CHAPTER 2

HISTORICAL

1. Botanical Aspect of *Cassia angustifolia* Vahl.

Cassia angustifolia Vahl (Fig. 1) or commonly known as Indian or Tinnevelly senna is in the family of Leguminosae and subfamily of Caesalpinoidae. Its local name in various countries are Sennesblatter (German); Sene de Tinnevelly (French) (Youngken, H.W. 1950.) Makhaam-Khaek (Thai) (เต็ม สมิตินันท์, 2523) Bombay senna, Indian senna, Lotus senna, Medicinal senna, Tinnevelly senna (English) Bhuikhakhasa (Hindi); Siah Yip (Malaya); Sonachindi (Persian); Bhumiari (Sanskrit); Kattunilavirai (Tamil); (Kirtikar K.B. 1975).

The name "Senna" is from the Arabic "sena", the native name of the drug; "Cassia" is from the Hebrew qetsiah, meaning to cut off, and refers to the fact that the bark of some of the species was once peeled off and used; "angustifolia" is Latin and refers to the narrow-leaved.

Cassia angustifolia is indigenous to Somaliland, Arabia peninsula, Sind and the Punjab, and

Fig. 1 *Cassia angustifolia* Vahl

Thai name : Ma-khaam-khaek

a. flowering branch

b. inflorescence

c. floret

d. pod

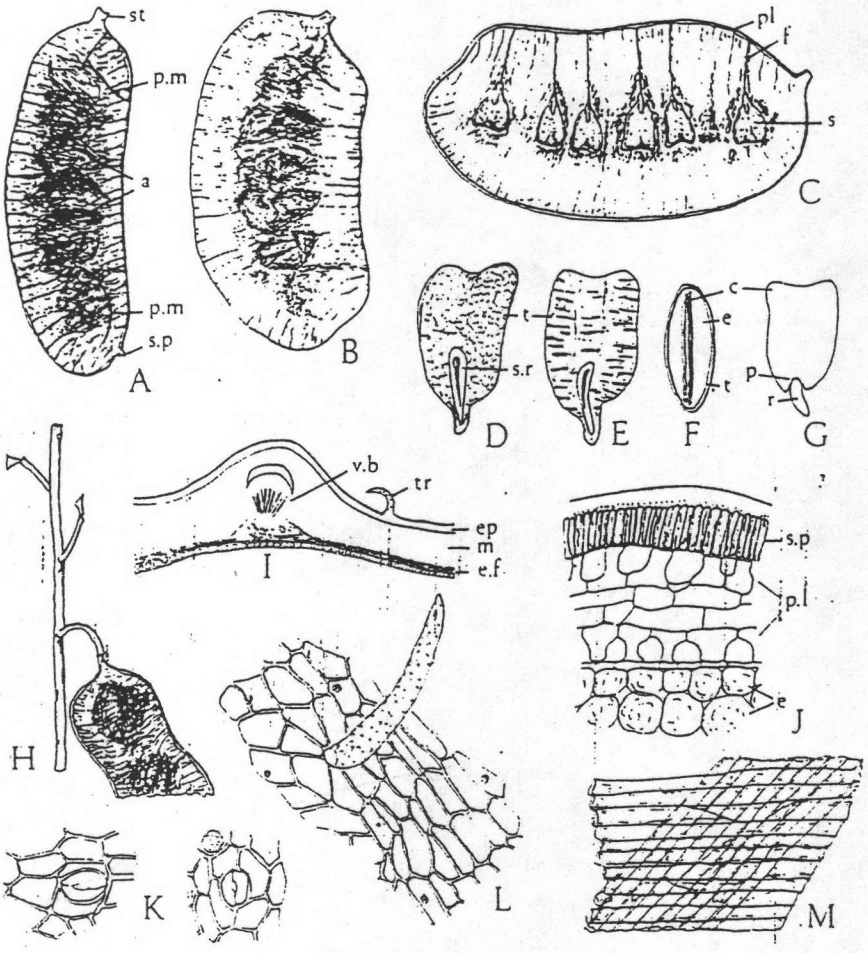
e. seed (After Aimon, *et al.*, 1986)

is cultivated in Southern India (Tinnevelly) (Trease and Evans, 1989; Claus, E.P. 1949; Youngken, 1950). It was introduced into Thailand in 1977 and is now cultivated in some provinces of the central region such as Lop buri and Saraburi (Aimon, S. *et al.* 1986). This plant is erect shrub which is up to 1.5 m in height. The stems are white and branched. The leaves are compound leaves which are stipulate and paripinnate with 10-16 oblong-lanceolate, acute, pale green leaflet. The size of leaflet is about 0.7 cm wide and 3 cm. long. The inflorescence flowers are showy in terminal or subterminal racemes with distingly peduncles. The flowers contain 5 glabrous, very obtuse and imbricated calyxes; 5 pale yellow and imbricate corollas; 10 unequal stamens; stalked ovary which contain many ovules; incurved style and terminal stigma. The bracts are membranous. The pod is thin narrowly, oblong, raniform, glabrous, short-stalked and flexible. The size of pod is about 1 cm wide and 5 cm long and sutures very thin which contained 6-12 seeds. The seeds are obovate, compressed (Kirtikar,1935). Cotyledons are plane. The medicinally important parts of *C. angustifolia* are the leaves and pods.

Senna is cultivated on wet lands resembling rice paddies; in fact, rice is often one crop of the season, and senna is a later crop of the same season. The poor grades of senna are grown on dry land without irrigation.

Senna is graded according to the size of the leaf and the color of the leaflets : blue-green leaves are best, yellowish leaves are poorest (Tyler V.E. 1988). *C. angustifolia* may be brown either on dry land or in wetter conditions as a successor to rice. Being a legume, it usually adds nitrogen to the soil. After soaking in water the pod are readily opened and about 6-12 wedge-shaped seed are disclosed, each attached to the dorsal surface of the pod by a thin funicle (Fig. 2 C). Under a lens the testas of the Tinnevelly show a general reticulation only (Fig. 2 D,E). The pericarp of the pod bears unicellular hairs and stomata of a type similar to those found on senna leaves; portions of the fibrous layer of endocarp are vary evident in the powder (Fig. 2 K,L,M)

The name "Senna" can be refered to two species of *Cassia spp.*, one is *C. angustifolia* Vahl which described above and the other is *C. senna* L. (*C. acutifolia* Delile). The name "acutifolia" is Latin and refers to sharply pointed leaflets. Most of chemical components in both species are the same. For macroscopical characters. the leaves of *C. acutifolia* are pale green but the leaves of *C. angustifolia* are yellowish-green and more elongated and narrow and there are some difference between the leaves of these two species as shown in Table 1.



(After Aimon, *et al.*, 1986)

Fig. 2 Senna fruits. A, Tinnevelly fruit; B, Alexandrian fruit; C, Alexandrian pod opened to show seeds (all x 1); D, seed of Alexandrian fruit; E, seed of Tinnevelly fruit; F, transverse section of seed; G, isolated embryo with one cotyledon removed (all x 4); H, stem with Tinnevelly fruit attached (x1); I, transverse section of pericarp (x90); I, transverse section of seed coat; K, fragments of epidermis with stomata; L, fragment of epidermis with trichome; M, Fibrous layers from endocarp in surface view (all x 100). a, brown areas of pericarp covering seeds; c, cotyledons; e, endosperm; e.f, fibrous endocarp; ep, epicarp; f, funiculus; m, mesocarp; p, plumule; pl, placenta; p.l, parenchymatous layers of testa; p.m, press marks from other pods; r, radicle; s, seed; st, stalk; s.p, (j) subepidermal palisade; s.p, (A), stylar point; s.r, spathate ridge; tr, trichome; v.b, vascular bundle partially enclosed by fibres.

In 1753 Linneus gave the name *Cassia senna* to a North African plant and recently it has been shown that this is the earliest name for the species commonly called *C. acutifolia* Del., the source of Alexandrian senna (Andrews, 1952). Linneus in fact did refer to his species as "Cassia alexandrinus". Later Vahl (1790) distinguished a closely allied species from Arabia and named it *Cassia angustifolia* and this has long been recognized as the source of Tinnevelly senna. Bentham (1871) maintained this distinction basing his arguments mainly on the shape of the pods. Recently Brenan (1958) has stated that these distinguishing characters are quite inconstant and that there seems no reason for recognizing the two names, not even as varietal ones. He suggests they should both be referred to a *Cassia senna* L. and in fact the present B.P. 1963 and B.P.C. 1963 use this new nomenclature although the National Formulary (1965) still retains the older name. However pharmacognosists have long distinguished the two commercial varieties on the basis of pod and seed characters (Trease, 1931) and certain microscopical characters of the leaf (George, 1943; Rowson, 1943). In order to test the reliability of these charactes, as a basis for retaining the two varieties as separate species, it was examined a selection of each variety grown in as varied enviroments as possible. The examination of authenticated material grow in very varied conditions and herbarium material from Kew showed the surface markings of the testa and the stomatal index are

surprisingly constant for each variety and enable both to be satisfactorily distinguished. It is therefore concluded that the two varieties represent two valid species whose names should be *Cassia senna* L. for Alexandrian senna and *Cassia angustifolia* Vahl for Tinnevelly senna (Fairbrain and Shrestha, 1967).

C. acutifolia is known in commerce as Alexandria senna growing wild in Somaliland, Arabia and India. Most of the commercial supply of the drug is collected from plants cultivated in southern India (Tinnevelly). Alexandria Senna is harvested in April and in September by cutting off the tops of the plants about 6 inches above the ground and drying them in the sun, after which the stems and pods are separated from the leaflets by mean of the sieves. Tinnevelly senna is gathered by hand and dried in the sun. The senna which has passed through the sieves is then "tossed" in shallow trey. The leaves working to the surface and heavier stalk fragments and sand sinking to the bottom. The leaves are then graded, partly by means of sieves and partly by hand-picking into 1. whole leaves, 2. whole leaves and half-leaves mixed, and 3. siftings (Trease and Evan 1989;Claus, E.P. 1949).

The whole leaves are those usually sold to the public, while the other grades are used for making galenicals. Alexandria Senna drug is packed, somewhat loosely, in bale and sent by rail to Port Sudan where it

is conveyed either to Port Said, Cairo, Alexandria or Red sea. Cairo is the trading market for Alexandria senna. About 75 percent of that reachs this country by ship, the other 25 percent comes indirectly through London. Tinnevelly senna is exported from Tuticorin, Madras and Calcutta and is shipped to the United States directly. The product arrives in bales packed under pressure and burlap covered.

Tinnevelly senna has been cultivated successfully in California and this state produces some of the present supply (Youngken, H.W. 1950). The Tinnevelly variety is more largely used, although the Alexandrian is said to be more highly esteemed (Claus, E.P. 1949). The pods of Tinnevelly senna are longer and narrow than the Alexandrian senna and the brown area of pericarp surrounding the seeds is greater. The remain of the style are distinct in the Tinnevelly but not in Alexandrian. Other characters of these two species are summarized in Table 1.

Table 1 Comparison of Alexandrian and Tinnevelly senna leaves

Alexandrian senna	Tinnevelly senna
Macroscopical characters	
- Seldom exceed 40 mm in length	- Seldom exceed 50 mm in length
- Greyish-green	- Yellowish-green
- More asymmetric at base	- Less asymmetric at base
- Rather more broken and curled at the edges	- Seldom broken and usually flat owing to compression
- Few press markings	- Often shows impressions due to the mid-vein of other leaflets
Microscopical characters	
- Hairs more numerous, the average distance between each being about three epidermal cells	- Hairs less numerous, the average distance between each being about six epidermal cells
- Most of the stomata have two subsidiary cells only	- The stomata having two or three subsidiary cells respectively are in the ratio of about 7 to 3
- Vein-islet number 25-29.5	- Vein-islet number 19.5-22.5
- Stomatal index 11.4-13.0	- Stomata index 17.1-20.0

Table 1 (Continued)

Alexandrian senna	Tinnevelly senna
Chemical tests	
- Ether extract of hydrolysed acid solution of drug gives with methanolic magnesium acetate solution: a pink colour in daylight, a pale greenish-orange in filtered ultraviolet light	- The same test give : an orange colour in daylight, a yellowish-green in filtered ultraviolet light
TLC test for distinctive naphthalene derivatives	
- 6-Hydroxymusizin glycoside present	- Tinnevellin glycoside present

2. Microscopical of *Cassia angustifolia* Pods

2.1 Anatomy and Histology

Transverse sections of the pericarp of the pods show the following tissue passing from periphery toward the center (Fig. 3a)

1. Epidermis, of cuticularized rectangular cells with occasional stomata and few unicellular warty-walled trichomes

2. The outer parenchymatous layers, consisting of polygonal cells, some containing calcium oxalate prisms; scattering sclereids and vascular bundles
3. Sclerenchymatous layers, consisting of 1-2 layers of fibers crossing one another obliquely
4. The inner parenchymatous layers, consisting of 2-4 layers of thin-walled parenchymatous cells.

