CHAPTER II

HISTORICAL

1. Distribution

The largest group of natural quinones is made up of anthraquinones. Some of them have been important as dyestuffs and others as purgative and for skin diseases. Nearly half of anthraquinones have been found in fungi and lichens and about similar number in higher plants. Recently it has been reported their first appearance in bacteria.[4] In animal a few occur in insects (Coccidae only) and in feather stars (Crinoidea).[5]

Anthraquinones are distributed fairly widely in moulds especially in *Aspergillus* and *Penicillium spp.* They are uncommon in higher fungi but are found more frequently in lichens.[6] The higher plant families rich in this type of compounds are :-

Monocotyledoneae

Family	Genus
Liliaceae (Asphodeloideae)	Aloe, Asphodeline, Asphodelus, Bulbine, Eremurus[7]

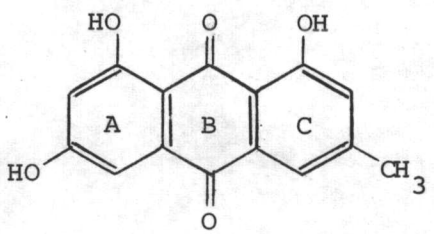
Dicotyledoneae

Family	Genus
Bignoniaceae	Tabebuia[6]
Caesalpiniaceae	Cassia[6]

Family	Genus
Papilionaceae	Andira[6]
Polygonaceae	Polygonum, Rheum, Rumex[6]
Rhamnaceae	Maesopsis[8], Rhamnus[6]
Rubiaceae	Coelospermum, Coprosma, Damnacanthus Galuim, Hymenodictyon, Morinda, Oldenlandia,[6] Prismatomeris,[9] Rubia[6]
Scrophulariaceae	Digitalis[6]
Verbenaceae	Tectona[6]

The following families contain some anthraquinones :- Anacardiaceae, Apocynaceae, Asclepiadaceae, Caryophyllaceae, Compositae, Ericaceae, Euphorbiaceae, Lythraceae, Rhizophoraceae, Saxifragaceae,[10] and Solanaceae (*Fabiana imbricata* Ruiz et Par.)[11]

Emodin is a typical anthraquinone of the most widely distributed ones, being found in higher fungi, lichens and higher plants.[13]

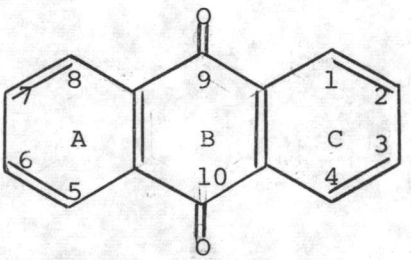


Emodin

A large proportion of the natural anthraquinones are biosynthetic variations of this basic structure.[12]

2. Chemical Nature of Anthraquinones

Anthraquinone compounds are distributed widely in nature. They are red yellow or orange yellow colouring matter. Anthraquinone is a tricyclic benzene ring structure having the quinoid double bond. So its structure is a diketone produced by oxidation of anthracene, all of these anthraquinone substances may be regarded as products formed by oxidation reduction hydrolysis and condensation from hydrocarbon anthracene. The fundamental anthraquinone structure is shown below with the ring numbering system.



According to biosynthetic pathways anthraquinones can be classified into two groups.

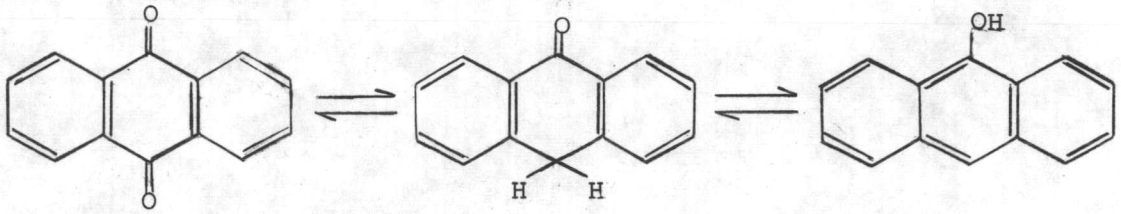
a. Anthraquinones with substitutions in rings A and C : These anthraquinones are found in fungi and higher plants. In fungi they are emodin, endocrocin, and islandicin. In higher plants these anthraquinones distribute in Caesalpiniaceae, Papilionaceae, Polygonaceae, and Rhamnaceae e.g. aloe-emodin, chrysophanol, emodin.

