68

1.2 Anthraquinones

Anthraquinone	Botanical source	Plant part	Reference
Aloe-emodin and its glycosides	*Cassia acutifolia* Delile.	leaf,pod, young plant	22,23, 24,25
	C. angustifolia Vahl.	leaf,pod	26,27
	C. alata Linn.	leaf	28
	C. garrettiana Craib.	leaf	29
	C. obovata Collad.	leaf,pod	30
	C. obtusa Roxb.	leaf	31
	C. occidentalis Linn.	seed	32
	C. tora Linn.	seed	32,33

Anthraquinone	Botanical source	Plant part	Reference
Aloin	*Cassia fistula* Linn.	pulp	74
Barbaloin	*C. fistula* Linn.	heartwood, pulp	58,74
Cassialoin	*C. garrettiana* Craib.	heartwood	37
Cassiamins (A, B & C)	*C. siamea* Lamk.	bark,leaf	20
Cassianin	*C. siamea* Lamk.	bark,leaf	20,71
Chrysophanol (Chrysophanic acid) and its glycosides	*C. acutifolia* Delile.	leaf,young plant	24,25
	C. angustifolia Vahl.	pod	26
	C. alata Linn.	leaf,seed, root	28,34,35
	C. frondosa Hook et Arn.	whole plant	36
	C. garrettiana Craib.	heartwood	37
	C. goratensis Fresen.	root,seed	38,39
	C. javanica Linn.	heartwood	65
	C. jaegeri Keay	leaf	40
	C. marylandica Linn.	leaf	41
	C. obovata Collad.	leaf,pod	30
	C. obtusa Roxb.	leaf	31
	C. occidentalis Linn.	root,seed	32,42
	C. reticulata Willd.	leaf	43
	C. siamea Lamk.	leaf	20

Anthraquinone	Botanical source	Plant part	Reference
Chrysophanol (Chrysophanic acid) and its glycosides	*Cassia sophera* Linn.	flower, seedling	44,45
	C. tora Linn.	seed	32,33,75
Emodin and its glycosides	*C. acutifolia* Delile	leaf	25
	C. angustifolia Vahl.	leaf	79
	C. auriculata Linn.	leaf	46
	C. frondosa Hook et Arn.	whole plant	36
	C. jaegeri Keay	leaf	40
	C. mimosoides Linn.	leaf,seed	47,48
	C. obtusa Roxb.	leaf	31
	C. occidentalis Linn.	flower, root	42,49 61
	C. podocarpa Guill & Perr.	leaf	51
	C. reticulata Willd.	leaf	43
	C. sophera Linn.	seed, seedling	45,50
	C. tora Linn.	seed	33,83
Emodic acid	*C. mimosoides* Linn.	seed	47
Fistulin rhamnoside	*C. fistula* Linn.	flower	72
Fistulic acid	*C. fistula* Linn.	pod	73
Germichrysone	*C. sophera* Linn.	seedling	45
Hydroxy anthracene and its derivatives	*C. chrysocarpa* Desv.	epicarp	63

Anthraquinone	Botanical source	Plant part	Reference
Hydroxy anthracene and its derivatives	*Cassia hoffmanseggii* Mart. ex Benth.	leaf	63
	C. splendida Vog.	pod	63
Hydroxymethyl-anthraquinone	*C. ovata* Merat.et lens. ex Geiger.	leaf	64
	C. spectabilis DC.	leaf,stem	16b
Obtusifolin and obtusin	*C. tora* Linn.	seed	75,76
Chryso-obtusin	*C. tora* Linn.	seed	76
Aurantio-obtusin	*C. tora* Linn.	seed	76
Physcion and its glycosides	*C. goratensis* Fresen.	root,seed	38,39
	C. jaegeri Keay.	leaf	40
	C. marylandica Linn.	leaf	41
	C. mimosoides Linn.	root,seed	47
	C. obtusa Roxb.	leaf	31
	C. occidentalis Linn.	flower, root,seed	42,49 52
	C. sophera Linn.	seed, seedling	45,50
	C. spectabilis DC.	leaf,stem	16b
	C. tora Linn.	seed	33,75
Rhein and its glycosides	*C. acutifolia* Delile.	young plant leaf,pod	22,23, 24,25, 27
	C. angustifolia Vahl.	leaf,pod	26,27, 53,79

Anthraquinone	Botanical source	Plant part	Reference
Rhein and its glycosides	*Cassia alata* Linn.	leaf	28,34,61
	C. auriculata Linn.	leaf	53
	C. fistula Linn.	bark,flower heartwood, leaf,pulp	54,55,56 57,58,72
	C. frondosa Hook et Arn.	whole plant	36
	C. marginata Roxb.	flower	65
	C. obovata Collad.	leaf,pod	30
	C. occidentalis Linn.	seed	32
	C. reticulata Willd.	leaf	59
	C. sieberiana DC.	leaf	60
	C. tora Linn.	leaf,seed	32,33
Nodososide	*C. nodosa* Buch. Ham. ex Roxb.	flower	66
Sennosides A & B	*C. acutifolia* Delile	leaf,pod	61
	C. angustifolia Vahl.	pod,leaf	26,61,62
	C. fistula Linn.	leaf	55
	C. obovata Collad.	leaf,pod	30
Total sennosides			
(4.23%)	*C. angustifolia* Vahl.	leaf	53
(0.15%)	*C. auriculata* Linn.	leaf	53
(1.80%)	*C. fistula* Linn.	leaf	53

Anthraquinone	Botanical source	Plant part	Reference
Total sennosides			
(0.20%)	*Cassia javanica* Linn.	leaf	53
(0.07%)	*C. siamea* Lamk.	leaf	53
(0.07%)	*C. sophera* Linn.	leaf	53
(0.14%)	*C. tora* Linn.	leaf	53
Siameadin and Siameanin	*C. siamea* Lamk.	bark	71
Anthraquinone derivatives	*C. corymbosa* Larranaga.	leaf	57
	C. grandis Linn.	leaf	14
	C. speciosa Shrad.	leaf	67
Torosachrysone	*C. sophera* Linn.	seedling	45

1.3 Chromones

Chromone	Botanical source	Plant part	Reference
5-acetonyl-7-hydroxy-2-methyl chromone	*Cassia siamea* Lamk.	flower, leaf	20,78

1.4 Flavonoids

Flavonoid	Botanical source	Plant part	Reference
Apigenin	*Cassia jaegeri* Keay.	leaf	40
Butein	*C. marginata* Roxb.	leaf	85
Catechin	*C. marginata* Roxb.	leaf	85
Kaempferol and its glycosides	*C. acutifolia* Delile.	leaf	27
	C. angustifolia Vahl.	leaf	27,79
	C. alata Linn.	leaf	28
	C. auriculata Linn.	flower	81
	C. fistula Linn.	flower	72
	C. javanica Linn.	flower	80
	C. marginata Roxb.	leaf,flower	65,82
	C. nodosa Buch. Ham. ex Roxb.	leaf	82
	C. obtusa Roxb.	leaf	31
	C. tora Linn.	flower	83
Leucocyanidin	*C. javanica* Linn.	flower	80
Luteolin	*C. mimosoides* Linn.	leaf,seed	47,48
Leucoanthocyanidin (Proanthocyanidin)	*C. jaegeri* Keay.	leaf	40
	C. sieberiana DC.	leaf,root	60,94
Auricassidin	*C. auriculata* Linn.	flower	89
Auriculacacidin	*C. auriculata* Linn.	bark	89