2.2 Powdered Drug

The powder is yellowish brown with a faint odor and a bitter taste. The diagonostic characters are (Fig. 3b)

1. Abundant fragments of interlacing fibers, sometimes with adhering sclereids
2. Abundant sclereids of various sizes and shapes.
3. Fragments of groups of fibers with and without calcium oxalate prism sheath
4. Conical, unicellular and warty-walled trichomes
5. Some fragments of the outermost layer of pericarp with polygonal cells, occasional paracytic or anomocytic stomata and cicatrices
6. Some fragments of parenchymatous cells from pericarp

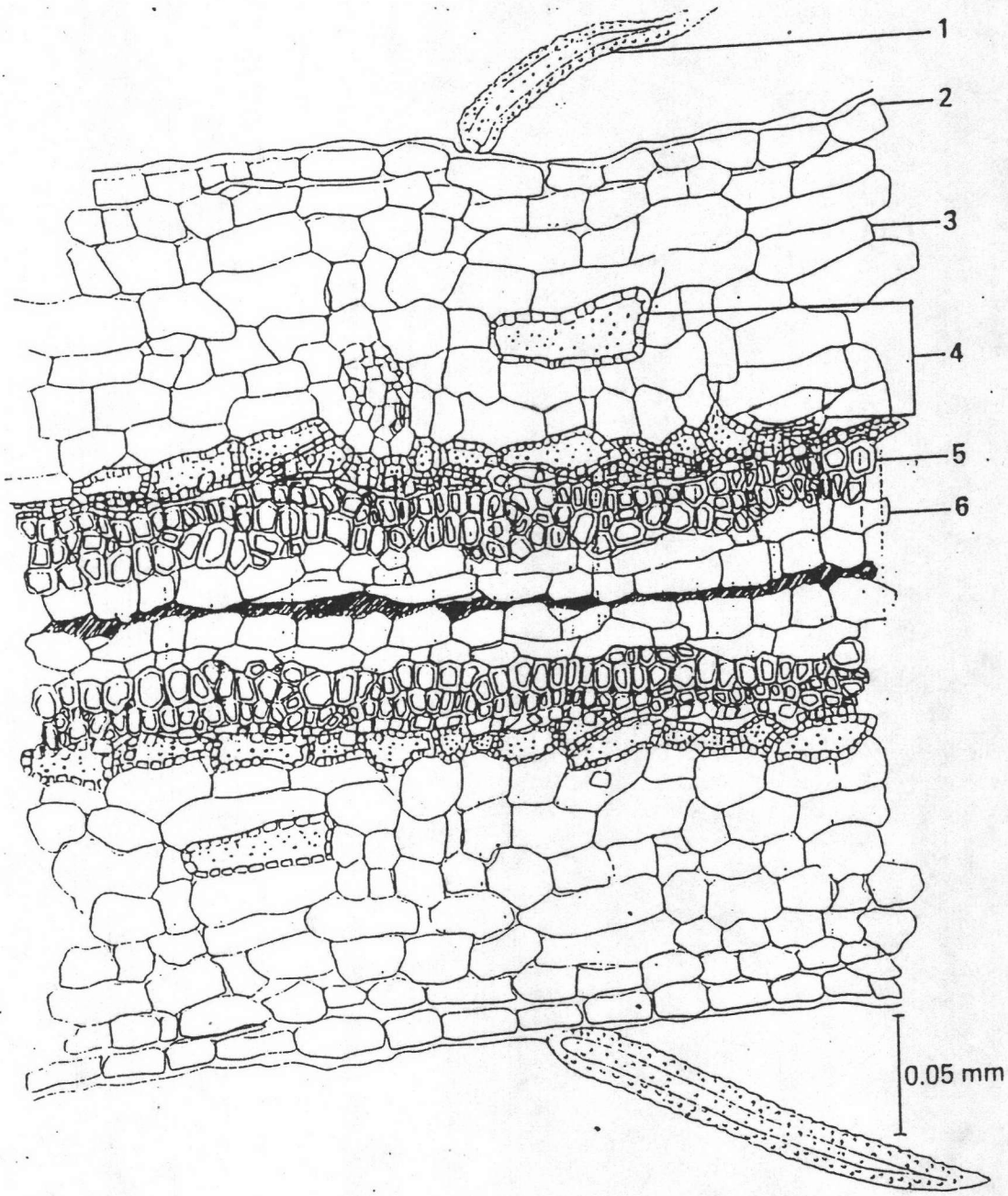


Fig. 3a Transverse Section of *Cassia angustifolia* Vahl Pod

1. trichome
2. epidermis
3. parencymatous layers with sclereids and vascular bundles
4. sclereids
5. layer of interlacing fibers
6. parenchyma (After Aimon, *et al.*, 1986)

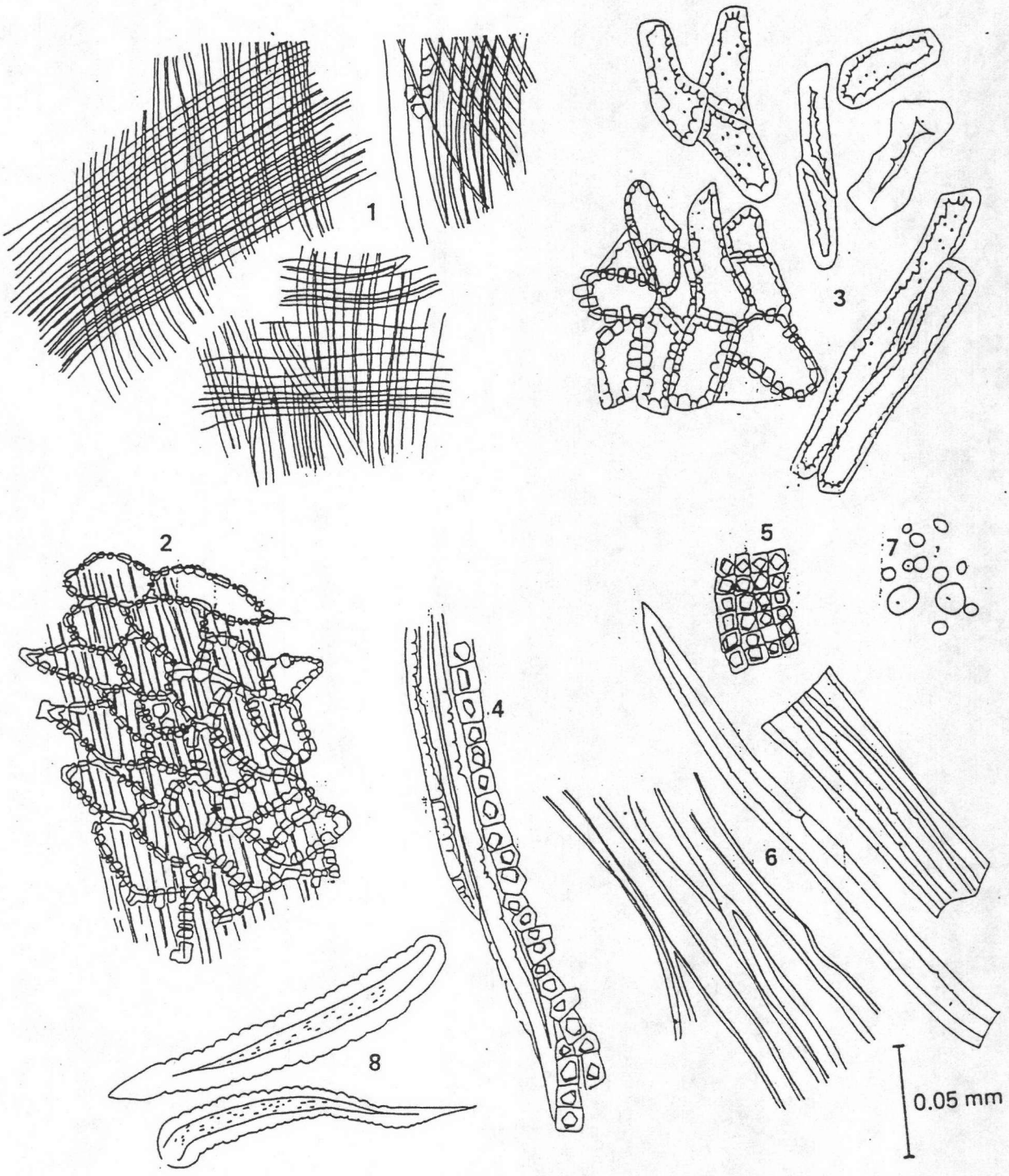


Fig. 3b Powdered *Cassia angustifolia* Vahl Pod

1. fragments of interlacing fibers
2. part of sclerenchymatous layers of pericarp showing a layer of sclereids with adhering fibers
3. sclereids
4. part of a group of fibers with calcium oxalate prism sheath
5. part of calcium oxalate prism sheath
6. parts of groups of fibers
7. starch granules
8. covering trichomes

(After Aimon, *et al.*, 1986)

7. Some fragments of the seed coat in surface and sectional views showing small epidermal cells with thick cuticle, non-lignified, columnar sclereids and thick-walled parenchymatous cells containing oil droplet
8. Some fragments of the cotyledons in sectional view showing the epidermis, palisade and spongy mesophyll
9. Few starch granules

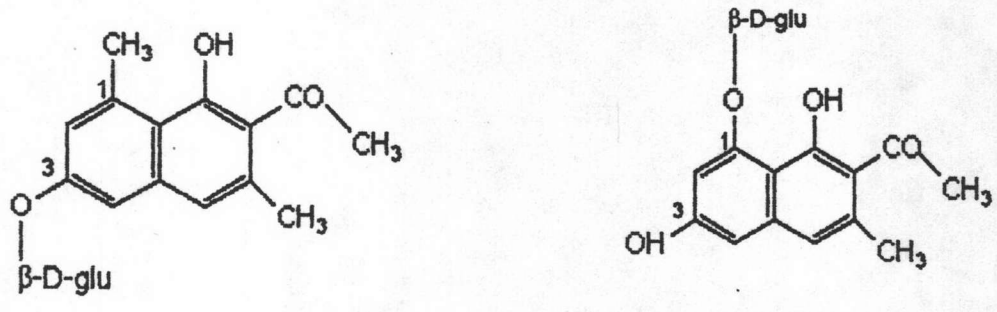
3. Chemical Constituents of *Cassia angustifolia* Vahl

3.1 Chemical Constituents in Various Plant Parts

There have been many reports on the chemical constituents of *C. angustifolia*. Sennoside A and sennoside B are believed to be the chief laxative principles of the plant (Stoll *et al.*, 1941). Other anthraquinones such as aloe-emodin, rhein (occuring both free state and combined glycoside form), coloring matters such as kaempferol, kaempferin and isorhamnetin, and phytosterol glycoside, myricyl-alcohol, mucilage, resin, starch etc. are also present in this plant.

Sennoside A and sennoside B can be both hydrolyzed to give two molecules of glucose and the aglycones, sennidin A and sennidin B. Sennidin A is dextrorotation and B is its mesoform formed by intramolecular compensation. This activity has not been fully explained by the isolation of these constituents. Later work notably by Fairbairn, Friedrich, Friedmann, Lemli and their associates (Friedrich *et al.*, 1973), has demonstrated the presence of many other (some pharmacologically active) components. These includes sennoside C and D which are the glycosides of heterodianthrones involving rhein and aloe-emodin; palmidin A; aloe-emodin dianthrone-diglycoside, rhein-anthrone-8-glycoside, rhein-8-diglucoside, aloe-emodin-8-glucoside, aloe-emodin- anthrone-diglucoside, possibly

rhein-1-glucose, and a primary glycoside having greater potency than sennosides A and B and distinguished from them by the addition of two glucose molecules. Two naphthalene glycosides isolated from senna leaves and pods are 6-hydroxymusizin glucoside and Tinnevellin glucoside (Lemli *et al.*, 1981) (Fig. 4). The former is found in Alexandria senna and the latter is in Tinnevelly senna. This difference has been use as a distinguishing feather of the commercial varieties (Table 1). List of compounds found in various parts of *Cassia angustifolia* are shown in Table 2. The active constituents of the pods are located in the pericarp. The seed contain very little sennoside. Zenk's group has reported that the cotyledon of 3-day-old seedlings contain sennosides in the amounts equivalent to those in the leaves (Zenk, Atzorn and Weiler, 1981). Sennoside content varies from about 1.2-2.5%. Preparations of the powdered pericarp e.g. Senna Tablet BP, standardized in term of sennoside B, is now commonly prescribed.