b. Anthraquinones with substitutions only in ring C : Members of this group are found mainly in Bignoniaceae, Rubiaceae,

Scrophulariaceae and Verbenaceae e.g. alizarin, rubiadin.

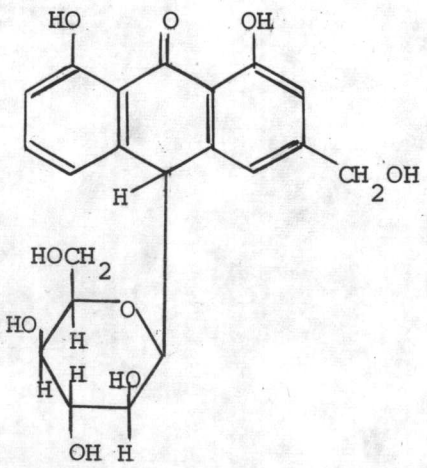
The hydroxylated anthraquinone probably do not often occur in plants as such but rather glycosides. Treatment of the plants to obtain the commercially desirable products has the effect of hydrolysing the glycosides, and in some case producing additional oxidation change. All of these anthraquinones are high melting crystalline compounds soluble in the usual organic solvents. The problem of the form in which these anthraquinones actually exist in plants remains a knotty one, and there are apparently several possibilities.[13] Since the native precursors generally break down readily under the influence of enzymes or extraction procedures, reports of the appearance of free anthraquinones must be regarded cautiously. Many of the anthraquinones occur as glycosides with the sugar residue linked through one of the phenolic hydroxyl groups. Several different sugars are found in such glycosides. Thus alizarin occurs as 2-primoroside, rubiadin from madder (*Rubia tinctorum* Linn.) as 3-glucoside, and from *Galium* spp. as 3-primoroside, morindone from *Coprosma australis* Robinson as 6-rutinoside.[13]

In many cases, it appears that the native glycosides have their aglycones as a reduced form of the anthraquinone, known as anthrone. The sugars in these reduced glycosides may be linked as usual through phenolic oxygen in the outside rings or they may be attached at C-9 to enol form of anthrone, anthranol.



anthraquinone anthrone anthranol

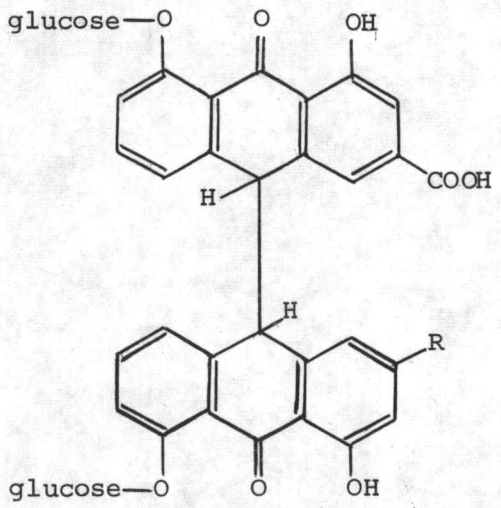
Enzymatic (or chemical) hydrolysis of a C-9 glycoside of anthranol is followed by oxidation of the anthrone to an anthraquinone if oxygen is present. If the sugar is linked at some other position, anthranol glycosides may be directly oxidised to anthraquinone glycosides. Although aloe-emodin occurs in *Rheum spp.* as an ordinary glycoside in aloes it is found as barbaloin, an unusual compound in which a glucose-like group is linked by a carbon-carbon bond to a partially reduced anthraquinone (anthrone).[13]