Flavonoid	Botanical source	Plant part	Reference
Goratensidine	*Cassia auriculata* Linn.	leaf	89
	C. goratensis Fresen.	leaf	92
Fistucacidin	*C. fistula* Linn.	bark, heartwood, sapwood	54,58,90, 91
Margicassidin	*C. marginata* Roxb.	flower	93
Leucopelargonidin	*C. fistula* Linn.	flower	54
	C. sieberiana DC.	root	94
	C. tora Linn.	root	83
Leucofisetinidin	*C. marginata* Roxb.	leaf	85
Pelargonidin (Anthocyanin)	*C. sieberiana* DC.	leaf	86
Quercetin and its glycosides	*C. javanica* Linn.	flower	80
	C. marginata Roxb.	flower	65
	C. sieberiana DC.	leaf	60
Isoquercetin	*C. sieberiana* DC.	leaf	60
Rhamnetin and its glycosides	*C. javanica* Linn.	flower	80
	C. sophera Linn.	flower	81
Isorhamnetin and its glycosides	*C. acutifolia* Delile	leaf	27
	C. angustifolia Vahl.	leaf	79
	C. obtusa Roxb.	leaf	31

Flavonoid	Botanical source	Plant part	Reference
Xanthones	*Cassia alata* Linn.	seed,root	28,35
	C. occidentalis Linn.	root	42
	C. reticulata Willd.	leaf	88
	C. tora Linn.	seed	83

1.5 sterols

Sterol	Botanical source	Plant part	Reference
Phytosterol	*Cassia acutifolia* Delile.	leaf	27
	C. angustifolia Vahl.	leaf	27
	C. occidentalis Linn.	root	84
	C. nodosa Buch. Ham. ex Roxb.	flower	87
Stigmasterol	*C. spectabilis* DC.	leaf,stem	16b
γ-sitosterol	*C. nodosa* Buch. Ham. ex Roxb.	flower	86,87
	C. siamea Lamk.	seed	97
β-sitosterol and its glycosides	*C. absus* Linn.	seed	95,96
	C. angustifolia Vahl.	seed	97
	C. alata Linn.	leaf,root, seed	28,35
	C. auriculata Linn.	flower, leaf,seed	46,81,97
	C. fistula Linn.	bark,seed	69,97

Sterol	Botanical source	Plant part	Reference
β-sitosterol and its glycosides	*Cassia jahnii* Britton et Rose.	flower,leaf	95
	C. javanica Linn.	bark,flower heartwood	77,80
	C. laevigata Willd.	seed	97
	C. obtusa Roxb.	leaf	31
	C. occidentalis Linn.	flower	49
	C. siamea Lamk.	flower,leaf	20,78
	C. sophera Linn.	seed	97
	C. spectabilis DC.	leaf,stem	16b
	C. tora Linn.	seed	97

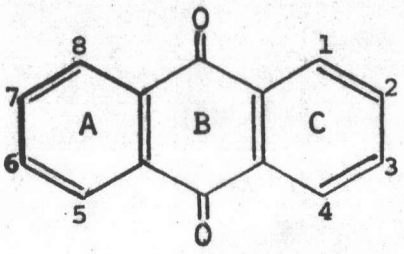
2. Chemical nature and identification of anthraquinones

The largest group of natural quinones is made up of anthraquinones which are the most important pigments in this group. They consist almost entirely of polyhydroxy or alkoxy derivatives.(98) Some of them from many Rubiaceous plants have been used as dyestuffs and others such as from *Cassia, Rhamnus, Rheum* genera are used as purgatives and for skin diseases.(99)

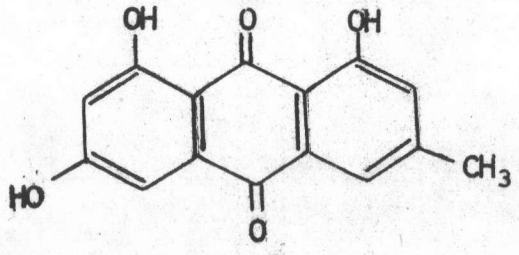
Anthraquinone compounds are distributed fairly widely in moulds, especially in *Aspergillus* and *Penicillium* species. They are uncommon in higher fungi, but are found more frequently in lichens. In higher plants they are located chiefly in heartwood, bark, root, occasionally in stem, seed and fruit. The plant families, which are

rich in this type of compounds, are Rubiaceae, Rhamnaceae, Polygonaceae, Caesalpiniaceae (especially in *Cassia*), Bignoniaceae, Verbenaceae and Liliaceae.(98)

The fundamental structure of anthraquinones composes of three benzene rings and the numbering system is as follows:



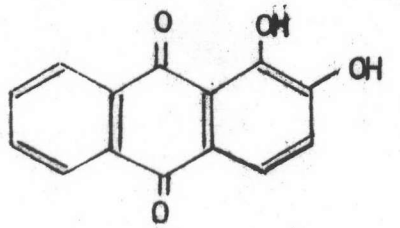
Emodin is probably the most widely distributed anthraquinone being found in moulds, higher fungi, lichens, flowering plants, and insects. A large proportion of the natural anthraquinones are biosynthetic variations of this basic structure.(98)



Emodin

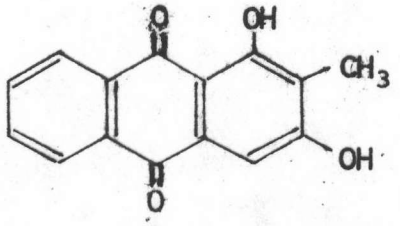
Most of anthraquinones from higher plants are hydroxylated at C-1 and C-2, and the hydroxylated anthraquinones probably do not often occur as such but rather glycosides. Treatment of the plants to obtain the commercially desirable products has the effect of hydrolysing the glycosides, and in some cases, producing additional oxida-

tive changes. Many anthraquinone glycosides occur with the sugar residue linked through one of the phenolic hydroxyl groups and several different sugars are found in such glycosides. Thus, alizalin occurs as a 2-primoveroside (ruberythric acid), rubiadin from madder (*Rubia tinctorum* Linn.) as a 3-glycoside and from *Galium* spp. as a 3-primoveroside, morindone from *Coprosma australis* Robinson as a 6-rutinoside and from *Morinda persicaefolia* Buch.-Ham. as a 6-primoveroside.[99]



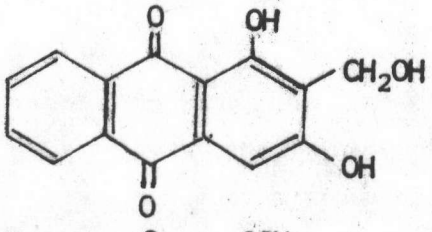
alizarin

(*Rubia tinctorum* Linn.)



