Historical

Coumarins

Introduction to Coumarins

Coumarin is a large group of naturally occurring compounds possessing a $2H$-1-benzopyran-2-one nucleus (Gray and Waterman, 1977). Since 1820, when Vogel for the first time isolated coumarin, the parent compound, present in the seeds of *Coumarona odorata* Aubl. (syn. *Dipteryx odorata* Willd.) or tonka bean, from which its name is derived. About 300 coumarins have been isolated from Gramineae, Orchidaceae, Leguminosae, Umbelliferae, Guttiferae, Rutaceae, Labiatae and other families of lesser importance (Seshadri and Vishwapaul, 1973). Coumarins have been found to be distributed extensively in varied types of flora and all parts of plant. They have also been reported from microorganisms (Asao, Buchi, Abdel-Kader, Chang, Wick & Wogan, 1963, 1965) and animals (Lederer, 1949). Lederer (1949) had demonstrated the presence of 3,4-benzocoumarins in the scent gland of the beaver. The simple hydroxylated and methoxylated coumarins in free state or as glycosides occur widely in different plant families, but as the structure complexity of the compound increases, they seem to be restricted more and more to familial occurrence. The coumarins content of the plants may vary in different stages of their growth. Some plants contain the maximum amount at the time of sprouting and the younger leaves are richer in coumarin content than the older ones.

The role of these compounds in the plant economy has not yet been fully elucidated (Tandon and Rastogi, 1979).

Coumarins characteristically fluoresce (usually blue) in ultraviolet light (Lutzman, 1940). Those derived from umbelliferone exhibit their blue fluorescence when irradiated with visible light but, if the 7-hydroxyl group is free, the fluorescence often disappears or turns green in alkaline solution. The relations between fluorescence and structure are far from clear, but the phenomenon is of great use in combination with chromatographic techniques (Dean, 1963). Coumarin has a sweet smell and bitter taste (Harborne, 1972).

Classification of coumarins

No completely satisfactory classification of the coumarins is possible at present. Steck and Mazurek (1972) had divided coumarins into two types. One is "normal" type, which has an oxygen function at C-7 and hydrogen at C-3 and C-4 (unsaturated pyrone ring), The other is "abnormal" type, which either lacks the C-7 oxygen or possesses pyrone ring substituents. However Seshadri and Vishwapaul (1973) had classified coumarins into five groups as follows.
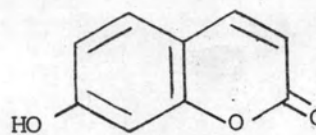
1. Simple coumarins

In this type of coumarins, only the benzene ring in the benzopyran nucleus could be substituted, e.g. coumarin (1), umbelliferone (7-hydroxycoumarin) (2), scopoletin (3) and aesculetin (4) (Gibbs, 1974).
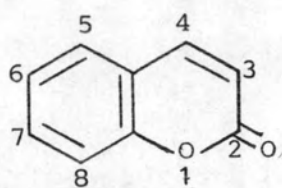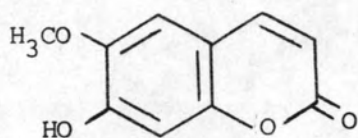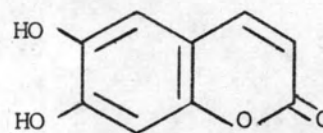
Coumarin
(1)

Umbelliferone
(2)

Scopoletin
(3)

Aesculetin
(4)

2. Furanocoumarins

This type of coumarins has furan ring fused with the coumarin nucleus at the various position on benzene ring to form linear or angular structure. Rings fused in a linear fashion are more common than in one of the several possible angular modes (Dean, 1963). Normally the furan rings does not carry any substituents. Considering the fusion of furan ring, furanocoumarins can be classified into six subtypes (Seshadri and Vishwapaul, 1973).