Tinnevellin glycoside

6-Hydroxymusizin glycoside

Fig. 4 Structure of Tinnevellin Glycoside in Tinnevelly Senna and 6-Hydroxymusizin Glycoside in Alexandria Senna

Table 2 List of Compounds Found in Various Parts of *Cassia angustifolia* Vahl.

Plant part	Chemical Substance	Reference
Root	Sennoside B	Singh and Rao, 1982
	Chrysophanol	Kalashnikova, 1983
	Physcion	Kalashnikova, 1983
	Emodin	Kalashnikova, 1983
	Aloe-emodin	Kalashnikova, 1983
	Rhein	Kalashnikova, 1983
	Chrysophanein	Kalashnikova, 1983
	Physcionin	Kalashnikova, 1983
	Glucoaloe-emodin	Kalashnikova, 1983
	Emodin 8-O-β-glucopyranoside	Kalashnikova, 1983
	Glucorein	Kalashnikova, 1983
	Sennidin C	Kalashnikova, 1983
Stem	Sennoside B	Singh and Rao, 1982
Whole stem	Sennoside A	Lemli and Cuveele, 1975
	Sennoside B	Lemli and Cuveele, 1975
	D-Arabinose	Lemli *et al.*, 1976
	D-Fructose	Lemli *et al.*, 1976
	D-Galactose	Lemli *et al.*, 1976
	D-Glucose	Lemli *et al.*, 1976
	D-Mannitol	Wojciechowski, 1937
	B-sitosterol	Lemli *et al.*, 1976

Table 2 (Continued)

Plant part	Chemical Substance	Reference
Whole stem	D- Rhamnose	Lemli *et al.*, 1976
	Rhein anthrone monoglucoside	Lemli and Cuveele, 1975
Leaf	Sennoside	ก้าวไปกับสมุนไพร, 2530
	Sennoside A	ก้าวไปกับสมุนไพร, 2530
	Sennoside B	Stahl and Brombeer, 1978
	Sennoside C	Stahl and Brombeer, 1978
	Sennoside D	Stahl and Brombeer, 1978
	Sennoside E	Ohshima and Takahashi, 1983
	Sennoside G	ก้าวไปกับสมุนไพร, 2530
	Anthrone glucosides	ก้าวไปกับสมุนไพร, 2530
	Dianthrones	ก้าวไปกับสมุนไพร, 2530
	Aloe emodin	Stahl and Brombeer, 1978
	Aloe emodin-8-O-β-D-glucoside	ก้าวไปกับสมุนไพร, 2530
	Chrysophanic acid	Stahl and Brombeer, 1978
	Emodin	Oshio *et al.*, 1978
	Emodin 8-O-β-D-glucoside	Oshio *et al.*, 1978

Table 2 (Continued)

Plant part	Chemical Substance	Reference
Leaf	Anthraquinone	ก้าวไปกับสมุนไพร, 2530
	Oxalic acid	ก้าวไปกับสมุนไพร, 2530
	Physcion	Stahl and Brombeer, 1978
	Physcionin	Stahl and Brombeer, 1978
	Isorhamnetin	ก้าวไปกับสมุนไพร, 2530
	Kaempferol	Murti *et al.*, 1940
	Rhein	Murti *et al.*, 1940
	Sennoside	ก้าวไปกับสมุนไพร, 2530
	Sennoside A	ก้าวไปกับสมุนไพร, 2530
	Sennoside B	Roder, 1982
	Sennoside C	Roder, 1982
	Sennoside D	Roder, 1982
	Tinnevellin glucoside	Lemli *et al.*, 1981
	Tinnevellin glycoside	Lemli *et al.*, 1983
	Aloe emodin anthrone diglucoside	ก้าวไปกับสมุนไพร, 2530
	Aloe emodin anthrone monoglucoside	ก้าวไปกับสมุนไพร, 2530
	Chrysophanol anthrone monoglucoside	ก้าวไปกับสมุนไพร, 2530
	Flavanol	Chopra, R.N; 1956

Table 2 (Continued)

Plant part	Chemical Substance	Reference
Leaf	Emodin anthrone monoglucoside	ก้าวไปกับสมุนไพร, 2530
	Physcion anthrone monoglucoside	ก้าวไปกับสมุนไพร, 2530
	Rhein anthrone diglucoside	ก้าวไปกับสมุนไพร, 2530
	Galacturonic acid	ก้าวไปกับสมุนไพร, 2530
	Sucrose	ก้าวไปกับสมุนไพร, 2530
	Cellulose	ก้าวไปกับสมุนไพร, 2530
	d-(+)-Tartaric acid	ก้าวไปกับสมุนไพร, 2530
	Pinitol	ก้าวไปกับสมุนไพร, 2530
	Elements	ก้าวไปกับสมุนไพร, 2530
flower	Sennoside B	Atzorn *et al.*, 1981
Pod	Sennoside A	Stahl and Brombeer, 1978
	Sennoside B	Stahl and Brombeer, 1978
	Sennoside C	Stahl and Brombeer, 1978
	Sennoside D	Stahl and Brombeer, 1978
	Aloe emodin	Stahl and Brombeer, 1978

Table 2 (Continued)

Plant part	Chemical Substance	Reference
	Chrysophanic acid	Stahl and Brombeer, 1978
	Physcion	Stahl and Brombeer, 1978
	Physcionin	Stahl and Brombeer, 1978
	Rhein	Stahl and Brombeer, 1978
	Rhein glucosides	ก้าวไปกับสมุนไพร, 2530
	Quinones	ก้าวไปกับสมุนไพร, 2530
	Anthraquinones	ก้าวไปกับสมุนไพร, 2530
Pod	Emodin anthrone monomethyl ether	ก้าวไปกับสมุนไพร, 2530
	Chrysophanic anthrone	ก้าวไปกับสมุนไพร, 2530
	Chrysarobin	ก้าวไปกับสมุนไพร, 2530
seed	Sennoside	Zenk, 1975
	Tinnevellin glucoside	ก้าวไปกับสมุนไพร, 2530
	Protein,	ก้าวไปกับสมุนไพร, 2530
	Fat and Fatty acid	ก้าวไปกับสมุนไพร, 2530
	Starch	ก้าวไปกับสมุนไพร, 2530
	Mucilages	ก้าวไปกับสมุนไพร, 2530

3.2 Formation and Distribution of Anthraquinone Derivatives in Senna.

In the young senna seedlings, chrysophanol is the first anthraquinone formed, then aloe-emodin appears and finally rhein. This antogenetic sequence is in keeping with the expected biogenetic order which involves the successive oxidation of the 3-methyl group of chrysophanol. In the presence of light glycosylation follows and later the glycosides are translocated to the leaves and flowers (Trease and Evan, 1978). During fruit development, the amounts of aloe-emodin glycoside and rhein glycoside fall markedly, and sennosides accumulated in the pericarp (Trease and Evan, 1978). Fresh leaves of *Cassia acutifolia* contain anthrone glycosides only. By drying between 20 and 50°C these are enzymatically converted to dianthrone forms (sennosides) (Lemli and Cuveele, 1978). However, sennoside formation is not entirely an artifact arising through drying but that these compounds, together with the monoanthrones, and their oxidized forms (anthraquinones) are part of a redox system of possible significance to the living cell. (Zenk, 1981).

It has been reported that the distribution of sennoside B in a flowering *C. angustifolia* plant (sample dried at 60°C) was flower 4.3%, leaves 2.8%, pericarp 2.4%, stem 0.2%, root 0.05%. Within the flowers the anthers and filaments contained 7.2%, carpels and

ovaries 5.8%, petal 5.2%, sepal 4.7% and flower stalks 3.2% (Zenk, Weiler and Atzorn). Alexandrin senna fruit (Alexandrin senna pods) of the BP is the dried fruit of *Cassia acutifolia* containing not less than 3.4% of hydroxyanthracene glycoside calculated as sennoside B. Tinnevelly senna fruit (Tinnevelly senna pods) of the B.P. and European Pharmacopoeias is the dried fruit of *C. angustifolia* containing not less than 2.2% of hydroxyanthracene glycoside calculate as sennoside B. Senna leaf (B.P., European Pharmacopoeia) is the dried leaflets of *Cassia acutifolia* or of *Cassia angustifolia* or a mixture of both species containing not less than 2.5% of hydroxyanthracene glycoside calculate as sennoside B.

3.3 The Uses of *Cassia angustifolia*

Cassia angustifolia has been used since the ninth or tenth century, its introduction into medicine being due to the Arabian physicians, who used both the leaves and the pods. The plant is useful in the treatment of constipation, loss of appetite, liver complaints, abdominal troubles, splenic enlargements, dyspepsia, typhoid, jaundice, anaemia, leprosy, poisoning symptoms, foul breath bronchitis, tumours.

Senna leaflets and pods are extensively used as a purgative. The purgative action is claimed to be due to the irritating effect of the anthraquinone derivative upon the muscles of the colon, producing increased

peristalsis. (Youngken H.W. 1950) It is useful purgative for either habitual constipution or occasional use. It lacks the astringent after-effect of rhubarb. Despite the availability of a number of synthetics, sennoside preperations remain among the most important pharmaceutical laxative. (Trease and Evans 1989).

The glycosides are hydrolysed by colonic bacteria (Reynolds, 1989) in the intestinal tract and the active anthraquinones liberated into the colon; the stomach and small intestine are not normally affected. Senna fruit may be administered in the form of an infusion, 4 to 12 pods being soaked in about 150 ml of warm water for about 12 hours, but standardised preparations of senna are now generally used. Since purgation occurs 8 to 10 hours after administration, senna preperation should preferably be given at bedtime. A concentrated senna extract, introduced into the lumen of the bowel through a colostomy, has no effect on the motility of the colon. The same extract incubated with faeces or a culture of *Escherichia coli* stimulated peristalsis in the colon. The activated senna appeared to act by contact stimulation of the submucosal nerve plexus which stimulated the deeper intramuscular plexus. Sublaxative doses of senokot were spasmolytic and might be of use in diverticular disorders of the elderly. The action of senna on the colon was probably due to stimulation of the myenteric plexus, direct stimulant activity and water and electrolyte

secretion. A comparison was made between 50 nursing mothers who were given senokot granules either in the morning or at bedtime on the first post-partum day with a control of 50 others who received liquid paraffin or a mixture of liquid parafiin with magnesium hydroxide mixture. The senna laxative was effective in 49 of 50 mother and their infants' bowel habits were not affected by the senna. Treatment with this form of standardised senna was more effective than the control treatment in overcoming puerperal constipation. The need for enamas of suppositories was almost entirely eliminated for a comparison of the laxative effects in antenatal patients and nursing mothers of a standardised senna preparation (Senokot) with other laxatives (Reynold, 1985).

3.4 Adverse Effects and Precaution

Damage to the myenteric plexus was found in a specimen taken from a middle-aged woman who had regularly taken purgatives; similar damage occurred in mice given senna for a few weeks. It was suggested that the pharmacological action of senna was on myenteric neurones and that they could be damaged by prolonged treatment with senna. Artificially high concentrations of urinary estrogens could be recorded in patients taking purgatives containing senna (Reynold, 1985)

4. Anthraquinones : The Chemistry and Distribution

4.1 Chemistry of Anthraquinones

Anthraquinone and the derivatives may be dihydroxyphenols (such as chrysophanol),trihydroxy phenols (such as emodin) or tetrahydroxy phenols (such as carminic acid). Othergroups are often present, for example, methyl in chrysophanol, hydroxymethyl in aloe-emodin and carboxyl in rhein and carminic acid. When such substances occur as glycosides, the sugar may be attached in various positions. See formulae for carminic acid and glucofrangulin. (Trease and Evans, 1989) Some examples of anthraquinone derivative are given in Table 3.

Anthraquinone itself has been obtained from several natural sources but was probably an artefact in all cases (Thomson, 1971). Most of the anthraquinone derivatives are red-yellow, orange-yellow or orange-red coloring compounds, varying from yellow to brown which may sometimes be observed *in situ* (e.g. in the medullary rays of rhubarb and cascara) (Trase and Evans, 1989). The anthraquinone crystals are high-melting point compounds, they are soluble in organic solvent and usually soluble in hot water or dilute alcohol. Anthraquinones containing a free carboxylic acid group (e.g. rhein) can be separated from other anthraquinones by extraction from an organic solution with sodium bicarbonate solution. (Trease and Evans, 1989). Anthraquinone composes of three benzene rings having the quinoid double bond. Most of the

naturally occurring anthraquinones are hydroxylated at the C-1 position.