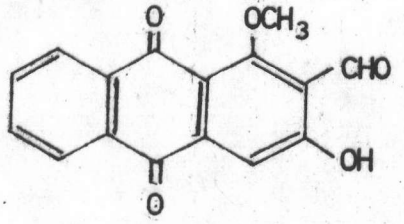
rubiadin

(*Rubia* and *Galium* spp.)



lucidin

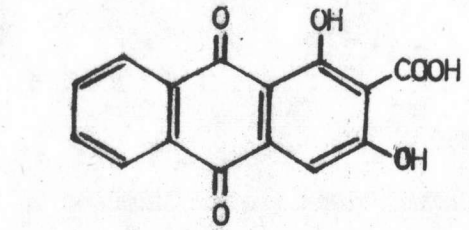
(*coprosma* spp.)



damnacanthal

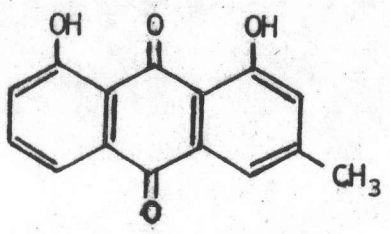
(*Damnacanthus* spp.)

Figure 3 Some naturally occurring anthraquinones.[99]

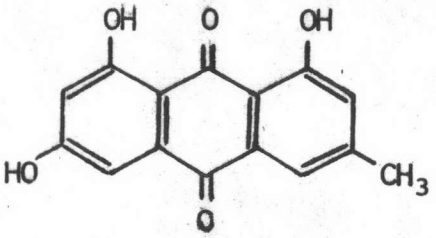


munjistin

(*Rubia* spp.)

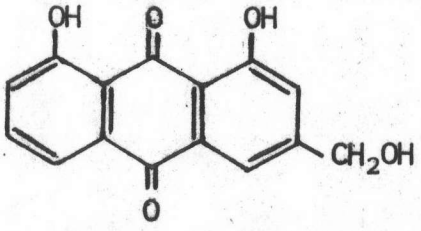


chrysophanol, chrysophanic acid (*Rheum* and *Rumex* spp.)



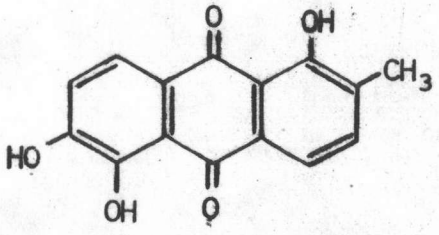
emodin, frangula-emodin

(*Rheum* and *Rumex* spp.)



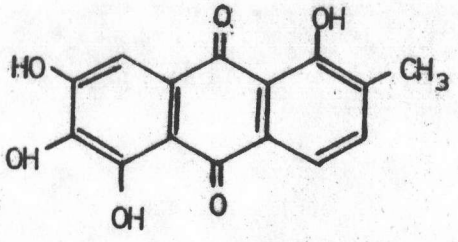
aloe-emodin

(*Aloe*, *Rheum* and *Rhamnus* spp.)



morindone

(*Morinda* spp.)

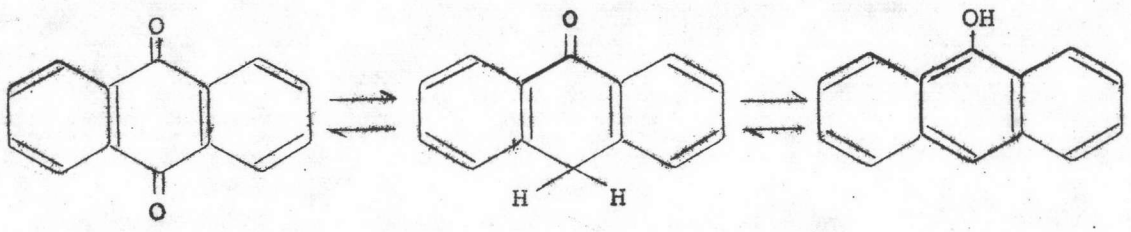


copareolatin

(*Coprosma areolata* Cheesem.)

Figure 3 (Continued)

All of these anthraquinones are high-melting crystalline compounds, soluble in the usual organic solvents. They are usually red in colour, but different ones range from yellow to brown. They dissolve in aqueous alkali with the formation of red-violet colours. In many cases it appears that the glycosides may have their aglycones as a reduced form of the anthraquinone known as anthrone. The sugar in these reduced glycosides may be linked as usual through phenolic oxygens in the outside rings or they may be attached at C-9 to the enol form of anthrone, anthranol.(99)



anthraquinone anthrone anthranol

Enzymatic (or chemical) hydrolysis of a C-9 glycoside of anthranol is followed by oxidation of the anthrone to an anthraquinone if oxygen is present. If the sugar is linked at some other positions, anthranol glycosides may be directly oxidised to anthraquinone. The complexity of anthraquinone derivatives as they occur naturally in plants have been studied, more than one type of derivatives may be present and frequently the nature of the constituents varies with the age of the plant. For example, in common rhubarb (*Rheum undulatum* Linn.) young leaves contain mostly anthranol glycosides, whereas older leaves have glycosides of anthraquinones.(99)

The physiological activity of several of these anthracene derivatives has made them important cathartics for many hundreds of years. Only recently, it has been shown that the free anthraquinones or the glycosides are ineffective, and the pharmacologically important compounds are free anthranols.[99] The fact that pharmacopoeiae recommend storage of some purgative plants for periods up to one year before use, is explained by the necessity for slow hydrolysis of the glycosides to free anthranols. If storage is too long, however the anthranols are oxidised to anthraquinones. Anthraquinones are active as cathartics only because they are reduced to anthranols by intestinal bacteria. Nothing is known regarding any function of these various anthracene derivatives in plants. The ease with which the reaction anthraquinone $\rightleftharpoons$ anthrone may be brought about in the laboratory, has raised the possibility that these compounds may somehow participate in hydrogen transfer or oxidation-reduction reactions.[99]