2.1 Psoralene type (linear)
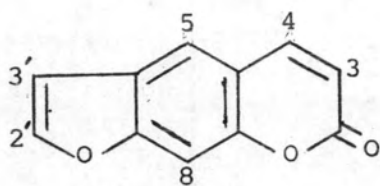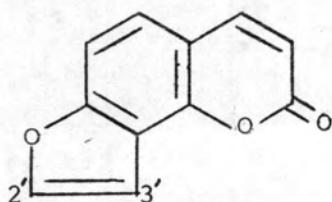

(Psoralen nucleus)
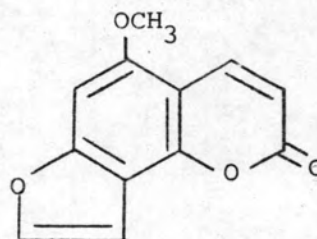
Psoralen (5)
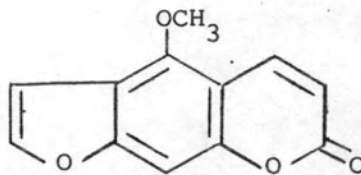
Bergapten

(6)

2.2 Angelicin type (angular)
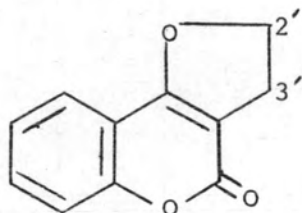

(Angelicin nucleus)

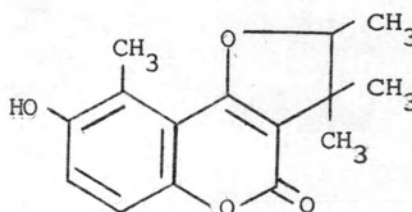Angelicin (7)

Isobergapten

(8)

2.3 Dihydrofuranocoumarin (4,3) type
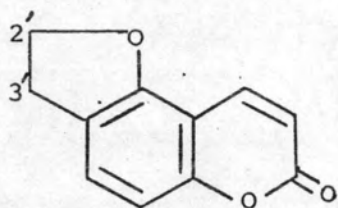

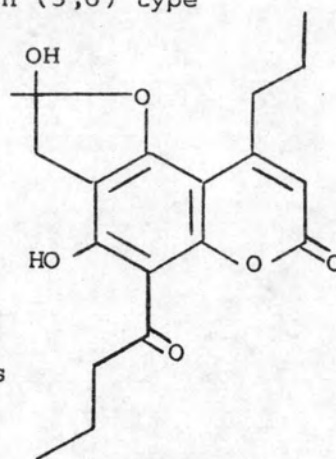Dihydrofuranocoumarin (4,3) nucleus

(9)

Glaupalol

(10)

2.4 Dihydrofuranocoumarin (5,6) type

Dihydrofuranocoumarin (5,6) nucleus
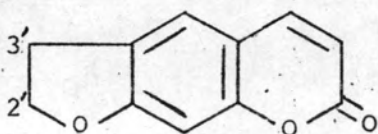
(11)

Furomammea A

(12)

2.5 Dihydrofuranocoumarin (7,6) type

Dihydrofuranocoumarin (7,6) nucleus

(13)
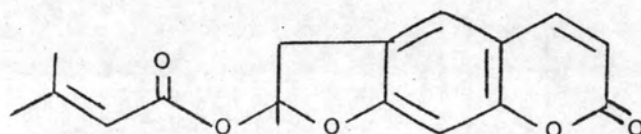
Prantschimgin

(14)

2.6 Dihydrofuranocoumarin (7,8) type
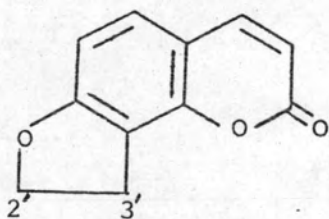
Dihydrofuranocoumarin (7,8) nucleus

(15)
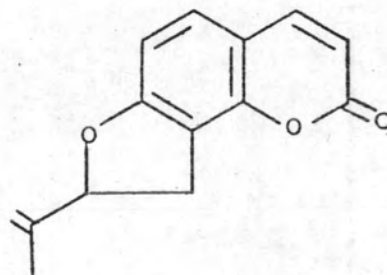
Angenomalin

(16)