The fundamental structure and the numbering system of anthraquinone is shown in Fig. 6.

The anthraquinones can be classified into two groups according to their biosynthetic pathways. The first group consists of anthraquinones with substitution in ring A and C. They are found in fungi and higher plants. In fungi, emodin (Fig. 5h), endocrocin and islandicin Fig. 5b have been found. In higher plants anthraquinones are distributed in Leguminoceae (Subfamily Caesalpiniaceae and Papilionaceae), Polygonaceae and Rhamnaceae e.g. emodin (Fig. 5h) aloe-emodin and chrysophanol (Fig. 7a). The other group consists of anthraquinone with substitution only in the ring C. They are found mainly in Bignoniaceae, Rubiaceae, Scrophulariaceae and Verbenaceae e.g. alizarin, lucidin and rubiadin (Robinson, 1967)

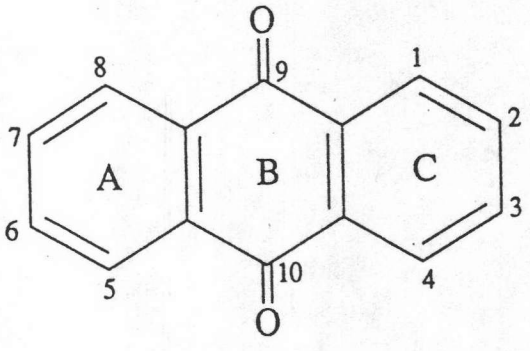
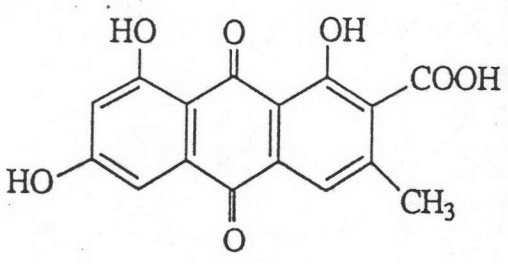


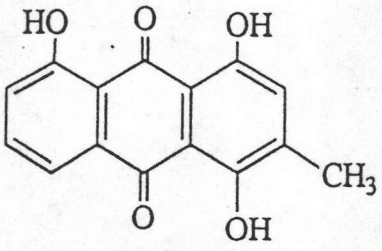
Fig. 5 The Structure and the Numbering System of Anthraquinones

Table. 3 Anthraquinone glycosides and aglycones

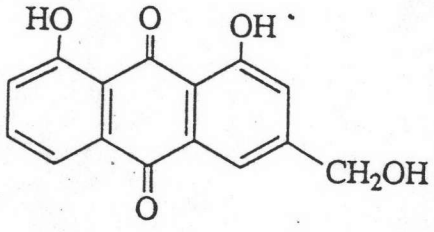
Glycoside	Aglycone	Sugar	Aglycone OH groups	Other group	Occurrence
Ruberythric acid	Alizarin	Primeverose	1,2	-	*Rubia tinctoria*
Rubiadin primeveroside	Rubiadin	Primeverose	1,3	2-methyl	*Rubia tinctoria*
Rubiadin glucoside	Rubiadin	Glucose	1,3	2-methyl	*Rubia tinctoria*
Chrysophanein	Chrysophanol	Glucose	1,8	3-methyl	*Rheum* and *Rumex* spp.
Rheochrysin	Physcion	Glucose	1,8	3-methyl, 6-methyl	*Rheum* spp.
Glucorhein	Rhein	Glucose	1,8	3-carboxylic acid	*Rheum*, *Rumex* and *Cassia* spp.
Gluco aloe-emodin	Aloe-emodin	Glucose	1,8	3-hydroxymethyl	*Rheum* and *Cassia* spp.
Glucochrysaron	Chrysarone	Glucose	1,2,7	6-methyl	*Rheum rhaponticum*
Glucofrangulin	Emodin	Glucose-rhamnose	1,3,8	6-methyl	*Rhamnus* spp.
Frangulin	Emodin	Rhamnose	1,3,8	6-methyl	*Rhamnus* spp.
Morindin	Morindone	Primeverose	1,5,6	2-methyl	*Morinda* spp. (Rubiaceae)
-	Islandicin	-	1,5,8	6-methyl	*Penicillium islandicum*
Carminic acid	-	Glucose	1,3,4,6	5-carboxylic acid 8-methyl	Cochineal



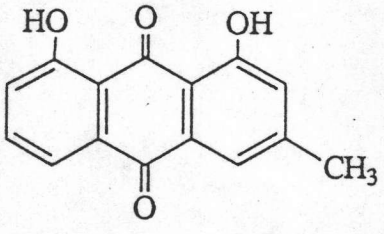
Endocrocin (a)



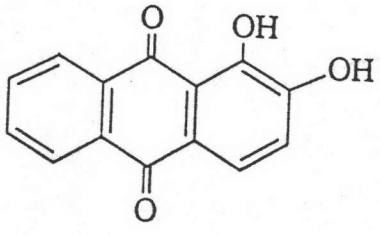
Islandicin (b)



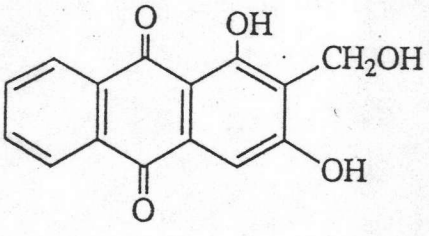
Aloe-emodin (c)



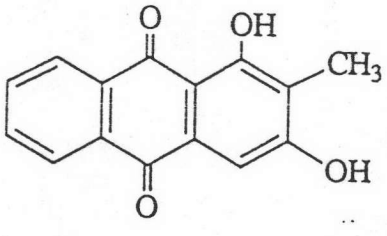
Chrysophanol (d)



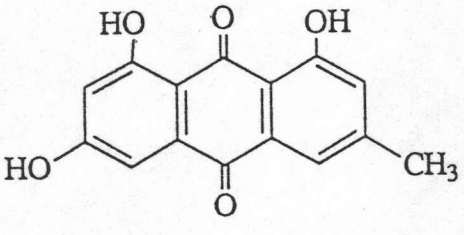
Alizarin (e)



Lucidin (f)

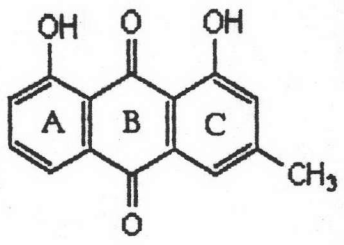


Rubiadin (g)

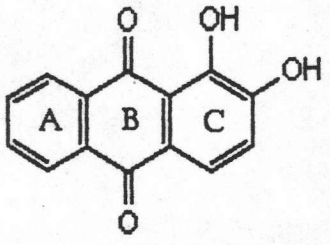


Emodin (h)

Fig. 6 Structures of some anthraquinones



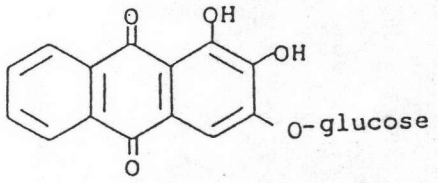
chrysophanol (a)



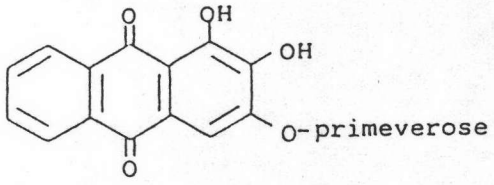
alizarin (b)

Fig. 7 The structure of chrysophanol and alizarin

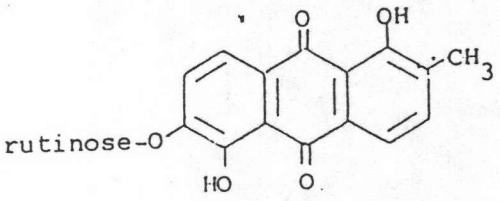
The anthraquinones actually existed in plants are apparently in several forms. They are often found as anthraquinone glycosides rather than hydroxylated anthraquinones or aglycones. Reports of the appearance of free anthraquinones must be regarded cautiously. Many anthraquinones occur as glycosides with the sugar residue linked through one of the phenolic hydroxyl groups. Several different sugars are found in such glycosides. For example, alizarin (Fig. 7b) occurs as a 3-glucoside (Fig. 8a) in *Rubia tinctorum* Linn. (madder) and as a 3-primeveroside (Fig. 8b) in *Galium* species; and morindone occurs as a 6-rutinoside (Fig. 8c) in *Coprosma australis* Robinson and as a 6-primeveroside in *Morinda persicaefolia* Ham. (Robinson, 1967).



Alizarin-3-glucoside (a)



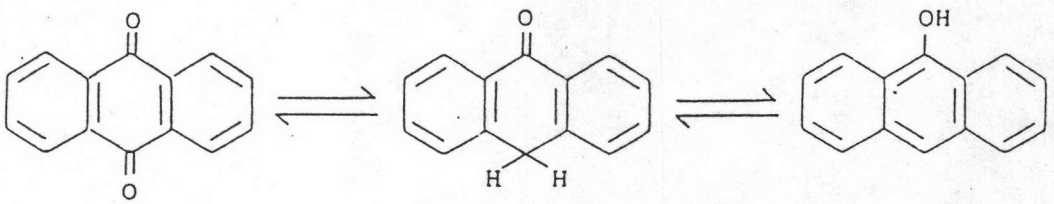
Alizarin-3-primeveroside (b)



Morindone-6-rutinoside (c)

Fig. 8 Some sugars found in anthraquinone glycoside

The aglycones of several anthraquinone glycosides may exist naturally in reduced forms, one of which is anthrone (Fig. 9b). The sugar in these reduced glycosides may be linked as usual through phenolic oxygens in the outside ring or they may be attached at C-9 of the enol form of anthrone, anthranol (Fig. 9c) (Robinson, 1967).



Anthraquinone (a) Anthrone (b) Anthranol (c)

Fig. 9 Various From of Anthraquinone

Enzymatic or chemical hydrolysis followed by oxidation of anthranol glycoside would give anthrone and anthraquinone. If the sugar is linked at some other positions except C-9 and C-10, anthranol glycosides may be directly oxidised to anthraquinone glycosides. Thus, the anthraquinone glycosides are divided 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).

A group of glycosides is known as the sennosides. They are the glycosides of sennidin dianthrone. Sennosides A and B are the 8,8'-diglucosides of rhein-rhein dianthrone, and sennosides C and D are the corresponding diglucosides of aloe-emodin-rhein dianthrone. The dianthrone carbon-carbon bond is liable to oxidative cleavage far more easily than the normal carbon-carbon bond. This is due to the stability of the resulting anthrone radical. Oxidation is carried out by using a free radical oxidising agent such as ferric chloride. (Fig. 10)

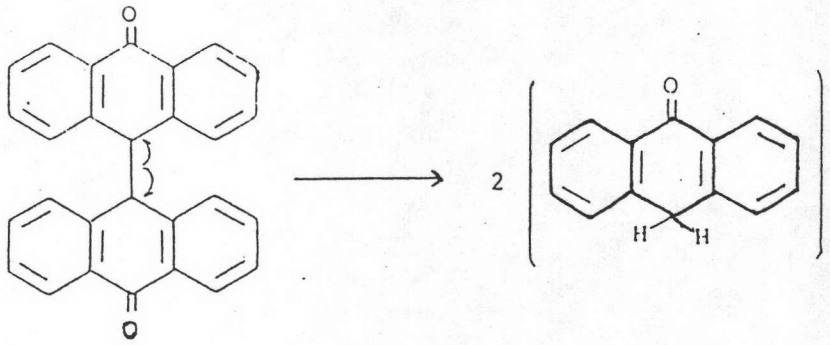
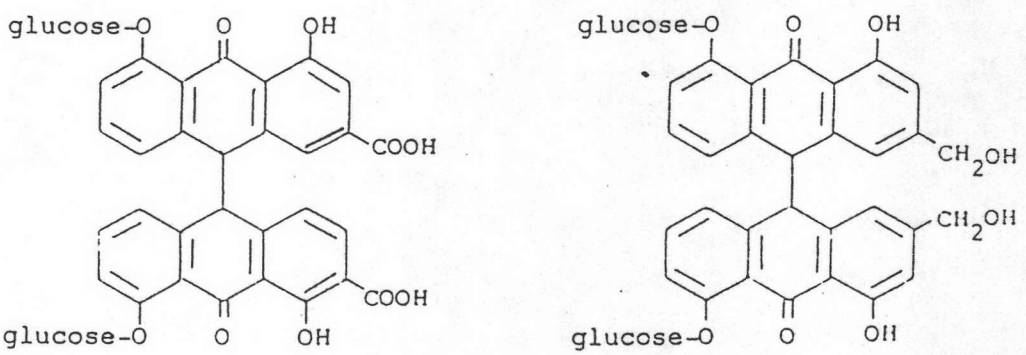


Fig. 10 Free Raical Oxidation of Dianthrone

The difference between the aglycone of sennosides A and B (Fig. 11a) is one of optical activity and the same relationship can be applied for the aglycones of sennoside C and D (Fig. 11b).