Barbaloin

Other compounds apparently similar to barbaloin occur in other species of aloes. Unlike other glycosides, they are stable toward acid hydrolysis, but may be split with ferric chloride to form aloe-emodin.[14]

Still more complex are the sennosides, the active cathartics of senna. These compounds are dianthrones; the chemistry of the sennoides has been reviewed by Stoll and Becker.[15] It seems at least possible that other anthraquinones are derived from such natural precursors by oxidative splitting. For instance, the native glycosides from the bark of *Rhamnus frangula* Linn. may be dianthrone glycosides which are oxidised on storage to form aloe-emodin and its glycosides.[16]

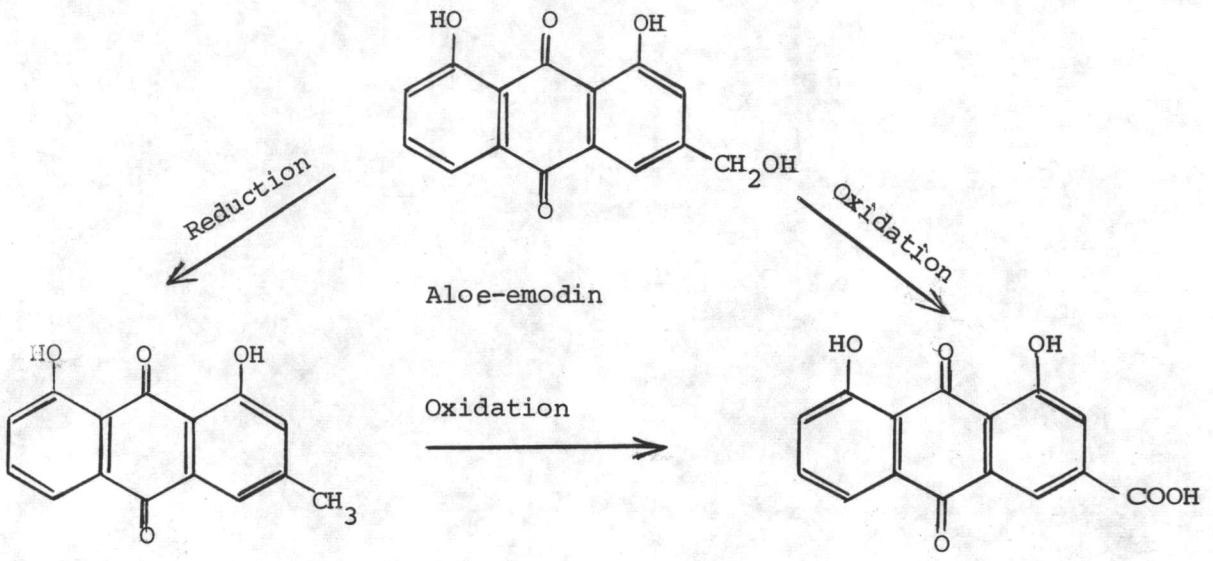


Sennosides A and B R = —COOH

Sennosides C and D R = $-CH_2OH$

Sennoside A is the dextrorotatory isomer and sennoside B is the meso isomer.[17] In the isomeric sennosides C and D, sennoside C is the (-) isomer, and sennoside D is the (+) isomer.[18,19]

Aloe-emodin, chrysophanic acid and rhein are related structures according to the following scheme.[20]



Chrysophanol

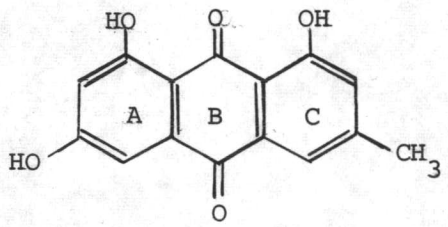
Rhein

For identification of anthraquinone derivatives the Bornträger reaction is routinely used. The Bornträger reaction can also be made the basis of a quantitative colorimetric determination, anthraquinones show a broad absorption peak at 440 nm, whereas the reduced forms absorb at about 360 nm with no significant absorption at 440 nm. If the reduced anthraquinones are present, the 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 solution. The colours given with alcoholic magnesium acetate solution are characteristic of different hydroxylation patterns.[21]