Some plants contain a wide range of furanocoumarins of both
linear and angular fusion. *Angelica keiskei* (Miq.) Koidz (Umbelliferae)
for example, is a source of angelicin (7) as well as psoralen (5),
bergapten (6) and xanthotoxin (17) (Dean,1963)
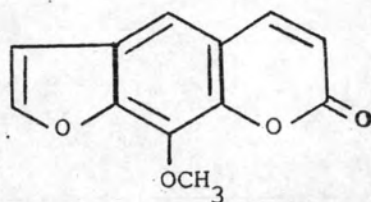
Xanthotoxin

(17)

3. Pyranocoumarins (2-Pyrano chromenes)

A few coumarins have a pyran ring fused to the benzene
ring. These coumarins may be called chromano-coumarins. The ring
may be variously placed (Gibbs, 1974). Considering the fusion of
pyran ring, pyranocoumarins may be classified into four subtypes
(Seshadri and Vishwapaul, 1973):

3.1 Xanthyletin type
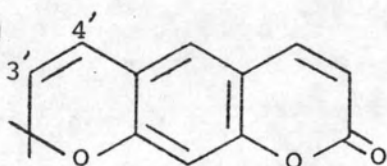
Xanthyletin nucleus

(18)
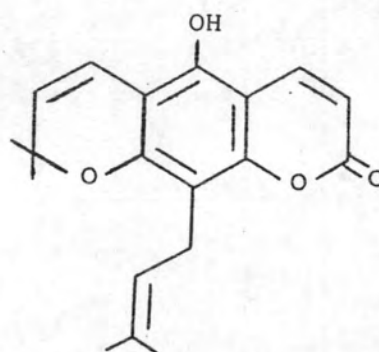
Trachyphyllin

(19)

3.2 Seselin type
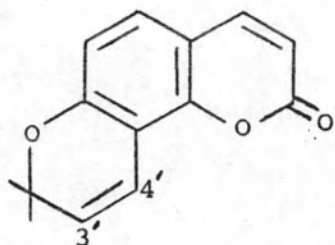
(Seselin nucleus)

Seselin (20)
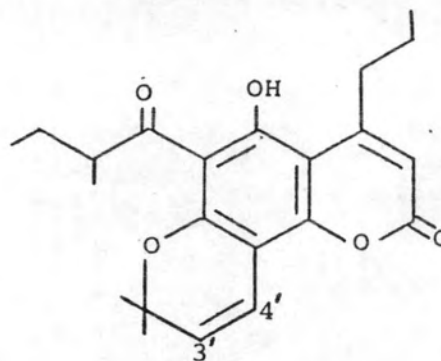
MAB 6

(21)

3.3 Dihydroxanthyletin type
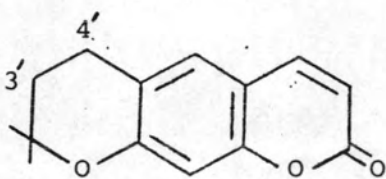
(Dihydroxanthyletin nucleus)

Dihydroxanthyletin (22)
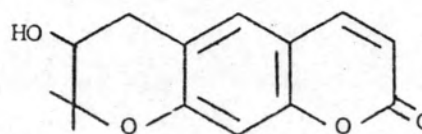
Decursinol

(23)

3.4 Dihydroseselin type
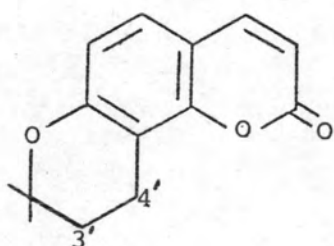
Dihydroseselin nucleus

(24)
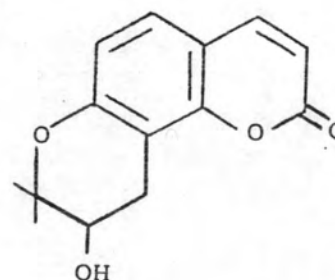
Lomatin

(25)

4. Phenylcoumarins

There is phenyl substitution at C-3 or C-4 of coumarin nucleus. Phenylcoumarins can be classified into seven subtypes (Tandon and Rastogi, 1979), (Seshadri and Vishwapaul, 1973):