Isolation procedures depend on whether free aglycones or the various glycosidic derivative are desired. For the first, extraction of the plant with rather non-polar solvents such as ether or benzene is effective. The sugar derivatives are extracted using water, ethanol, or water-ethanol mixtures. If anthrones or anthranols are to be isolated, care must be taken to avoid their oxidation by oxygen in the air. This oxidation is particularly rapid in alkaline solutions and leads to the formation of dianthrones and poly-anthrones as well as the anthraquinones. After extraction, a solution of glycosides may be concentrated under reduced pressure, to obtain crude crystals,

which may be purified by repeated crystallisation from acetone-water. The glycosides, on heating with acetic acid or 5% alcoholic hydrochloric acid are readily hydrolysed within one hour at 70°C. After hydrolysis, a 1+1 mixture of ethanol benzene is added and then diluted with 0.5% aqueous hydrochloric acid. A layer of benzene separated contains the aglycones. The aglycones obtained by hydrolysis or direct extraction from plant materials may be purified by extraction from benzene into diluted alkali and precipitated with acid. Aglycones with free carboxyl groups can be extracted from benzene using sodium bicarbonate solution and a second extraction with sodium hydroxide to remove any less acedic substances. This crude precipitate is crystallised from benzene or alcohol. Purification of the aglycones by column chromatography is successful if rather weak adsorbents are used, for example, magnesium oxide, polyamide, or calcium phosphate.[99]

For identification of anthraquinone derivatives, the Borntträger reaction is routinely used.[99] Some of the unknown material is boiled in diluted aqueous potassium hydroxide for a few minutes. This not only hydrolysed glycosides but also oxidises anthranols to anthraquinones. The alkaline solution is cooled, acidified with dilute hydrochloric or acetic acid and extracted with benzene. When the benzene phase is separated and shaken with dilute alkali such as ammonium hydroxide, the benzene layer loses its yellow colour and the alkaline phase becomes red if quinones are present. The test is not specific for anthraquinones, naphthoquinones also give a positive reaction. If partially reduced anthraquinones are present, the ori-

ginal solution does not turn red immediately on making alkaline, but turn yellow with green fluorescence and then gradually becomes red as oxidation occurs. If desired, the oxidation may be hastened by adding a few drops of 3% hydrogen peroxide. The Borntrager reaction can also be made the basis of a quantitative colorimetric determination. Direct spectral observations of benzene solution may also be made for characterisation of anthracene derivatives. Anthraquinones show a broad absorption peak at about 440 nm, whereas, the reduced forms absorb at about 360 nm with no significant absorption at 440 nm, 1,4-dihydroxyanthraquinones fluoresce in acetic acid solution.[99] The colours given with alcoholic magnesium acetate solution are characteristic of different hydroxylation patterns. Compounds containing, two hydroxy groups in the 1,3 position *(meta)*, i.e emodin, chrysophanol, or aloe-emodin give an orange-red or pink colour, those with two in the 1,4-position *(para)*, i.e. quinizarin, produce a purple, and those with two in the 1,2-position *(ortho)*, i.e. alizarin exhibit a violet colour. This colour reaction is specific, stable and very sensitive. Characterisation of anthraquinone derivatives by means of paper chromatography and electrophoresis have been developed by several workers.[99] Paper electrophoresis methods are described by Core and Kirch, Siesto and Bartoli.[99] Shibata, Takido and Tanaka reported the relationship between the Rf-values and the structures of several synthetic and natural hydroxy anthraquinones by using benzine (b.p. 45°- 70°C) saturated with 97% methanol as a solvent for one-dimensional ascending paper chromatography. The developed-paper

strip was sprayed with 0.5% methanolic magnesium acetate solution and heated at 90°C for five minutes[100] as shown below.

Rf-Values of hydroxyanthraquinones.[100]

(97% methanol saturated benzine as solvent at 24°- 25°C by using one-dimensional ascending method.)

Substance	Position of substituents in anthraquinone nucleus	Rf-value	Colour of spot
Chrysophanol	4,5-Dihydroxy-2-methyl	0.92	orange
Physcion	4,5-Dihydroxy-7-methoxy-2-methyl	0.89	orange
Quinizarin	1,4-Dihydroxy	0.89	purple
2-Methyl quinizarin	1,4-Dihydroxy-2-methyl	0.92	purple
Emodin	4,5,7-Trihydroxy-2-methyl	0.52	pink
Rubiadin	1,3-Dihydroxy-2-methyl	0.49	orange-yellow
Aloe-emodin	4,5-Dihydroxy-2-hydroxymethyl	0.15	orange
Rhein	4,5-Dihydroxy-2-carboxyl	0	orange
Alizarin	1,2-Dihydroxy	0.04	violet
Endocrocin	4,5,7-Trihydroxy-2-methyl-3-carboxyl	0	pink
Purpurin	1,3,4-Trihydroxy	0.03	purple
Dihydroxymethyl-anthraquinone	1,5-Dihydroxy-2-methyl	0.92	orange
	1,8-Dihydroxy-2-methyl	0.92	orange
Tetrahydroxy-anthraquinone	1,3,5,7-Tetrahydroxy-2-6-dimethyl	0.02	orange
	1,3,6,8-Tetrahydroxy	0.01	orange-pink

Aloe-emodin or rhabarberone is named chemically as 3-hydroxymethylchrysazin or 1,8-dihydroxy-3-hydroxymethylanthraquinone. Its molecular is $C_{15}H_{10}O_5$, molecular weight 270.23 and composing of C 66.67%, H 3.73%, and O 29.60%. It occurs in the free state and as glycoside in *Rheum* (rhubarb), in senna leaves (*Cassia acutifolia* Delile. and *Cassia angustifolia* Vahl.) and in various species of *Aloe*.[101] From toluene, it occurs as orange needles, m.p. 223°-224°C and sublimes in carbondioxide stream. Aloe-emodin is freely soluble in hot alcohol, ethyl ether, benzene with yellow colour, in ammonia water and in sulphuric acid with crimson colour.[101]

Ultraviolet absorption spectrum of Aloe-emodin in one normal sodium hydroxide solution, λ_{max} = 236 (log ε = 4.51), 282 (log ε = 4.05) and 500 (log ε = 3.96)nm. The infrared absorption spectrum of aloe-emodin exhibit strong bands at 1630 (C=O, chelated), 1670 (C=O, free), 1570 (-C=C-) and 3400 (-OH groups) cm^{-1}.[102]

Aloe-emodin triacetate ($C_{21}H_{16}O_8$) occurs as yellow needles from benzene, m.p. 175°-177°C.[101]

3. Biogenesis of anthraquinone.

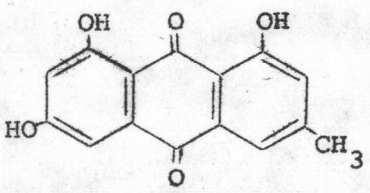
Anthraquinones consist almost entirely of polyhydroxy or methoxy derivatives with little variation in skeletal structure. Like other secondary metabolites, the anthraquinones are derived from a few key intermediates, principally acetate, shikimate and mevalonate by a series of reactions which lead to the formation of benzenoid compounds. It is assumed that the last stage involves the oxidation

of a phenol as in many laboratory synthesis. Nevertheless they arise by at least two biosynthetic routes.

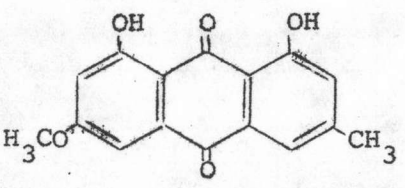
A) Acetate-malonate route.