Sennoside A and B (a) Sennoside C and B (b)

Fig. 11 Structure of Sennosides

Anthraquinones can be detected by the Borntrager test in which an organic solution containing the test material is shaken with an aqueous base. In bases, 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 an a measure of the amount of anthraquinone in the original material. Only free anthraquinone give a positive Borntrager 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 (Ross and Brain, 1977). 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 an orange-red or pink colour, and those with two in para position e.g. quinizarin, produce a purple. These colour reactions are specific and stable.

The most important group 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 groups, either free, as methyl ethers, or as glycosides. The free anthraquinones or glycosides are ineffectivek, and the pharmacologically important compounds are free anthranols. Anthraquinones are active as cathartics only because they are reduced to anthranols

by intestinal bacteria (Robinson, 1967).

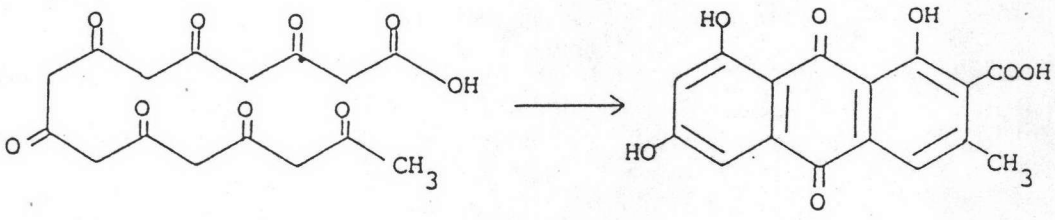
4.2 Biogenesis of Anthraquinone Compounds

Anthraquinones are derived from a few key intermediate, principally acetate, shikimate and mevalonate by a series of reactions which lead to the formation of benzenoid compounds, they arise by at least two biosynthetic routes. Fungal anthraquinones such as emodin (Fig. 5h) and chrysophanol (Fig. 7a) have structures in accord with the acetate hypothesis, while the anthraquinones found in higher plants, for example alizarin (Fig. 7b), have biosynthetic route *via* shikimate-mevalonate pathway.

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

4.2.1 Acetate-Malonate Pathway (Thomson, 1971)

The majority of the anthraquinones which are assumed to be elaborated by the acetate-malonate pathway is confined to emodin pattern. They are arised by suitable folding and condensation of a polyketide chain derived from eight acetate units. (Fig. 12) Numerous variations of this basic structure exist, resulting from O-methylation, side-chain oxidation, chlorination, dimerisation and the introduction or omission of nuclear hydroxyl groups, while in endocrocin (Fig. 5a) the terminal carboxyl group is retained.



Poly-β-ketomethylene compound (a) Endocrocin (b)

Fig. 12 Folding and Condensation of a Polyketide Chain

Emodin can be found in higher plants, it lacks only the carboxyl residue of endocrocin, a component of the lichen *Nephromopsis endocrocea* Asahina (Asahina and Fujikawa, 1935), but bears the same pattern of oxidation.

Bacterial and fungal anthraquinones have their formation *via* the acetate-malonate pathway. As such typical fungal anthraquinones as emodin and chrysophanol are also found in higher plants. Leistner and Zenk have proved that chrysophanol is produced in *Rumex alpinus* Linn. and *Rhamnus frangula* Linn. *via* the acetate-malonate route (Leistner and Zenk, 1969).

4.2.2 Shikimate-Mevalonate Pathway

Anthraquinones which are found in some higher plants especially in the order Tubiflorales have shikimate-mevalonate bio-synthetic route. They are substituted in only one benzenoid ring (ring C) and may be totally devoid of a carbon side chain or hydroxyl

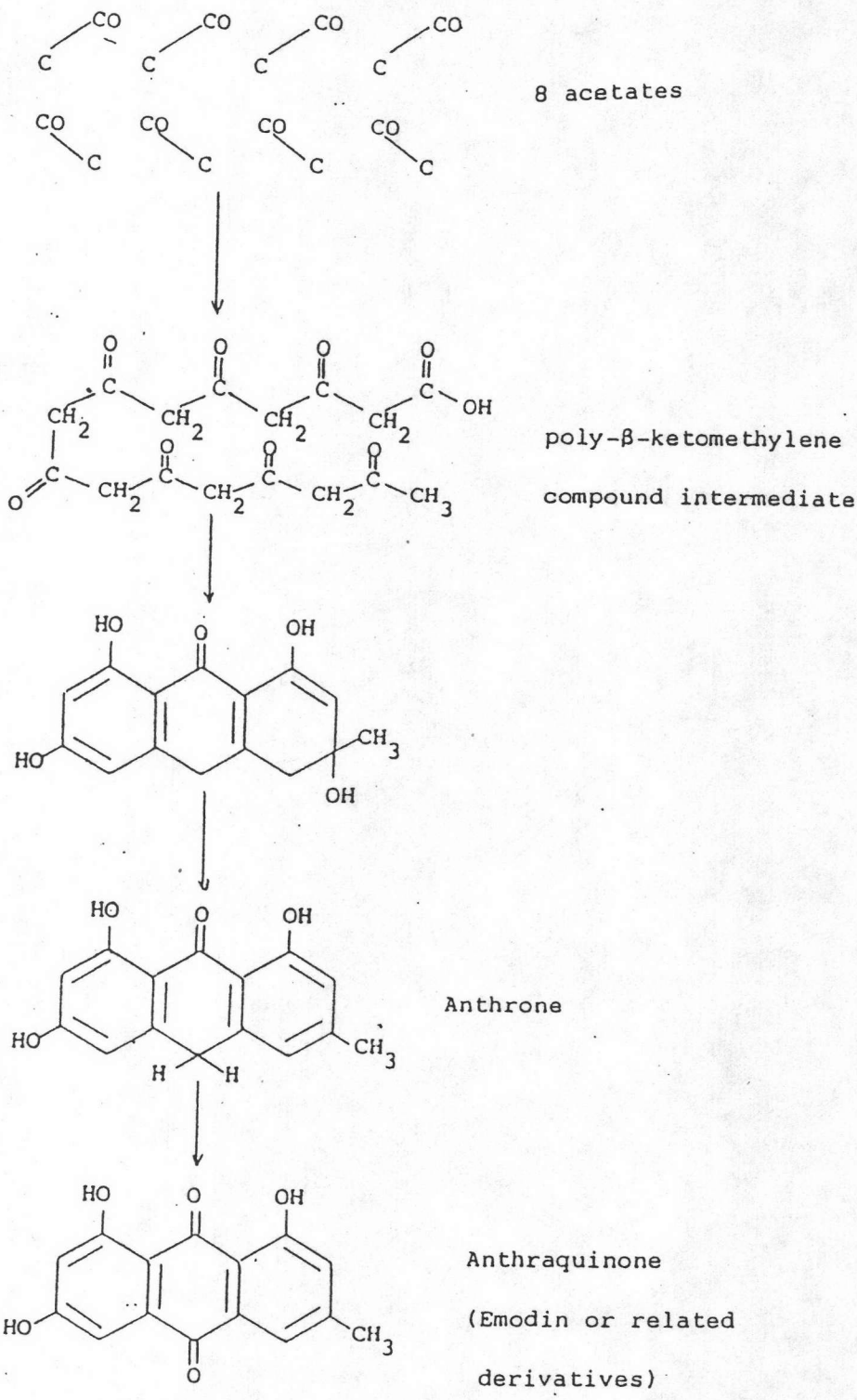
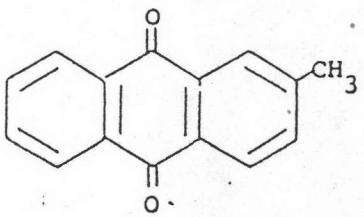


Fig. 13 Acetate-malonate pathway of anthraquinone.

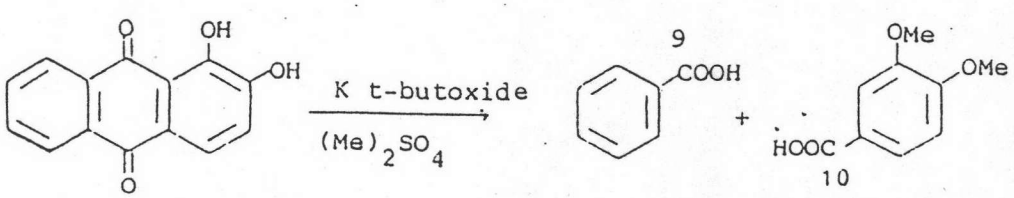
groups in ring A e.g. alizarin (Fig. 7b) and tectoquinone (Fig. 14). The majority of these anthraquinones occur in the Rubiaceae subfamily Rubioideae and, to a lesser extent, in the Bignoniaceae and Verbenaceae (Thomson, 1971).



Tectoquinone

Fig. 14 Structure of tectoquinone

Labelled precursors, carboxyl-14C-D-shikimic acid, in *Rubia tinctorum* Linn. roots, led to labelled alizarin. The distribution of radioactivity in the alizarin molecule was determined by degradation of the alizarin dimethylether which yielded benzoic acid (ring A plus C-atom 9) and veratric acid (ring C plus C-atom 10). The result of this degradation showed that the carboxyl group of shikimic acid is exclusively incorporated into C-atom 9 of alizarin.



(a) Benzoic acid (b) Veratric acid (c)

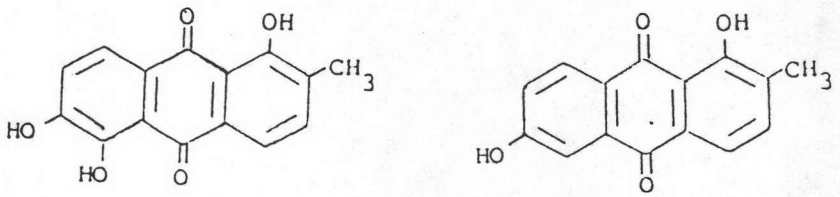
Fig. 15 Degradation of the alizarin dimethyl ether

After ^{14}C-2-glutamic acid feeding, it seemed that C-2 of glutamic acid give rise specifically to C-10 of naphthalene or alizarin anthraquinone. This naphthalene could be 1,4-dihydroxy-2-naphthoic acid which is linked to ɣ,ɣ-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 C-1 to C-4 are derived from ɣ,ɣ-dimethylallyl pyrophosphate. Decarboxylation and ring C closure would lead to anthraquinone such as alizarin (Leistner, 1973).

Leistner has shown the biosynthesis of alizarin in *Rubia tinctoria* 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 ß-keto-glutaric acid or derivative combines with shikimic acid, chorismic acid, or phrephenic acid to give o-succinylbenzoic acid which is then transformed to a nonsymetrical 1,4-naphthaquinone intermediate, and ɣ,ɣ-dimethylallyl pyrophosphate is then attached. Ring closure and further modification lead to alizarin (Leistner, 1973).