4.1 3-Phenylcoumarin type
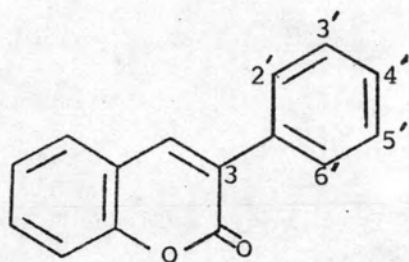
3-Phenylcoumarin nucleus

(26)
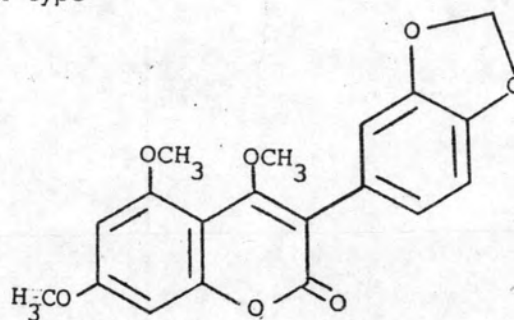
Derrusnin

(27)

4.2 4-Phenylcoumarin type
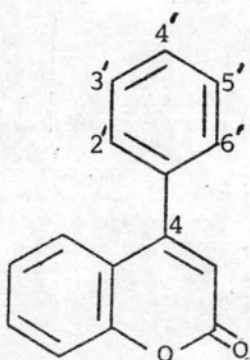
4-Phenylcoumarin nucleus

(28)
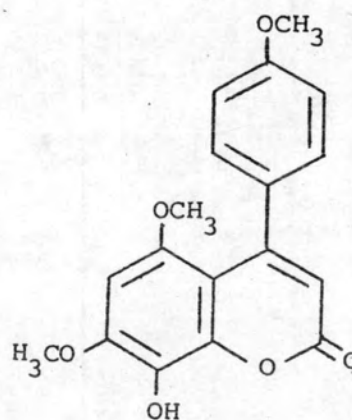
Exostemin

(29)

4.3   4-Phenyldihydroangelicin type
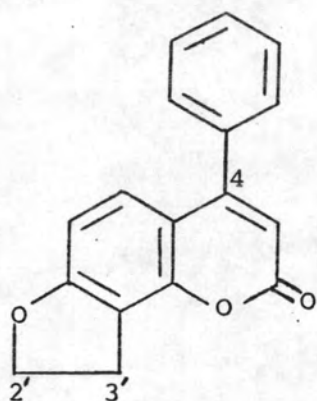
4-Phenyldihydroangelicin nucleus

(30)
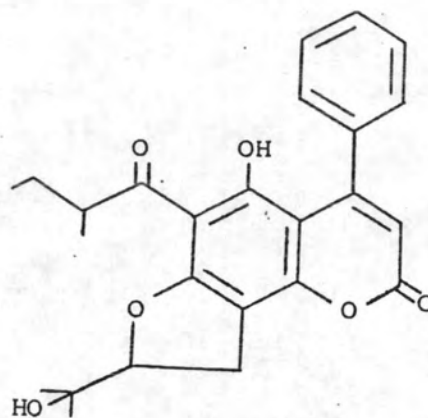
Phenylmammea A

(31)

4.4   3-Phenylxanthyletin type
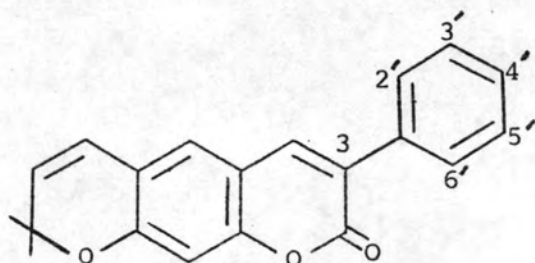
3-Phenylxanthyletin nucleus

(32)
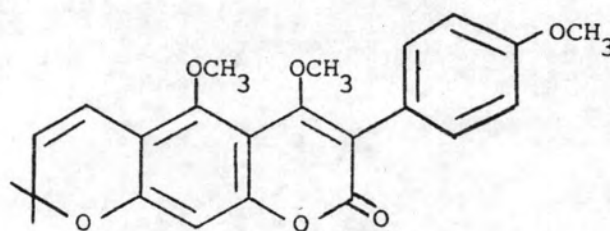
Robustic acid

(33)