Birch and Donovan revealed that many anthraquinones, like emodin had structures in accordance with the "acetate-hypothesis".[103,104]

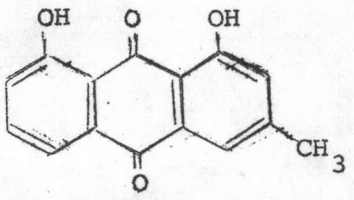
Birch *et al.*[105,108] and Gatenbeck[106,107] carried out labelling experiments with C^{14} acetate to establish the acetate derivation of helminthosporin, emodin, islandicin and cynodontin. Additional confirmation was obtained by using C^{14}, O^{18}-acetate as precursor.[107] Later investigations showed that aromatic polyketides were actually built up from a starter unit (usually acetate) and a chain of malonate units[109] formed by carboxylation of acetyl coenzyme A, this was confirmed in the case of islandicin[110] and the bianthraquinone "regulosin".[111] All the fungal anthraquinones are structurally consistent with their formation by the acetate-malonate pathway. As such typical fungal anthraquinones as emodin and chrysophanol are also found in higher plants, thus it is possible to assume that they are formed in the same way. The isolation of both anthrone of physcion from cultures of several *Aspergilli*, from the root bark of *Ventilago maderaspatana* Roxb. (Rhamnaceae), and the occurrence of bianthrones, on occasion, gave support to this view.[98] Recently, Leistner and Zenk have proved that chrysophanol is produced in *Rumex alpinus* Linn. and *Rhamnus frangula* Linn. via the acetate-malonate route.[98,112]



Emodin



Physcion

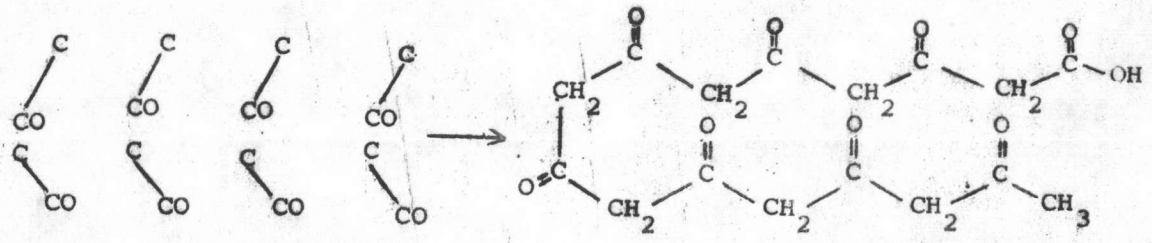


Chrysophanol

The majority of the anthraquinones which are assumed to be elaborated by the acetate-malonate pathway confirm to the emodin pattern. They are arised by suitable folding and condensation of a polyketide chain derived from eight acetate units. Resulting from O-methylation, side-chain oxidation, chlorination, dimerisation and the introduction or omission of nuclear hydroxyl groups, numerous variations of basic structure occurred.(98)

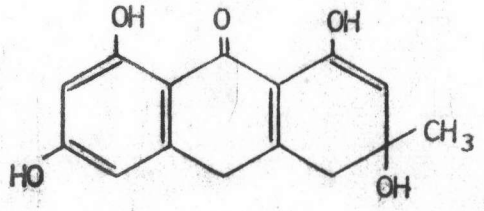
Such a scheme A shown on page 28 accounts perfectly for the structure of emodin, for other compounds, loss or rearrangement of hydroxyl groups and oxidation or removal of methyl group are required.(99)

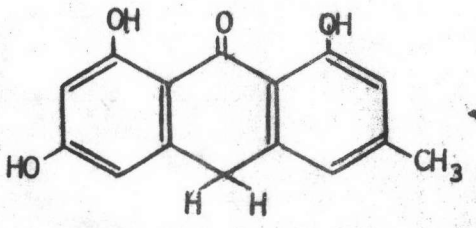
Scheme A: Acetate-Malonate Route.[98,99]



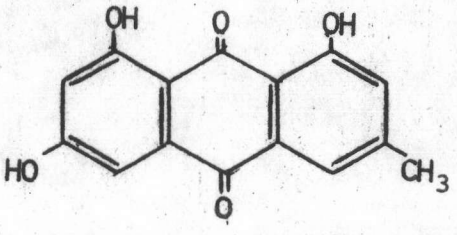
8-acetate

Poly-β-ketomethylene compound intermediate.





Anthrone

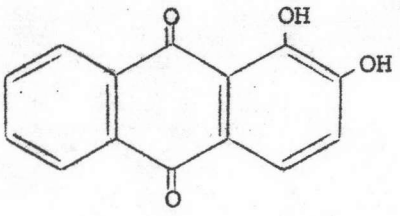


Anthraquinone

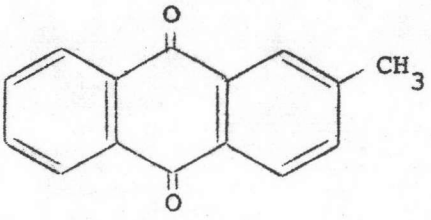
(Emodin or related derivatives)

B) Shikimate and Mevalonate route.

About half of anthraquinones which have Shikimate and Mevalonate biosynthetic route are found in higher plants. The majority of them occur in the Rubiaceae, sub-family Rubioideae, and to a lesser extent in the Bignoniaceae and the Verbenaceae.(98) They are substituted in only one benzenoid ring (ring C) and may be totally devoid of a carbon side chain or hydroxyl groups e.g. alizarin, tectoquinone.(98)