Morindone (Fig. 16a) and soranjidiol (Fig. 16b)

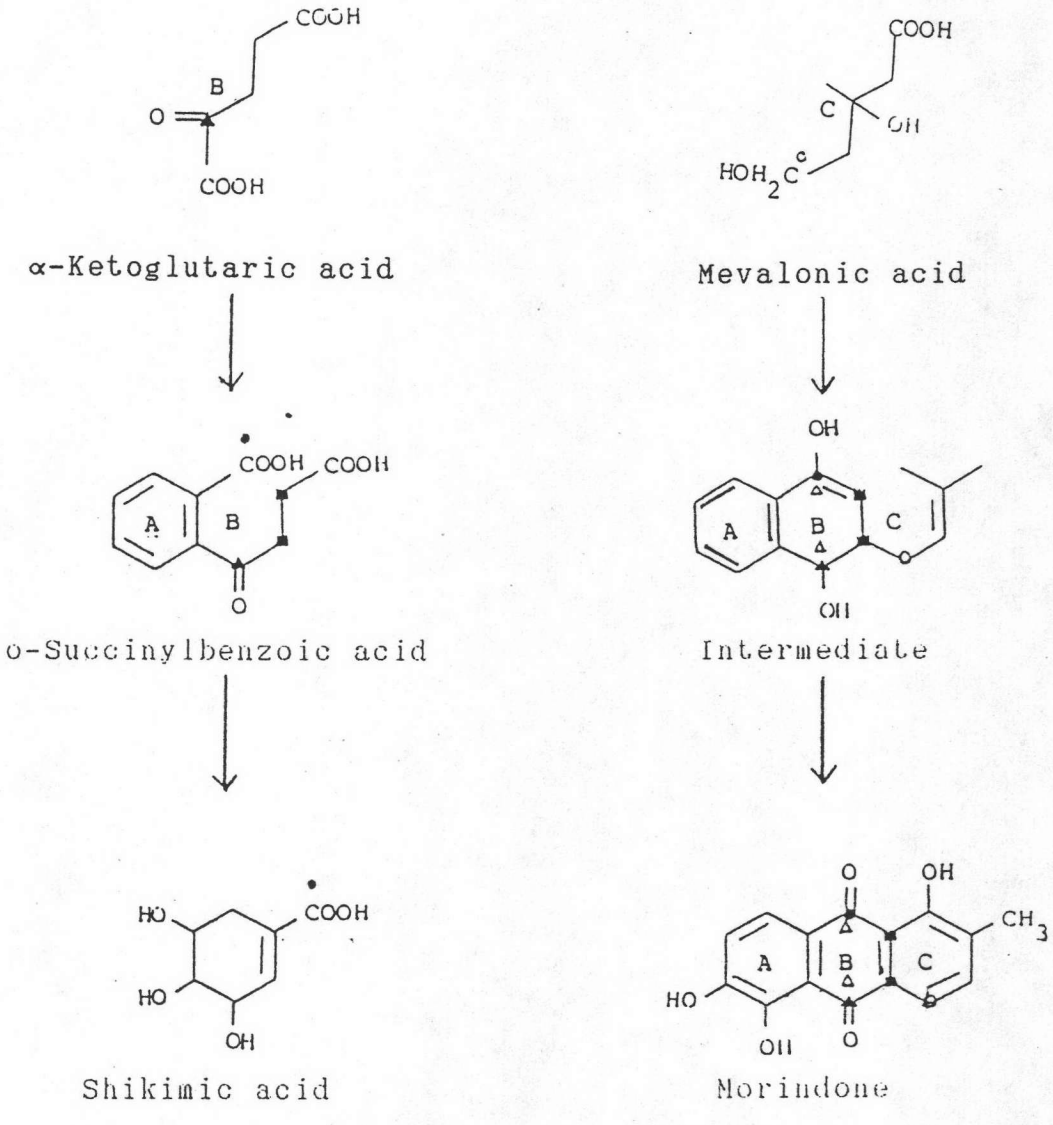
anthraquinones of *Morinda citrifolia* Linn. are hydroxylated in both ring A and ring C.



Morindone (a) Soranjidiol (b)

Fig. 16 Structure of Anthraquinones of *Morinda citrifolia* Linn.

Experiments carried out by Leistner (Leistner, 1973) showed that anthraquinones in morindone are 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 and are not derived from hydroxyl groups of shikimic acid.



▲ ^{14}C from α-Ketoglutaric acid

■ ^{14}C from o-Succinylbenzoic acid

● ^{14}C from Shikimic acid

O ^{14}C- from Mevalonic acid

Δ ^{14}C from 2-(γ,γ-Dimethylallyl)-naphthaquinone

Fig. 17 Migration of radioactivity from different precursors to morindone (Leistner, 1973)

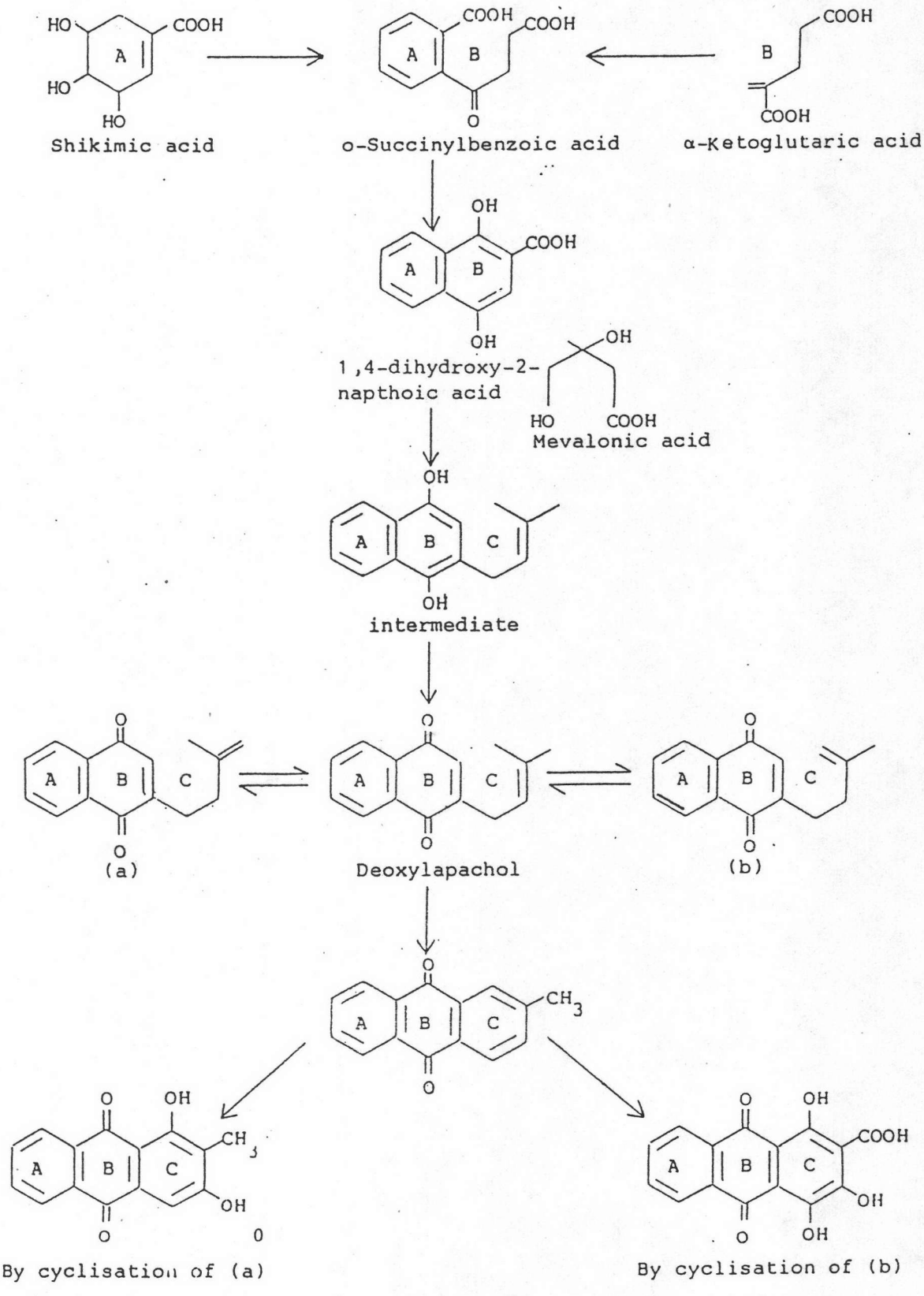


Fig. 18 Shikimate-Mevalonate Pathway of Anthraquinones.

4.3 Distribution of the Anthraquinones

Anthraquinones are the largest group of natural quinones. They are widely distributed in the plant kingdom as shown in Table 4 and some animal such as in insects (Coccidae only) and in feather star (Crinoidea). (Shibata, 1967). They are frequency yellow to red color because of its anthraquinone pigments. It was reported that the first appearance of anthraquinone was in bacteria (Thomson, 1971). In moulds, anthraquinones are mostly found in *Aspergillus* and *Penicillium.* More than twenty anthraquinones are related compounds have been isolated from *Penicillium isoindicum.* (Shibata, 1967). The distribution of anthraquinones in higher plants are mostly in the Rubiaceae, the account for half the total number. They are located chiefly in heartwood, bark and roots, sometimes they are in stem, seed and fruits. (Thomson, 1971). Anthraquinone 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* Moens (Rubiaceae) (Wijnsma et al., 1987). The bark of *Coprosma australis* Robinson contains 17% of its dry weight of anthraquinones. The root of *Rubia tinctoria* Linn. (madder) has been known to contain about twenty anthraquinones (Thomson, 1971). The other families also containing anthraquinone pigments are Rhamnaceae (*Rhamnus*,

Maesopsis, *Ventilago*), Polygonaceae (particularly *Rumex*, *Rheum* and *Polygonum*), Legumineceae, Subfamily Caesalpinoidae (*Cassia*), subfamily Papilionoidae (*Abrus*), Bignoniaceae (*Tabebuia avellanedae*), Verbenaceae (*Tectona grandis*) and Scrophulariaceae (*Digitalis* spp.). Anthraquinones are also found among monocotyledons especially in the Liliaceae and Xyridaceae. *Aloe, Asphodeline, Asphodelus, Bolbine, Enemerus and Polygonatum* represented the genera of the Liliaceae which contained anthraquinones, as well as *Xyris indica* Linn. and *X. semifuscata* Baker of the Xyridaceae. Some anthraquinones are found in Anacardiaceae Apocynaceae, Lythraceae, Rhizophoraceae, Saxifragaceae and Solanaceae.

The distribution of anthraquinone may be overlapping with the distributian of Naphthaquinone, [modern research indicate that the 1,8-dihydroxyanthraquinone derivatives frequently co-occur with 1,8-dihydroxynapthalene glycosides (Trease and Evans, 1989)] but this is only of significance in the Bignoniaceae and Verbenaceae where related naphthaquinones and anthraquinones co-exist in the same species.

Emodin is probably, the most widely distributed anthraquinone, being found in moulds, higher fungi, lichens, flowering plants (at least six families) and insects. The structural formular of emodin is shown in Fig. 6h.

Table 4 Distribution of Anthraquinone in some families.

Class (Division) Monocotyledones	
Subclass Monocotyledoneae	
Family	Genus
Liliaceae (Asphodeloideae)	*Aloe* *Asphodeline* *Asphodelus* *Bulbine* *Eremurus*
Class (Division) Dicotyledones	
Subclass Dicotyledoneae	
Family	Genus
Bignoniaceae Caesalpiniaceae Papilionaceae Polygonaceae	*Tabebuia* *Cassia* *Andira* *Polygonum* *Rheum*. *Rumex*

Table 4. (Continued)

Class (Division) Dicotyledones	
Subclass Dicotyledoneae	
Family	Genus
Rhamnaceae	*Maesopsis*
	Coprosma
	Damnacanthus
	Galium
	Hymenodictyon
	Morinda
Scrophulariaceae	*Digitalis*
Verbenaceae	*Tectona*

5. Sennosides

5.1 Chemistry of sennosides

Sennosides A and B ($C_{42}H_{38}O_{20}$ MW. 862.72) are dianthrone glycosides occured as the brownish powder, soluble 1 in 35 water, very slightly soluble in alcohol, chloroform and ether.The solubility increases tremendously in water misciable organic solvents containing in optimum of 30% w/w of water. Soluble in aqueous solution of sodium bicarbonate. Sennoside A can be slowly isomerized to sennoside B in $NaHCO_3$ solution at 80°C (Windholz, 1983). A 10% suspension in water has a pH of 6.3 to 7.3 (Windholz, 1983). Sennoside A is built up from the dextrorotatory aglycon sennidin A and D-glucose. Sennoside B is built up from the intramolecularly compensated meso-sennidin B and D-glucose (Windholz et al., 1983). Sennoside B has the same solubility characteristics as sennoside A, but is more soluble.

Sennosides are of medicinal interest because their strong laxative properties. Despite the availability of a number of synthetic laxatives, sennoside containing formulation are still among the most important pharmaceuticals of plant origin.

Sennosides can be extracted from a number of plant species. Among the commercial sources, *C. angustifolia* Vahl and *C. senna* Linn. are the most important.

Furthermore, sennosides have been extracted from *C. alata*, *C. fistula*, and *Rheum* species (Yasmin,1986). Sennosides alway be analysed in term of Sennoside B equivalent that included all sennosides. Not only sennoside A and B, there are also sennosides C, D, E and F.

5.2 Distribution

5.2.1 Occurrence of sennoside in *C. angustifolia*

Comparison of fresh and dry leaves from the same plant which was frozen in liquid nitrogen immediately after harvest and process as rapid as possible the fresh leaf extract gave 0.5 mg/g fresh weight and the dried sample gave 6 mg/g initial fresh weight of sennoside B-equivalents (Atzorn; 1981). In the fresh sample, only-8-glucosidorheinanthrone could be detected but in the dry leaf sample, both sennosides A and B were found. The amount of 8-glucosidorheinanthrone was almost indentical in both the dry leaf (0.32% base on initial fresh weight) and the fresh leaf sample (0.25%).