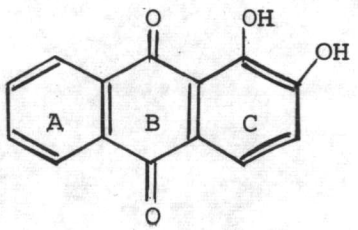
Compounds containing two hydroxy groups in meta position (1, 3) e.g. aloe-emodin, chrysophanol, or emodin give an orange-red or pink colour, those with two hydroxy groups in para position (1, 4) e.g. quinizarin, produce a purple colour and those with two hydroxy groups in the ortho position (1, 2) e.g. alizarin exhibit a violet colour.

3. Biogenesis

Anthraquinones form a large and compact group, nearly all of which are polyhydroxy (methoxy) derivatives with little variation in skeletal structure. Nevertheless they arise by at least two biosynthetic routes. Birch and Donovan revealed that many anthraquinone compounds, liked emodin had structure in accord with the acetate hypothesis. A ring related to alizarin seemed to be formed in some other way.[22,23]



Emodin

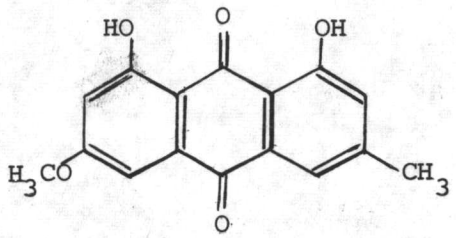


Alizarin

There are two possible biogenetic pathways of these compounds.[22,23]

a. Acetate-Malonate route. Birch and his colleagues and Gatenbeck carried out labelling experiments with (^{14}C)-acetate in order to establish the acetate derivation of helminthosporin, emodin, islandicin and cynodontin.[24,25,26,27] Additional confirmation was obtained by Gatenbeck using ^{14}C, or ^{18}C-acetate as precursor.[26] Later investigation 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); this was confirmed in the case of islandicin,[27] and the bianthraquinone rugulosin.[28] All the fungal anthraquinones are structurally consistent with their

formation by the acetate-malonate pathway. It seems reasonable to conclude that this is so. Typical fungal anthraquinones such as emodin and chrysophanol are also found in higher plants, therefore it is possible to assume that they are formed in the same way. The isolation of anthrone physcion from cultures of several *Aspergilli*[29] and from bark of *Ventilago maderaspatana* Roxb.[30] (Rhamnaceae) gave support to this view.