4.5   3-Phenylseselin type
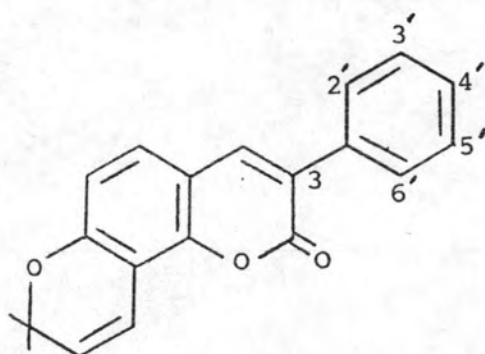
3-Phenylseselin nucleus

(34)
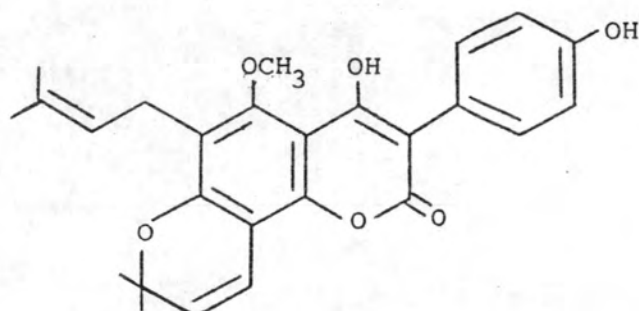
Scandenin

(35)

4.6   4-Phenylseselin type
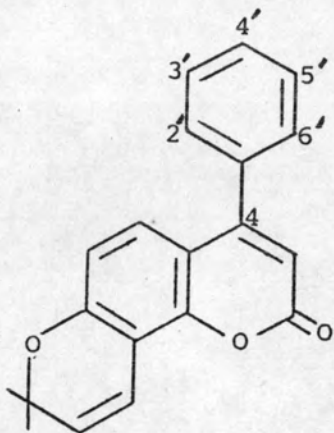
4-Phenylseselin nucleus

(36)
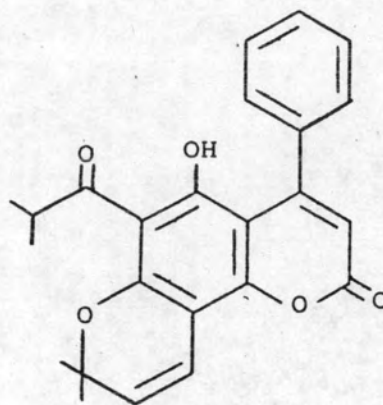
Mesuagin

(37)

4.7   Tripyran derivative type
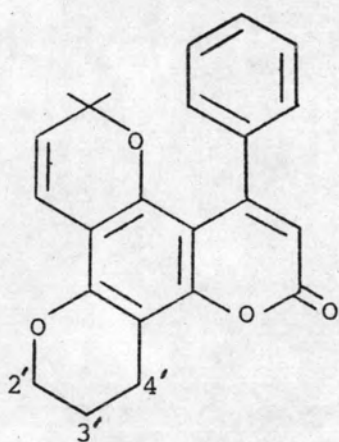
Tripyran nucleus

(38)
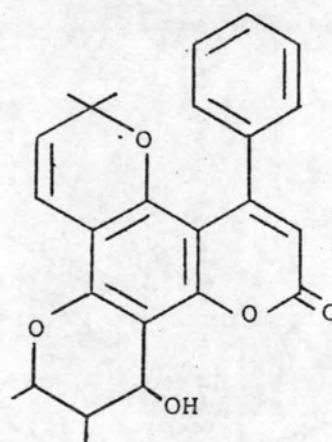
Inophyllum A

(39)