To study the relationship of sennoside content and drying process in more detail, two freshly harvested leaves of *Cassia angustifolia* Vahl were allowed to loose water gradually over a peroid of 14 days. Each day, one leaflet was removed from each leaf and extracted. Previous experiments had shown a homogeneous distribution of sennosides within the leaf when all leaflets were identically treated (Atzorn, 1981). Fig shows the levels

of sennosides steadily increased with progressing water loss. The maximum value reached, 5 mg sennoside B-equivalents per g. initial fresh weight after 14 days and 75% water loss, closely agrees with the levels in leaves dried rapidly at 60°C for 12 hrs. (water loss 80%, 6 mg/g initial fresh weight sennoside B) (Atzorn, 1981).

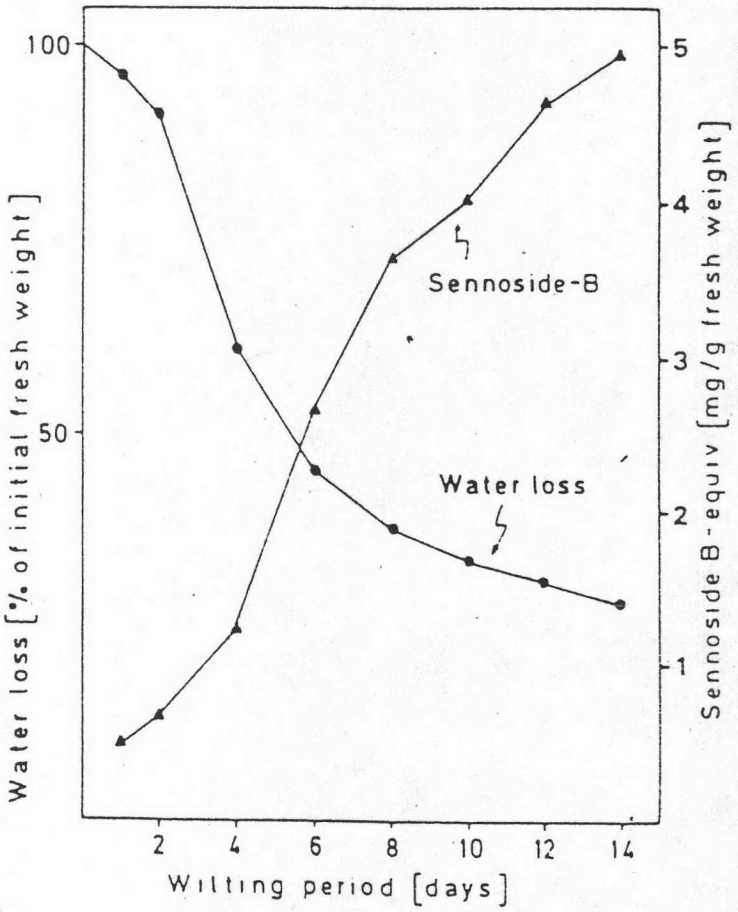


Fig. 19 Formation of sennoside B in relation to the Water-loss of leaves of *C. angustifolia*

5.2.2. Sennoside B formation during germination and early phase of development

Whereas sennoside B could not be extracted from dry, ungerminated seeds, the extractable amount per seedling steadily increased during germination and early growth (Fig. 20). When the radicula becomes visible, the first detectable traces of sennosides become apparent. This rise in sennoside closely parallels the dry weight increase of the seedling. The young plants, when the primary leaves are developed (day 17) already contain as much as 15 mg sennoside B/g dry weight, i.e. 50% of the average leaf concentration of a mature plant. After 27-32 days, average concentrations of the young plantlets have reached ca. 2% of dry weight. The distribution of sennoside B within the seedling (Fig. 20) show that even in the earliest stages analyzed (cotyledons not yet green, stage 3) the sennosides are located mainly in the leaves (cotyledons : 2-2.3% of dry weight), and only low concentrations are found in hypocotyl and root (0.12-0.18%). The cotyledons contain ca. 90% of the total sennoside B formed per seedling. Thus the build-up of the pool of sennoside precursors in the cell seems to be a rapid process linked to cell growth (Atzorn, 1981).

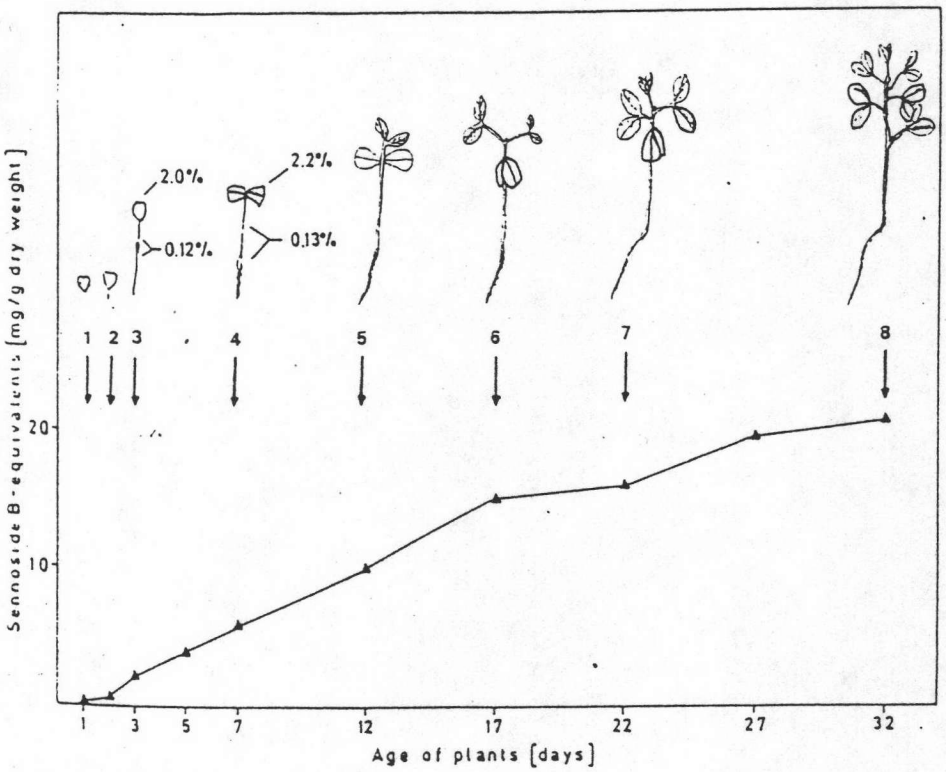


Fig. 20 Formation of sennoside B in germinating seeds and plantlets of *C. angustifolia*. For plantlet at day 3 and 7 of germination, the distribution of sennosides (on a % dry weight basis) within the plant is given. Sample were dried at 60°C overnight before extraction.

5.2.3. Distribution of sennoside B in a flowering *C. angustifolia* plants

All part of the plant were found to contain sennoside B after drying except for the ripe seeds which did not yield significant amounts of the compound (Atzorn, 1981). Highest amounts of sennosides could be extracted from the flowers of the plant (Fig. 21) and within the inflorescence, sennoside concentrations were found to increase in senescing flowers at early stage of

fruit development (Fig. 22). A fully developed flower (Table 5) showed highest sennoside levels in the carpels and in the anthers and stamina.

Table 5. Distribution of sennoside B within the flower of *Cassia angustifolia* Vahl Samples were taken after drying at 60°C overnight.

Part of flower	Sennoside B-equivalents (in % of dry weight)
Flower	3.2
Petals	5.3
Sepals	4.7
Carpels and ovary	5.8
Anthers and filaments	7.2

Whereas roots and stems show very little sennoside B, both leaves and fruits contain from 2-3% of the compound (fig. 21). Leaf samples from different plants, all gave between 2% and 2.9% of sennoside B- equivalents.

5.2.4. Variability of Sennoside B Extractable from Fruit of Individual Plants

The sennoside B in senna fruit was ranged from 1% to 4% of dry weight. (The average sennoside yield of fruit was 2.4% of dry weight (Fig. 23).

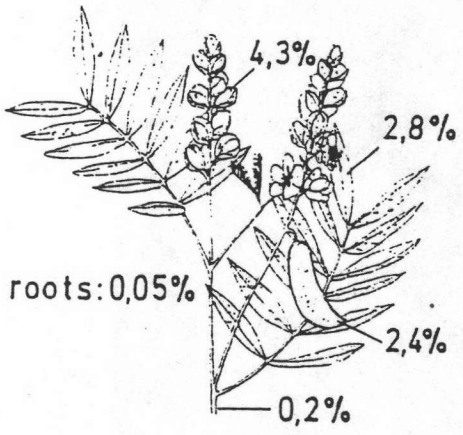


Fig. 21 Distribution of sennoside B (% dry wieght basis) in a flowering plant of *C. angustifolia*. Samples were dried at 60°C before extract.

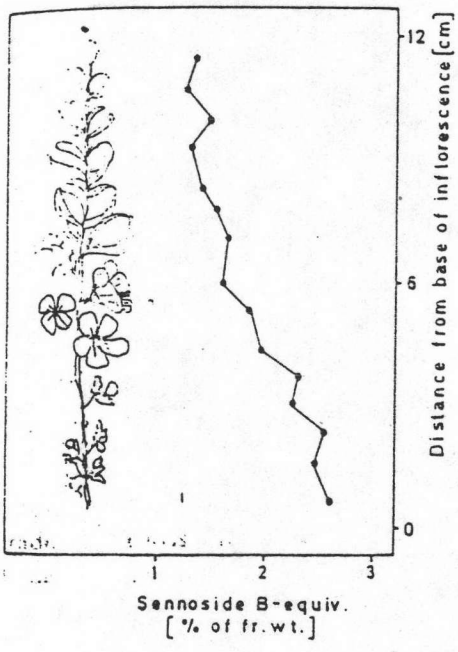


Fig. 22 Sennoside B distribution within the inflorescence of *C. angustifolia*. Sample were extracted fresh.

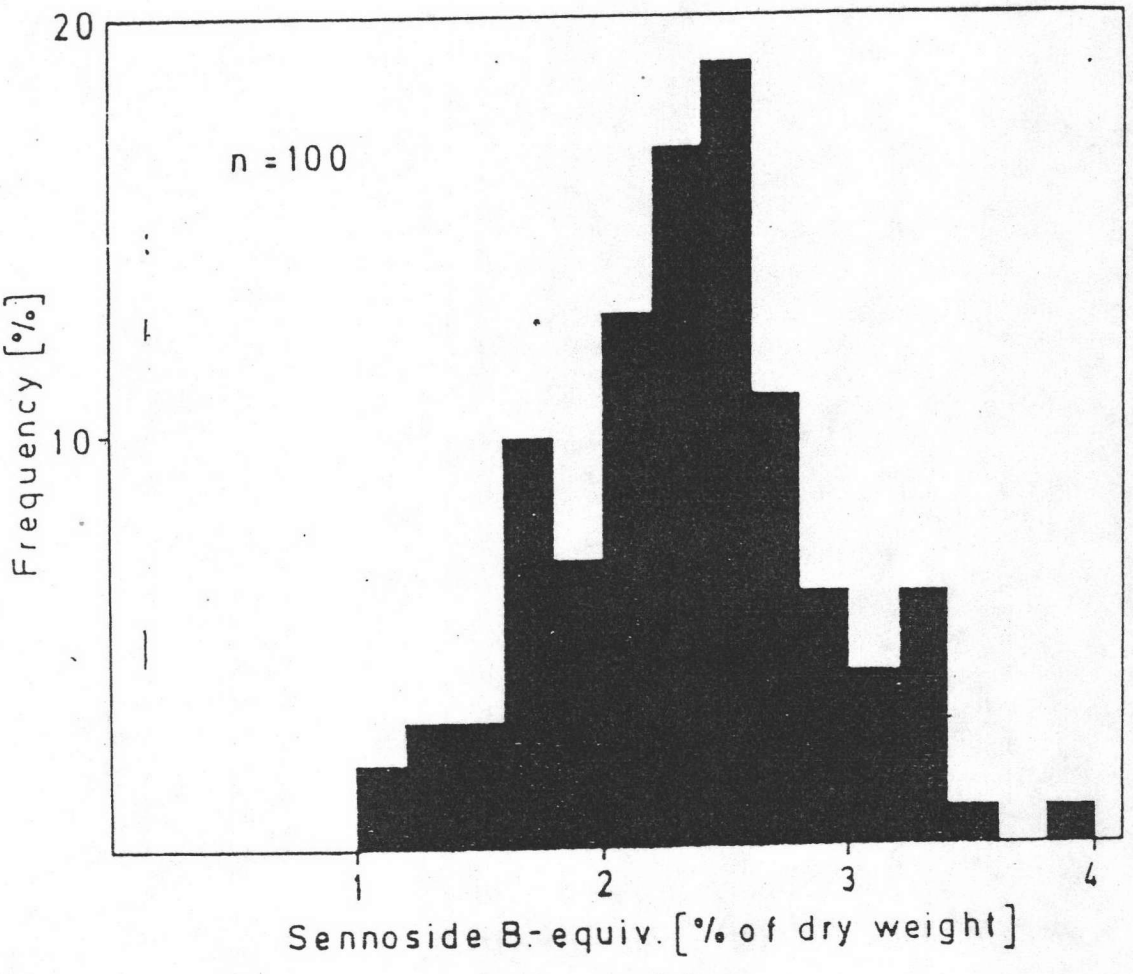


Fig. 23 Frequency distribution of sennoside B concentrations in fruits of *C. angustifolia* plants.