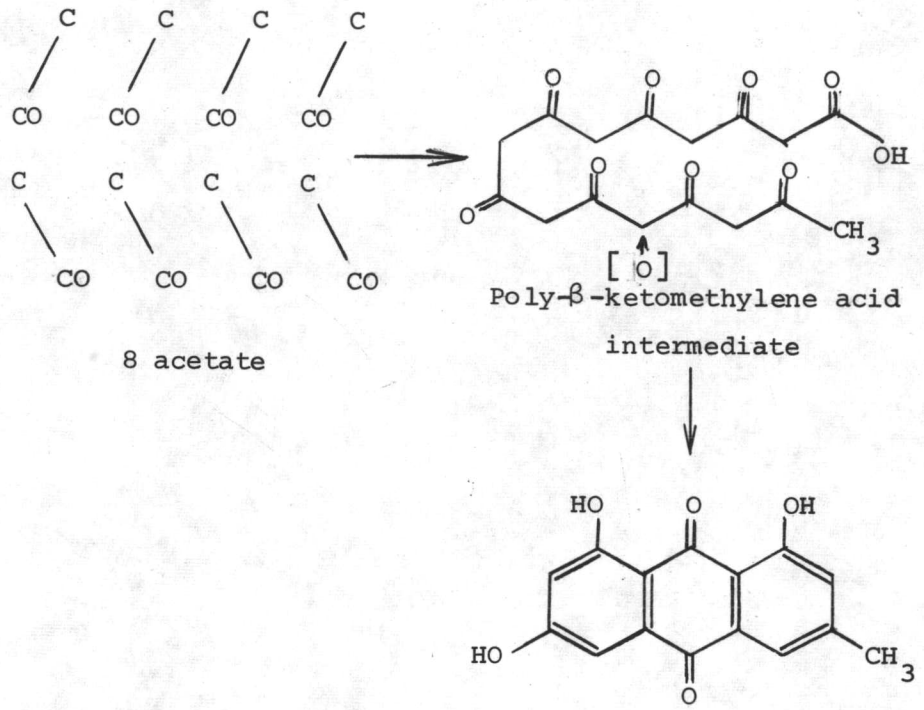


Physcion

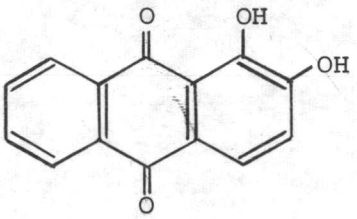
Leistner and Zenk[31] published a paper proving that chrysophanol is produced in *Rumex alpinus* Linn. via the acetate-malonate route. Fairbairn and Muhtadi fed radioactive acetate to *Rumex obtusifolius* Linn. plants and after seven days, aloe-emodin, chrysophanol and emodin were isolated and found to be radioactive.[32] Each was degraded to phthalic acid and the proportion of radioactivity present in the acids shown to be consistent with the acetate-malonate route earlier established for a related species of *Rumex*.[32] The majority of the anthraquinones which are assumed to be elaborated by the acetate-malonate route are conformable to emodin pattern. They are arised by suitable folding and condensation of a polyketide chain derived from eight acetate units. Resulting of O-methylation, side-chain oxidation,

chlorination, dimerisation and the introduction or omission of nuclear hydroxyl groups exist to numerous variations of the basic structure.[6]

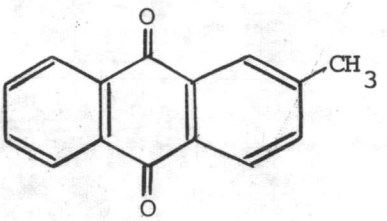
Scheme a Acetate-Malonate Route



b. Shikimate and Mevalonate route. Anthraquinones which have biosynthesis route via shikimate and mevalonate route are only found in higher plants. The majority of them occur in the Rubiaceae sub-family Rubioideae and to a lesser extent in the Bignoniaceae and Verbenaceae.[33,34,35] They are substituted in only one benzenoid ring (ring C) and are devoid of a carbon side chain or hydroxyl group e.g. alizarin, tectoquinone.