5.   Bicoumarins

Two coumarin nucleus units are bonded with or without methylene group.
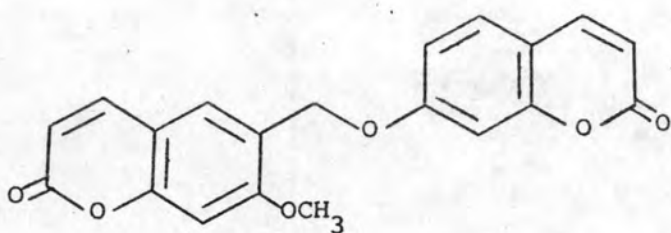
Lasiocephalin
(40)
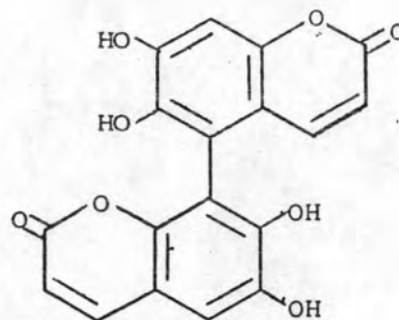
Isoeuphorbetin
(41)

Biosynthesis of Simple Coumarins

Coumarins are lactones of phenylpropanoid acid, a class
which derives from the shikimic acid pathway *via* the protoaromatic
amino acids, phenylalanine and in a few plants, tyrosine. The entire
carbon skeleton of the simple coumarins, i.e. those having no rings
additional to the benzopyrone nucleus, is phenylalanine derivative.
In the number of early biosynthesis experiments on these compounds in
plants it was shown that shikimic acid, phenylalanine and *trans*-
cinnamic acid are common precursors of this benzopyrone nucleus.
Cinnamic acid, however, represents a branch point in the elaboration
of coumarin itself and probably other coumarins lacking 7-oxygenation
on one hand and the 7-hydroxy coumarins based on umbelliferone on the
other the latter group constituting the vast majority of the class.
Ortho-hydroxylation of *trans*-cinnamic acid leads to coumarin itself,
via a light-catalysed *trans-cis* isomerization, and lactone ring
formation which can be formally represented as a dehydration (Edwards
and Stoker, 1967). The enzyme responsible, which is membranebound,
has been obtained from chloroplast. The mechanism involved is not

yet known (Goodwin and Mercer, 1983). *Para*-hydroxylation of *trans*-cinnamic acid is a necessary prerequisite for synthesis of the 7-hydroxycoumarins, *via ortho*-hydroxylation and lactonization as before. The *ortho*-hydroxylations of cinnamic and *para*-hydroxycinnamic acids are mediated by different enzymes (Gestetner and Conn, 1974), which are apparently seldom found together in the same species, so that coexistence of coumarin and the umbelliferone is an exceptional phenomenon (Brown, 1979).

It should be noted that a number of the simple coumarins are artifacts of isolation, and do not occur to any very significant extent in the free form in the intact plant cell. Thus coumarins herniarin, and umbelliferone have all been shown to occur in the form of glycosides of the corresponding *cis-o*-hydroxy (42). However, glucosidases are also present which gain excess to these substances upon disruption of the cells, and the liberated aglycones (43), lactonize spontaneously to the free coumarins (Kosuge and Conn, 1961). The pungent odour of coumarin associated with new-mown hay originates in this way (Brown, 1979).

In at least one case in the elaboration of microbial coumarin, novobiocin (44) by *Streptomyces nivens* Smith, Dietz, Sokokski & Sawage, lactone ring closure is not by dehydration but by an oxidative mechanism (Brown, 1979).
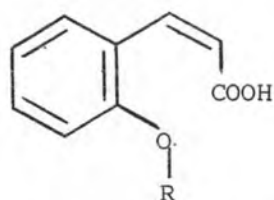
Cis-o-hydroxycinnamic acid   R = glucosyl   (42)

aglycone   R = H   (43)

Novobiocin

(44)

D – Glucose

COOH

5-Dehydroshikimic acid

(keto form)