6. Plant Tissue and Cell Cultures as Source of Phytochemicals.

In plant tissue and cell cultures there are some reactions lead to the formation of compound unique to a few species or even to a single cultivation.These reaction are classified under the term secondary metabolism and their products know as "secondary metabolite" (Luckner and Nover, 1977). These substances include alkaloids, anthraquinone, antibiotic, volatile oil, glycoside, resin, tannin and saponins. These products appear to be important in the interactions between the plant and its environment (Harborne, 1982). Their functions may be in protecting the plant from predators, pathogens, environmental stress or may be related to the reproduction machinery of the plant in promoting pollination (Rhodes *et al.*, 1987; Charlwood and Rhodes, 1990). Many of these unique plant secondary product have found economic importance as medicines, fragrances, insecticides, food coloring and flavors. Because of their short supply some of these compounds and expensive and there is considerable interest in finding alternative source of supply which avoid dependence on the whole plant.

Since plant cells isolated from field grown plants and cultivated *in vitro* have the potential to produce and accumulate chemicals similar to those produced by the whole plant, plant cell cultures have been considered as

alternative sources to agricultural production. In the last decade numerous studies with callus and suspension culture from various plants have been published describing the formation, and sometimes accumulation in rather high concentration, of many difference secondary metabolites. The accumulation of these products in cell culture has often been compared to the concentration of these compounds in the intact plant (Table 6) and many cases have been reported in which the accumulation in cell cultures was considerably higher than in the intact plants (Vasil, 1987).

Although more than 30 years of research has been devoted to achieving the feasibility of cell cultures as source of plant constituents, only a few cases have been successful in term of economic benefits. One of the major problems is the low production of secondary metabolites under *in vitro* conditions (Narong Chomchalow and Oradee Sahavacharin, 1981). This is expectable since such constituents are present in very small amount even in intact plants. Another problem includes a decline in the amount in succeeding subcultures as reported in *Nicotiana* callus (Tabata and Noboru, 1976). The nicotine content in the cultures were rapidly decreased to trace amounts in succeeding subculture in association with the decline of the root regenerating activity. Thus the lack of specialised cell structures in some cultures may be another reason for the absence of accumulated secondary

Table 5. Examples of Secondary Metabolite Production by Plant Tissue Culture (Constabel, 1987)

Plant	Metabolite	Culture method	Content (%dwt)	Content in plant (%dwt)	Ratio of content (cell culture /plant)	Reporter
Papaver somniferum	Sanguinarine	Liquid	2.9	-	-	Eilert et al., 1985
Dioscorea deltoidea	Diosgenin	Liquid	2	2	1	Kaul and Staba, 1968
Coffea arabica	Caffeine	Agar	1.6	1.6	1	Frischknecht et al., 1977
Coptis japonica	Berberine	Agar	7.4	7	1	Fukui et al., 1982
Macleayava cordata	Protopin	Agar	0.4	0.32	1.25	Koblitz et al., 1975
Coptis japonica	Berberine	Liquid	13	-	2	Sato and Yamada, 1984
Catharanthus roseus	Ajmalicine	Liquid	1	0.3	3	Zenk et al., 1977
Coleus blumei	Rosmarinic acid	Liquid	15	3.6	5	Razzaque and Ellis, 1977
Panax ginseng	Ginsengoside	Agar	27	4.5	6	Furuya et al., 1983
Lithospermum erythrorhizon	Shikonin	Agar	12	1.5	8	Tabata et al., 1978
Morinda citrifolia	Anthraquinones	Liquid	18	2.5	8	Zenk et al., 1975
Lithospermum erythrorhizon	Shikonin	Liquid	14	1.5	9.3	Fujita and Tabata, 1986
Cassia tora	Anthraquinone	Agar	6	0.6	10	Tabata et al., 1975
Nicotiana tabacum	Ubiquinone-10	Liquid	0.18	0.003	60	Matsumoto et al., 1981
Catharanthus roseus	Catharanthine	Liquid	0.24	0.002	77	Smith et al., 1987

metabolites. In fact, decreased secondary metabolite yields have sometimes been reported for cultures as a consequence of organogenesis. For example undifferentiated culture of *Dioscoria deltoidea* and *Agave wightii* yield 1-2% dry weight steroidal sapogenins, but when cultures differentiate to produce roots or bulbils, only trace amount of sapogenins are produce (Kaul and Staba, 1986; Sharma and Khanna, 1980).

7. Plant Tissue and Cell Cultures as Metabolite Model System

Plant tissue and cell cultures offer a number of advantages over intact plant for studies of metabolism. They are relatively easy to establish and maintain under strictly controlled nutritional and environmental conditions. They can be grown in either small containers (such as 250 ml erlenmeyer flasks) or large fermentors and chemostas, and thus the amount of biomass are sufficiently available as needed. As cell suspension cultures often consist of a relatively homogenous population of cells, they can be rapidly and uniformly exposed to exogenously added chemical agents, often a difficult task when dealing with the intact plant finally, because of their limited degree of differentiation cell cultures generally display simpler metabolite patterns.

From the mentioned advantages, the use of tissue

cultures for metabolite studies has so far been limited, mainly because of the special metabolite characteristic impose by the usual culture environment. In the case of secondary metabolism, the culture of many species do not produce significant amount of the compound characteristic of intact plants. This may sometimes be due to the loss of genetic information during prolong culture, but in many cases it has been shown that even long-term cultures remain totipotent (Chaleff, 1983; Davey, 1983). It is more likely that the failure of cultured cell to produce the pattern or level of secondary metabolites typical of the source plant is a consequence of the specific physiological and morphological state of cultured tissue.

The biosynthesis studies of rosmarinic acid in cell suspension cultures of *Anchusa officinalis* (Wanchai De-Eknamkul and Ellis, 1984). Shown that, cell cultures are valuable experimental system to elucidate the biosynthesis and enzymology of their secondary metabolites. This can be said that there are some plant cell cultures which the biosynthetic pathways for secondary metabolites can be expressed at a level and rate much higher than intact plant.

8. Source and Type of Plant Tissue and Cell Culture

The techniques of plant tissue and cell culture are the art of growing, aseptically and heterotrophically, isolated plant as explants on appropriate media. These techniques can be considered to be extended from the nutritional methods of microbiology to higher plants. The term "tissue culture" embraces procedures and practices which may be applied to plant materials from source that may cover the entire morphological range. This range may be from young leaves, immature seeds, embryos, buds, apical shoots or roots and protoplasts. Since these plant parts can be grown without the requirement of the essential organization that supports life, the term "in vitro" culture was introduced. As their are many different building materials within a plant, there are many different types of *in vitro* culture Fig. 24

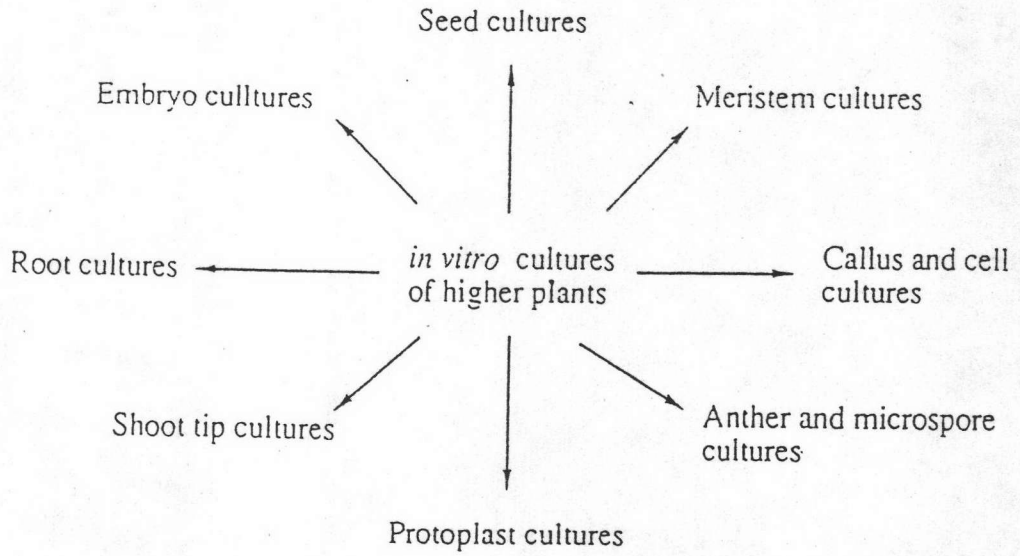


Fig. 24 Schematic representation of *in vitro* cultures of higher plants.

8.1 Callus Culture

Depending on the explant, callus proliferation may arise from the cambium, cortex, pith, secondary phloem or even xylem parenchyma. Before attemping to initiate a callus culture it is first necessary to sterilize the plant organ from which an explant is to be taken. If taken from a seedling it is usually more convenient to sterilize the seed before imbibition and allow it to germinate in aseptic condition. Then at a suitable stage the appropriate organ (cotyledon hypocotyl or root) can be excised with a sharp scalpel and transfer to a nutrient medium solidified with sugar.

The callus take anything from 3 to 8 week to reach a size when it may be subcultured by transferring a small pieces of tissue (50-100 mg) to fresh medium. Once well established, most callus cultures will require a regular subculture at approximately 4 week interval. In general 25°C is a suitable temperature for incubating cultures and exposure to low light intensities is often beneficial. The growth of callus is most conveniently assessed by measuring fresh and dry weight increase, although other parameters such as increases in cell number, cell volume and insoluble nitrogen have been used.

Microscopic studies have shown that callus tissue are often heterogeneous in cell composition. The diversity of the constituent cells varies according to

many factors, including the origin and age of the culture and the composition of the medium. Actively growing cultures usually contain a high proportion of vacuolated cells resembling parenchyma, and more localized groups of smaller dividing cells. The highly vacuolated cells have very diverse shape ranging from spheres to filaments with all stage is in between . The large number of morphological types of calli vary according to external appearance, texture and cellular composition. Some calli consist of compact tissues with small closely packed cells,while others consist of soft tissues with minimal cellular contact. The pigmentation of callus is also variable, even among isolated from the same species.

8.2 Cell Suspension Culture

Cell suspension cultures are usually initiated by transferring established callus tissue to a liquid medium. It consist of isolated cell and very small cell aggregates remaining dispersed as they grow in agitated liquid media. Ideally such suspensions should consist of single cells (rather than cell aggregates) which are physiologically and biochemically uniform. They represent a lower of organize than callus culture. For each cell culture there is a minimum inoculum size below which the culture will not grow. The lag phase of the culture increase in length as the inoculum size decreases towards the minimum level. Agitation rate of shakers should be in the range of 60-150 r.p.m. At the first subculture into

fresh medium, remove large clumps of initial inoculum either by transfering material with a pipette or syringe suitable orifice diameter to exclude large cell aggregates or alternatively by allowing the culture to sattle for a short time and then transfering the cells from only the upper part of the culture. Some callus cultures grow as compact, non-friable lumps and do not readily break up to form suspension. In this case, however, may be improved by modifying the culture medium, such as, increasing the concentrations of auxin, altering the ratio of auxin to cytokinin or adding low concentrations of cell-wall degrading enzyme such as cellulase and pectinase (Butcher and Ingram, 1976).

The media suitable for growing callus cultures for a particular species are also generally suitable for growing suspension cultures, providing that agar is omitted. However in some cases suspension are more exacting in their requirements, for example the concentration of auxin and cytokinins are often more critical.

Cell suspension cultures are widely use as model system for studying pathways of secondary metabolism, enzyme induction and gene expression, degradation of xenobiotics and as source of material for enzyme purification. They provide a relatively homogeneous population of cells, readily accessible to exogeneously

applied chemical and growing under defined, aseptic condition. The lack of chlorophyll and carotenoid pigments in most plant cell suspension cultures is of great benefit for work involving isolation of enzyme or secondary products.