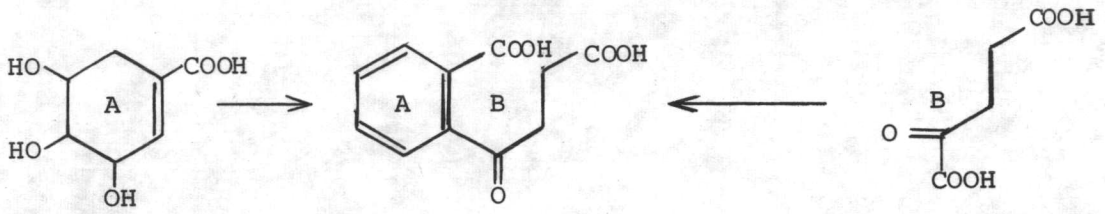
Alizarin



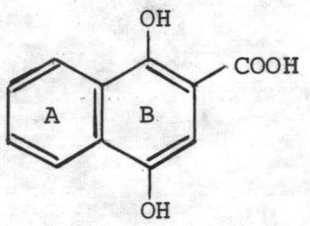
Tectoquinone

Significantly the anthraquinones present in Bignoniaceae[33,34] and Verbenaceae[35] are accompanied by C_{15} naphthaquinone notably deoxylapachol while the Rubiaceous plants contain a number of this type of compounds too.[36] The 4-methoxy-1-naphthol has also been found in Rubiaceae. These finding suggested that deoxylapachol is synthesised in vivo by prenylation of a naphthol precursor and followed by oxidation. Deoxylapachol can also be converted into tectoquinone in vitro either by boron trifluoride catalysis or by irradiation.[33] It seems likely that substituted ring C in this group of anthraquinone is derived from mevalonate. This was shown by feeding *Rubia tinctorum* Linn. plants with 2-^{14}C-mevalonate. Four radioactive pigments, rubiadin, pseudopurpurin, alizarin and purpurin were isolated.[37,38,39] All of these compounds have only substitution in ring C. Therefore it seems that ring C of the anthraquinones in Rubiaceous plants is formed as shown in scheme b and supposedly the same route is followed in Bignoniaceae and Verbenaceae.

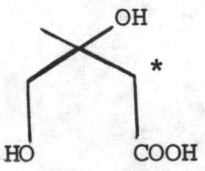
Scheme b Shikimate and Mevalonate Route



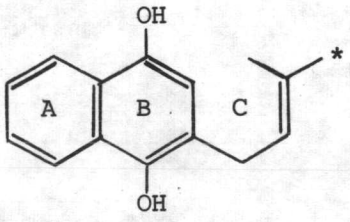
Shikimic acid O-Succinylbenzoic acid Glutamic acid



intermediate 1,4-dihydroxy-2-naphthoic acid

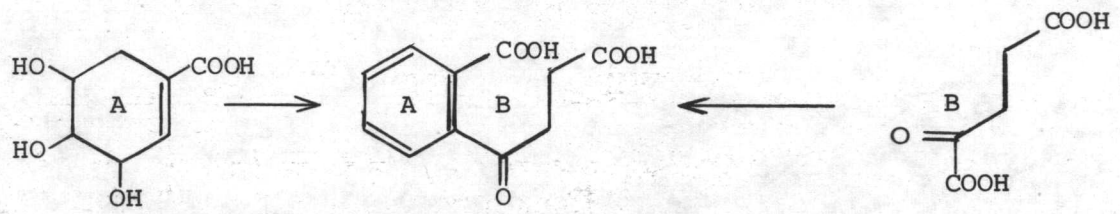


Mevalonic acid

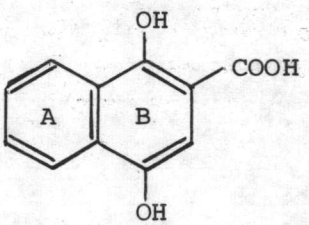


intermediate

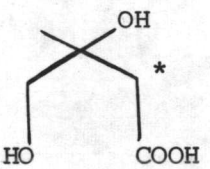
Scheme b Shikimate and Mevalonate Route



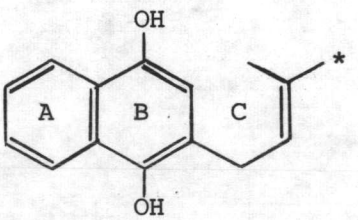
Shikimic acid O-Succinylbenzoic acid Glutamic acid



intermediate 1,4-dihydroxy-2-naphthoic acid



Mevalonic acid



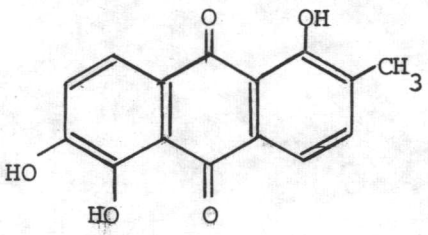
intermediate

The ring A of alizarin is derived from shikimic acid,[40] thus confirming the previous result.[41] The distribution of radioactivity in alizarin molecule after feeding of carboxyl ^{14}C-D shikimic acid was determined by degradation of the alizarin dimethylether which yielded benzoic acid and veratric acid.[42] The result of this degradation showed that the carboxyl groups of shikimic acid exclusively incorporated into C atom of alizarin. Campbell[43] 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[44] 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[45,46] 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.