Alizarin

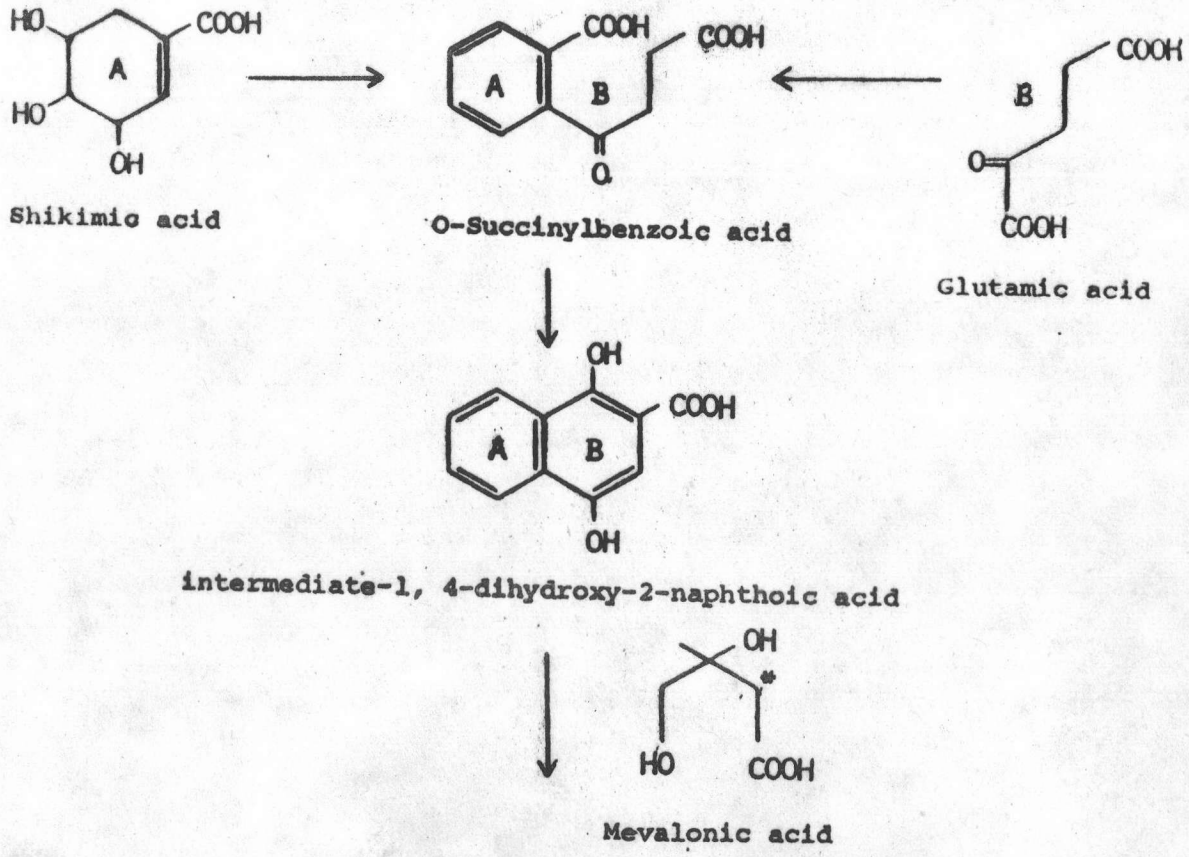


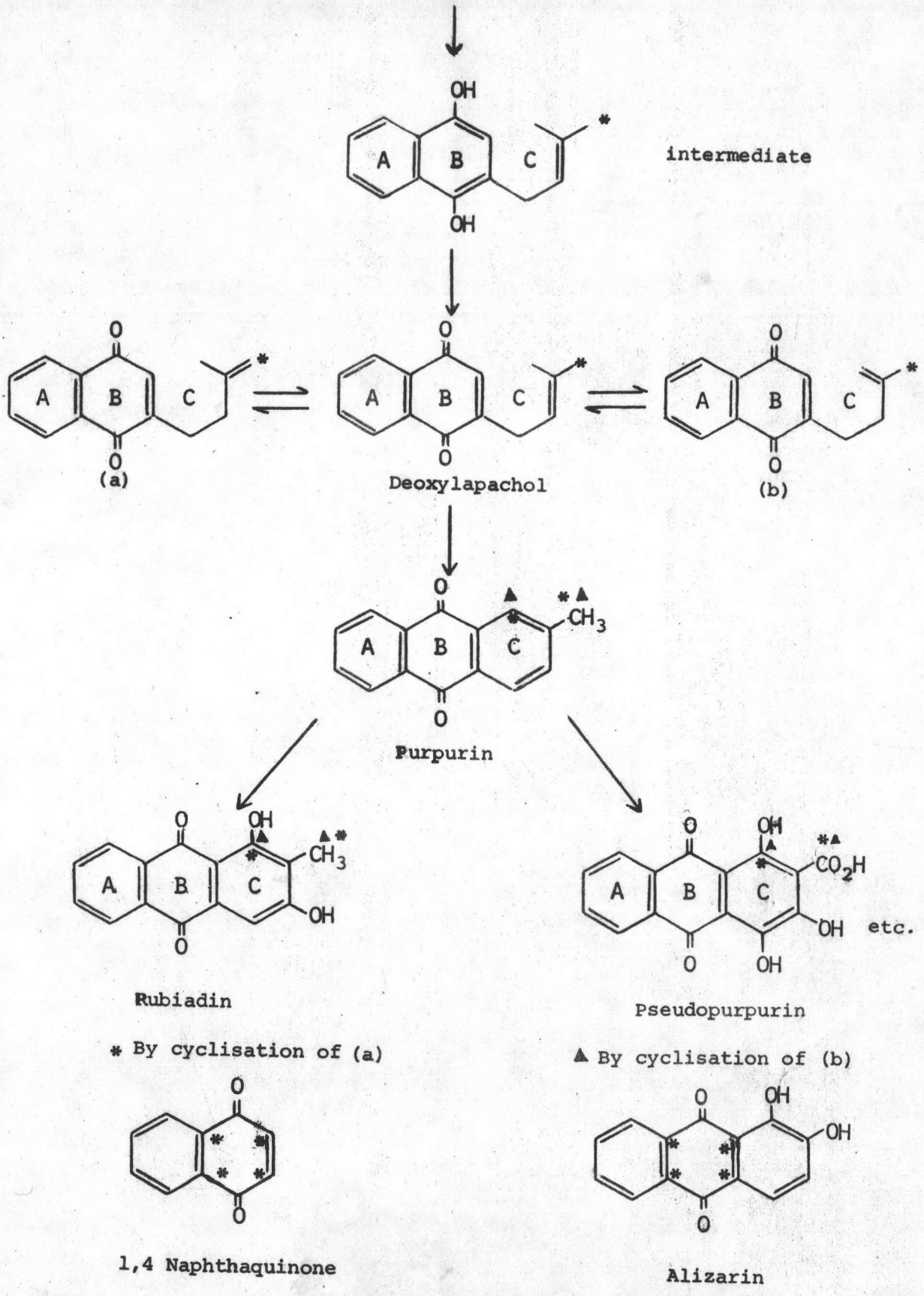
Tectoquinone

Leistner and Zenk have shown that *Rubia tinctorum* Linn. utilises shikimic acid to form ring A of alizarin and one of the carbonyl carbon atoms.(113) Significantly the anthraquinones present in Bignoniaceae and Verbenaceae heartwoods are accompanied by C^{15} naphthaquinones, notably deoxy-lapachol while the Rubiaceous plants contain a number of this type of compounds too.(98) The 4-methoxy-1-naphthol has also been found in Rubiaceae.(114) These finding suggested that deoxylapachol is synthesised *in vivo* by prenylation of a naphthol precursor and followed by oxidation, and it can also be converted into tectoquinone *in vitro* either by boron trifluoride

catalysis or by irradiation.[98,115] It seems likely that substituted ring C in this group of anthraquinones is derived from mevalonate.[98] This was established[113,116,117] by feeding *Rubia tinctorum* Linn. Plants with 2-C^{14}-mevalonate. Four radioactive pigments rubiadin, pseudopurpurin, alizarin and purpurin were isolated.[116] Appropriate degradation of pseudopurpurin established that the C^{14} was distributed between the side chain and C-1 in ring C. Therefore it seems that ring C of the anthraquinones in Rubiaceous plants is formed as in Scheme B (shown below) and presumably the same pathway is followed in the Bignoniaceae and Verbenaceae.[98]