NADPH → NADPH

+ H⁺   Δ + H⁺

NADP⁺   NADP⁺

COOH

HO⃰⃰   ⃰OH

OH
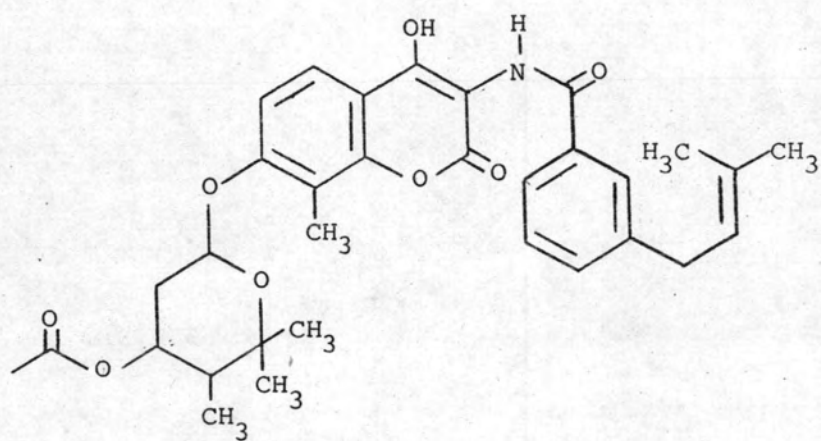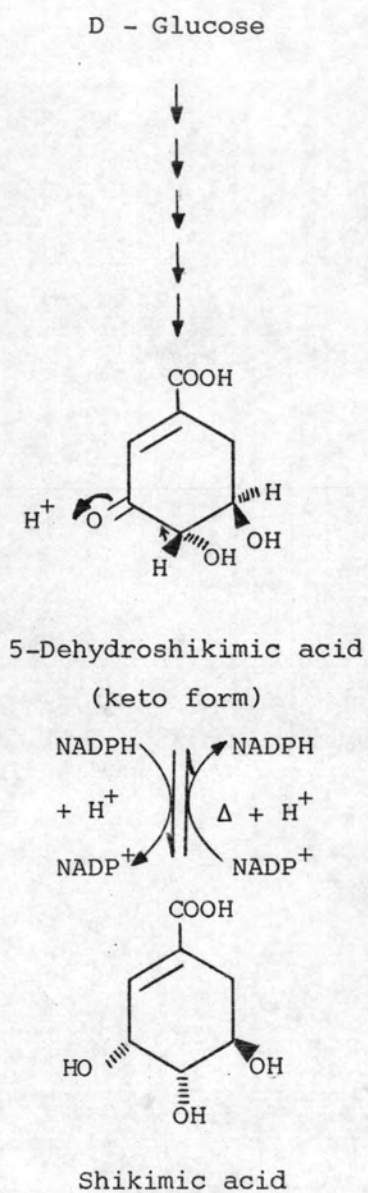
Shikimic acid