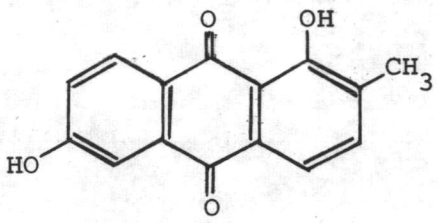
Recent work by Leistner[47] has shown the biosynthesis of alizarin in *Rubia tinctorum* Linn. by using tracer technique. Specific incorporation of labels from carboxyl-^{14}C-D shikimic acid, 2-^{14}C-D

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 phrephenic 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 and soranjidiol anthraquinones of *Morinda citrifolia* Linn. are hydroxylated in both ring A and ring C.



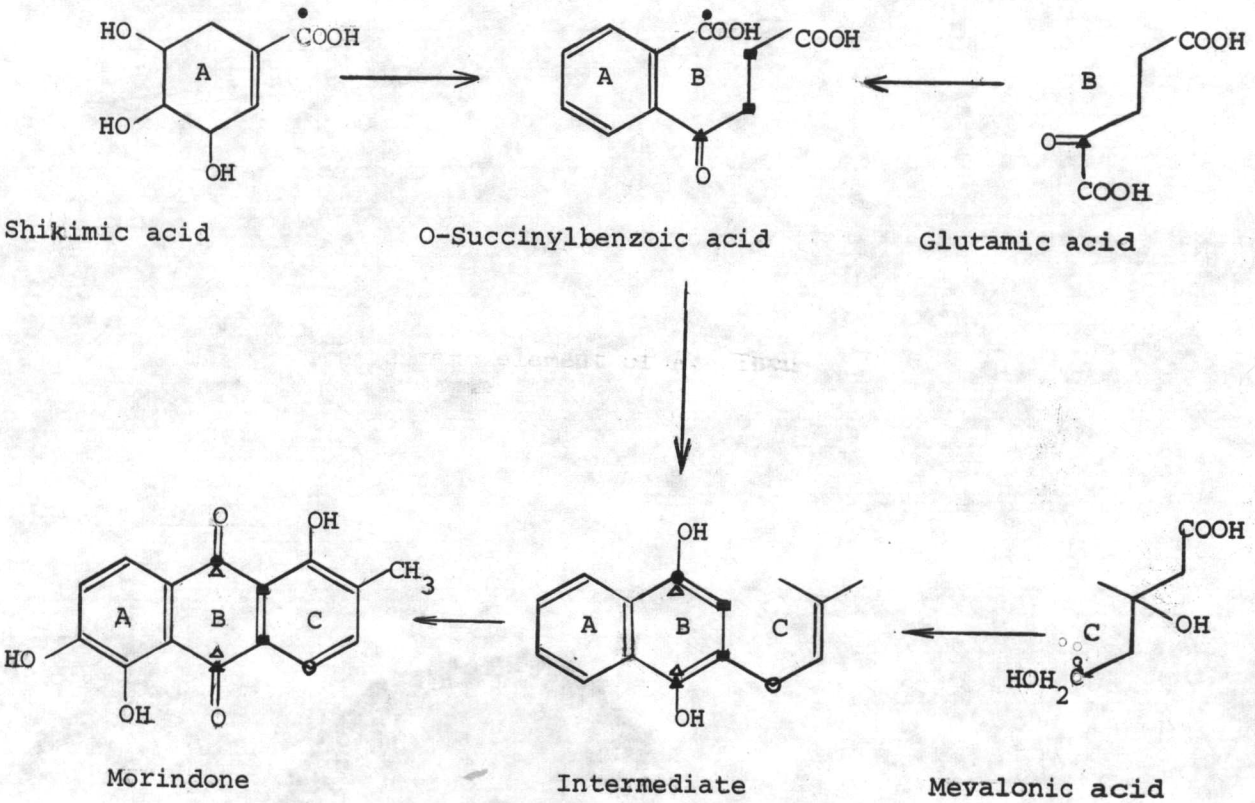
Morindone



Soranjidiol

Experiments carried out by Leistner[48] showed that anthraquinones in morindone are derived from shikimic acid via O-succinylbenzoic acid as the same biosynthetic pathway as alizarin. The hydroxy groups attached to ring A are introduced at the latter stage of biosynthesis and are not derived from hydroxy groups of shikimic acid (Scheme c)