Scheme B : Shikimate and Mevalonate Route.[98]



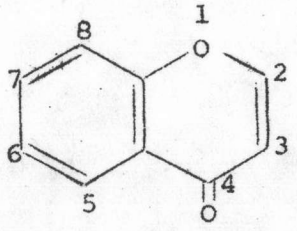

intermediate
Deoxylapachol
(a)
(b)
Purpurin
Rubiadin
Pseudopurpurin
etc.
* By cyclisation of (a)
▲ By cyclisation of (b)
1,4 Naphthaquinone
Alizarin

Therefore it can be considered that in fungi, anthraquinones of emodin type are synthesised via the acetate-malonate pathway while higher plants have developed two completely separate pathways for the synthesis of the anthraquinone carbon skeleton, *i.e.*, the shikimate and the polyacetate-malonate pathways.(112)

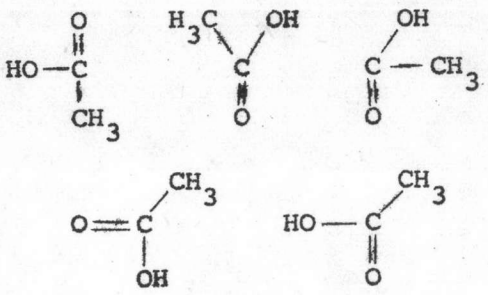
4. Chemical nature and classification of naturally occurring Chromones

Chromone is the parent compound of important vegetable colouring matters which are derived from flavone (2-phenyl chromone), flavonol (3-hydroxyflavone), flavanone (2,3-dihydroflavone) and isoflavone (3-phenylchromone). Chromone is the name given by Von Kostanecki, its chemical name is 5,6-benzo-γ-pyrones.(118) The chromones are interesting as the active ingredients of several plants used for centuries in folk medicine. Khellin has primary action as a vasodilator, it is valuable as an antispasmodic and for relieving the pain of renal colic, dental caries, angina pectoris, etc.(99)

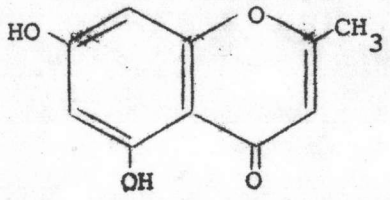
The chromone nucleus with its numbering system is as follows.



Naturally occurring chromones generally have a methyl group at C-2 and are oxygenated at C-5 and C-7. They may be derived from the condensation of five molecules of acetic acid (Figure 4), and it is possible that chromones could be formed by the addition of an acetate unit to a phenylpropane intermediate.(99)



Five acetic acids.



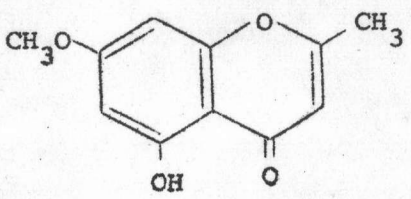
Chromone.

Figure 4 The formation of chromone from five molecules of acetic acid.

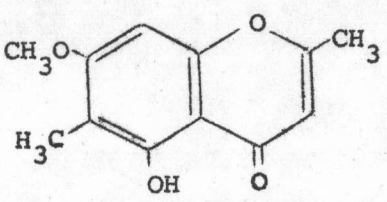
The naturally occurring chromones are classified into 3 types as followed.(118)

4.1 Hydroxy chromones (such as eugenin,eugenitin)

Eugenin is the simplest naturally occurring chromone. It is named chemically as 5-hydroxy-7-methoxy-2-methylchromone, and obtained from wild cloves, *Eugenia caryophyllata* thunb.(118)



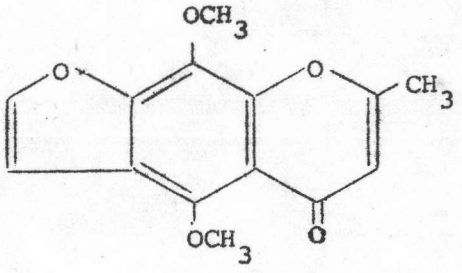
Eugenin.



Eugenitin.

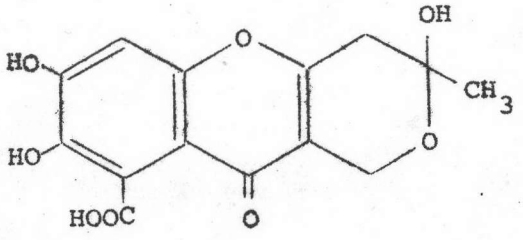
4.2 Furanochromones (Furochromones)

This type of chromone composes of khellin, visnagin, kellinin etc. Khellin is used in treating bronchial asthma and whooping cough.



Khellin

4.3 Pyranochromones (e.g. fulvic acid)



Fulvic acid

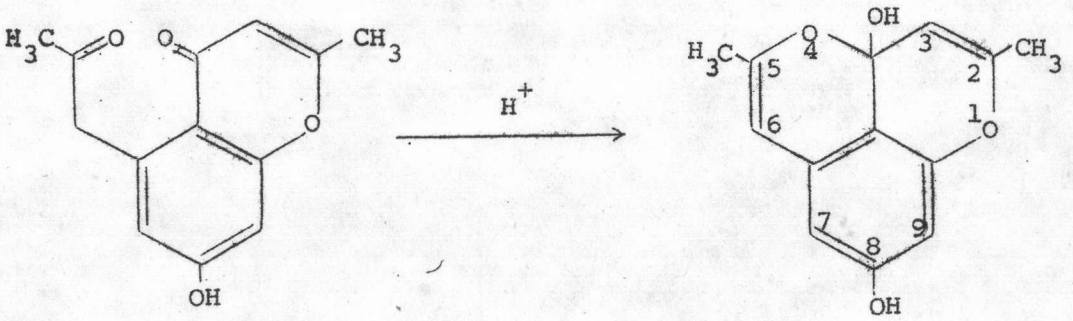
Fulvic acid is a yellow acidic metabolite produced by fungi including *Carpenteles brefeldianum* Dodge.[118]

Chromones are extracted from dried plant material by using organic solvents such as ethyl ether, chloroform, or acetone. They may be crystallised directly from these solvents or purified by chromatography on magnesium oxide or neutral deactivated alumina.[99]

Chromone with phenolic hydroxyl groups can be extracted from ethereal solution with dilute sodium hydroxide, and chromone glycosides are extracted by methanol.[99] Chromones are usually colourless but form yellow-orange oxonium salts in the presence of strong mineral acids. This colour reaction is useful for indicating the presence of chromones, but proof of structure rests on degradation and identification of split products. The ultraviolet absorption spectrum of chromones usually shows a main band at about 295 nm with weaker absorption at about 250 nm. Substitution at C-8 or presence of the furan ring may increase the absorption maximum to as much as 340 nm. Paper chromatographic separation of chromones can be made by using the mixture of water and 2-propanol as the solvent.[99]