Scheme c Migration of radioactivity from different precursors to morindone



▲ α-Ketoglutaric acid

■ O-Succinylbenzoic acid

o Mevalonic acid

△ 2-(γ,γ-Dimethylallyl)-naphthoquinone

● Shikimic acid

4. Anthraquinones previously isolated from *Cassia spp.*

Cassia acutifolia Delile (Alexandria Senna)

Cassia angustifolia Vahl (Indian Senna)

Plant part	Anthraquinone	Reference
leaf, fruit	Aloe-emodin Chrysophanol Physcion Rhein Rheum-emodin Sennoside A Sennoside B Sennoside C Sennoside D	49,50

Cassia alata Linn. f.

Plant part	Anthraquinone	Reference
leaf	Chrysophanol Rhein	51

Cassia fistula Linn.

Plant part	Anthraquinone	Reference
pulp	Rhein	52
	Sennoside A	
	Sennoside B	
flower	Fistulin (bianthraquinone glycoside)	53
fruit	Fistulic acid (1,4-dihydroxy-6,7-dimethoxy-2-methyl-anthraquinone)-3-carboxylic acid	54

Cassia javanica Linn.

Plant part	Anthraquinone	Reference
leaf	Aloe-emodin	55
	Chrysophanol	
	Rhein	
	Glucoside of Rhein	

Cassia marylandica Linn.

Plant part	Anthraquinone	Reference
flower	Chrysophanic acid	56
	Physcion	
	Glucoside of Emodol	
	Glucofrangulin	

Cassia mimosoides Linn.

Plant part	Anthraquinone	Reference
leaf	Emodin	57
	Emodin glucoside	
	Luteolin-7-glucoside	
seed	Emodin	58
	Emodic acid	
	Physcion	
root	Physcion	58

Cassia obtusa Roxb.

Plant part	Anthraquinone	Reference
	Aloe-emodin Chrysophanol Emodin Emodin Rhamnoside Physcion Physcion glucosyl-rhamnoside	59

Cassia obtusifolia Linn.

Plant part	Anthraquinone	Reference
seed	Aurantio obtusin Chryso-obtusin Obtusifolin Obtusin	60

Cassia occidentalis Linn.

Plant part	Anthraquinone	Reference
	Islandicin	61
	Helminthosporin	
	Xanthorin	
	Physcion	62
	Emodin	
	Chrysophanol	
seed	Chrysophanol	63
	1,4,5-Trihydroxy-7-methoxy-3-methyl-anthraquinone	
flower	Physcion	64
	Emodin	
	Physcion-1-β-D-glucopyranoside	

Cassia podocarpa Guill.

Plant part	Anthraquinone	Reference
	Emodin	65

Cassia reticulata Willd.

Plant part	Anthraquinone	Reference
flower	Rhein Emodin	66

Cassia siamea Lam.

Plant part	Anthraquinone	Reference
bark	Cassiamin C	67
root bark	Cassiamin A Cassiamin B	68

Cassia sieberiana DC.

Plant part	Anthraquinone	Reference
leaf	Rhein Rhein-8-glucoside	69

Cassia sinqueana Delile

Plant part	Anthraquinone	Reference
root	Chrysophanic acid Physcion	70

Cassia sophera Linn.

Plant part	Anthraquinone	Reference
flower	Chrysophanol	71

Cassia tora Linn.

Plant part	Anthraquinone	Reference
seed	Chrysophanol Physcion Emodin Rubrofusarin-6-β- gentiobioside Chryso-obtusin	72 6

Cassia tora Linn. (continued)

Plant part	Anthraquinone	Reference
root	Chrysophanic acid -9-anthraquinone	73
	1,3,5-Trihydroxy-6,7-dimethoxy-2-methyl-anthraquinone	74