5. Identification and biogenesis of barakol.

Barakol is 3a,4-dihydro-3a,8-dihydroxy-2,5-dimethyl-1,4-dioxaphenalene.[123] It was first isolated from the leaves of *Cassia siamea* Lamk. in 1969, and from dried flowers of the same plants in 1971. It is an artifact, the product of acid treatment of 5-acetonyl-7-hydroxy-2-methylchromone as shown in Figure 5.[119,120]



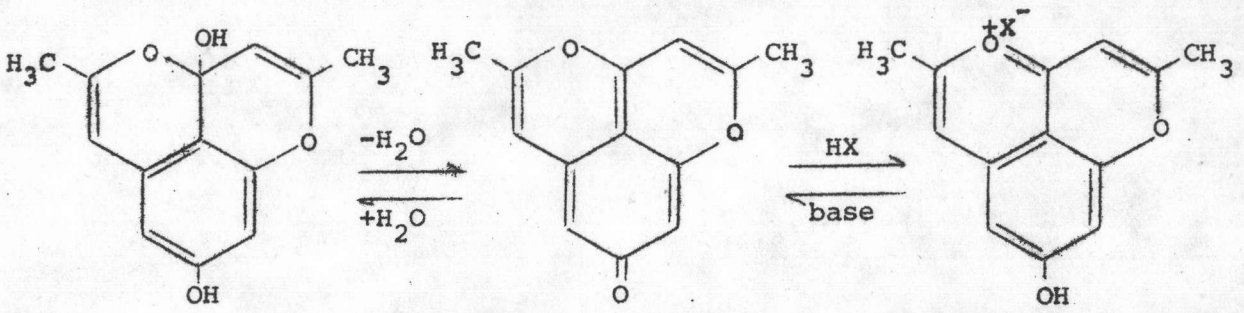
5-acetonyl-7-hydroxy-2-methylchromone

barakol

Figure 5 Acid treatment of 5-acetonyl-7-hydroxy-2-methylchromone into barakol.

Barakol occurs as pale lemon-yellow needles, m.p. 165°C (decomposed). It is soluble in methanol, ethanol and acetone, moder--ately soluble in chloroform and dichloromethane, and readily soluble in benzene, carbontetrachloride, ethyl ether and water. It has the molecular formula of $C_{13}H_{12}O_4$. Its solution turns brown and precipitates as dark brown polymeric material after warming for a short time. Chemical dehydration of barakol was readily achieved in a desiccator over phosphorus pentoxide or in vacuum. The resulting dark green amorphous material of anhydrobarakol ($C_{13}H_{10}O_3$) which is unstable is obtained. Anhydrobarakol is the methylene quinone derivative, it decomposes at 165°C and it can be reconverted into barakol by dissolution in aqueous methanol, as illustrated in Figure 6 (119) on page 37.

The strong basic character of barakol was demonstrated by the fact that crystalline hydrobromide and hydrochloride derivatives, $C_{13}H_{10}O_3$,HX, which are salts of the anhydro-base, could be prepared by addition of concentrated hydrochloric or hydrobromic acids to a methanolic solution of barakol, the ready reversible dehydration and salt formation occurred.(119)



Barakol Anhydrobarakol Anhydronium salt

Figure 6 Conversion between barakol and anhydrobarakol.

Attempts to verify the presence of a further hydroxy group in barakol by methylation or acetylation led to no identifiable products. Attempts to hydrogenate barakol were equally unsuccessful.(119)

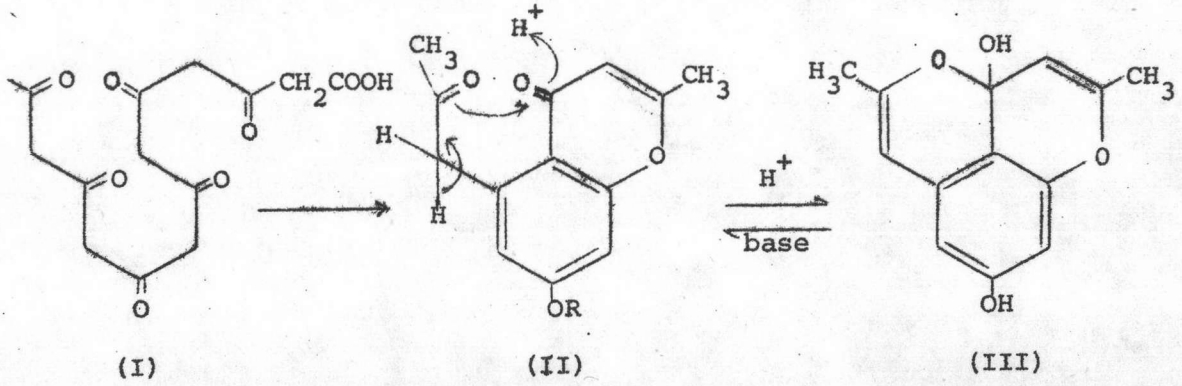
High resolution mass spectrometry of barakol exhibited a weak parent peak at *m/e* 232, with the base peak at *m/e* 214 corresponding to a loss of water from the molecular ion. The infrared spectra (Nujol) of barakol, anhydrobarakol and anhydrobarakol hydrochloride exhibit strong bands at 1670, 1670 and 1660 cm^{-1} respectively.(119)

The ultraviolet spectrum of barakol shows maxima at 241 and 384 nm (ε 34,700 and 13,000 respectively). The relative simple 100 MHz NMR spectrum in $CDCl_3$ of anhydrobarakol shows two methyl signals at τ 7.84 and 7.95, both exhibiting allylic fine coupling. Double irradiation studies demonstrated that high-field methyl protons were coupled to a vinyl proton (τ 3.97) and the low-field methyl protons were coupled to another vinyl proton (τ 4.23). The signals at τ 3.83

(1H,J 1.7 Hz) and 3.93 (1H,J 1.7 Hz) were assignable to an AB system due to two *meta*-coupled protons.[119]

Biogenesis:

A possible biogenesis of barakol from a polyketide (I) derived from seven-acetate units and involving the intermediacy of the compound (II) is illustrated in Figure 7. Cyclisation of (II) to barakol (III) can be regarded as either the interaction of an enolate anion with the chromone carbonyl or the nucleophilic attack of the chromonecarbonyl on the side-chain ketone, only the former is represented.



(I) (II) (III)

Figure 7 Biogenesis of barakol from a polyketide.

It seems reasonable that the reverse reaction, barakol (III) ⟶ II(R=H) might also occur. Mild basic treatment of barakol afforded an unstable compound, which on methylation gave a crystalline-O-methyl derivative, 5-acetonyl-7-methoxy-2-methylchromone.[119]