Figure 2   Formation of shikimic acid (Goodwin and Mercer, 1983)
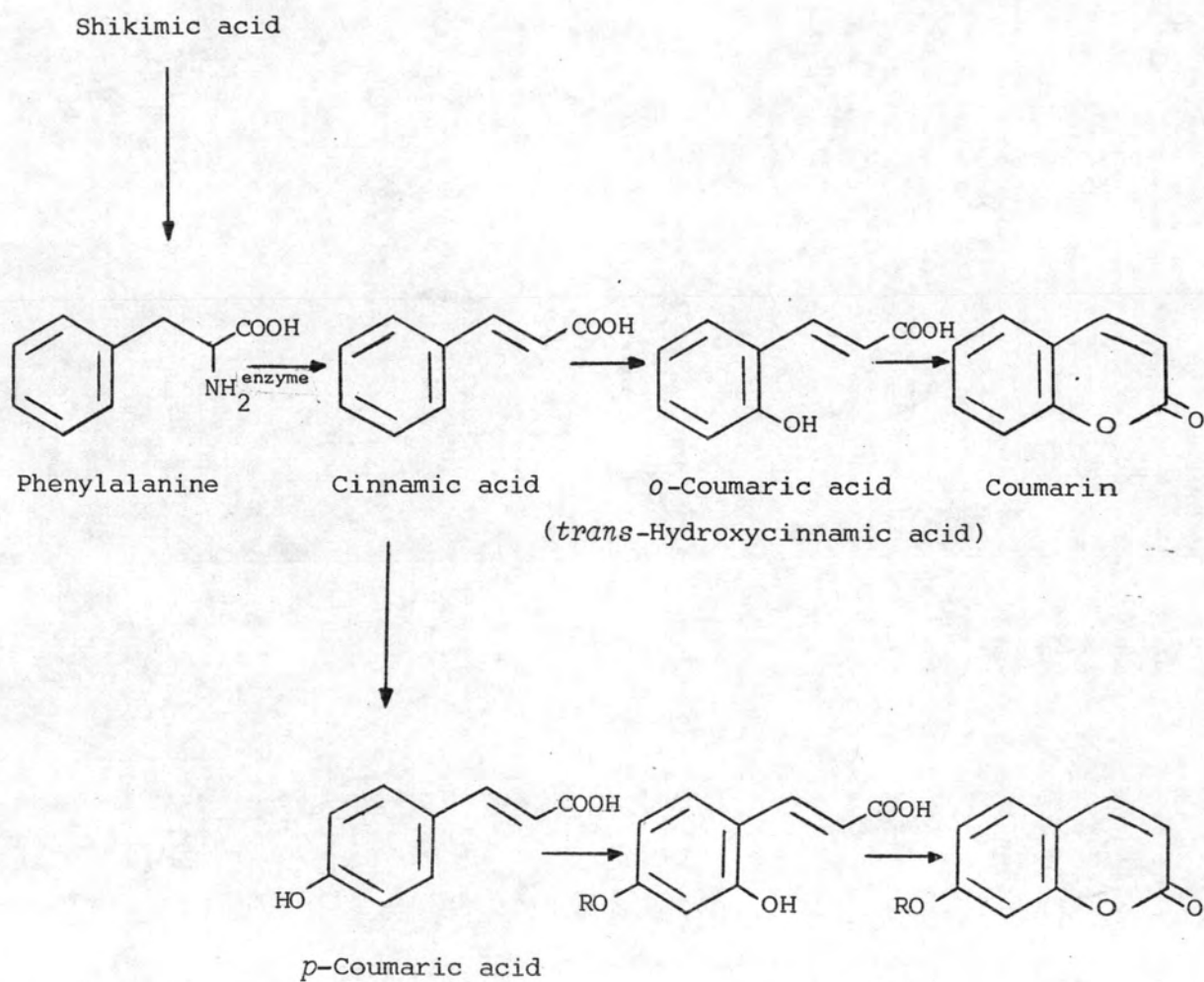
Figure 3 Pathways from *trans*-cinnamic acid to coumarins

(Street and Cockburn, 1972) (Brown, 1979)

Physiological Activities of Coumarins

Natural coumarins as a class can not be classified as a pharmacodynamic group, although varied types of activities have been encountered in some of these substances. A more synthetic analogues have been prepared and evaluated for physiological activities found in natural coumarins (Tandon and Rastogi, 1979). The least complex member of the class structurally, known simply as coumarin, is toxic to mammals, another microbial coumarin type is the aflatoxin (45) elaborated by *Aspergillus flavus* Lk., these metabolites are potent hepatotoxins and are among the most intense carcinogens yet discovered (Ciegler, Detroy & Lillehoj, 1971).
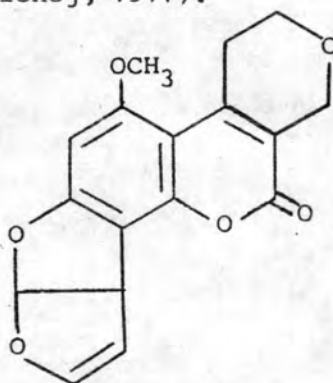
Aflatoxin type

(45)

1. Anticarcinogenic activity

The compounds act as inducers of aryl hydrocarbon hydroxylase (AHH) system and that this increased activity gives protection against neoplasia induced by polycyclic aromatic hydrocarbons (PAH). Further, these inducers of AHH have been found to be present in certain vegetable species and dietary components, some of them have been identified as coumarins, flavones, aromatic isothiocyanates, selenium salts and indoles. Although the mechanism

by which these inhibitors of chemical carcinogenesis inhibit
PAH-induced neoplasia is not known, the induction of increased AHH
activity reflected the formation of phenolic metabolites of PAH.
Probably the relative amounts of these metabolites of detoxification,
as compared to those leading to the formation of ultimate carcinogenic
species, control the response to a particular dose of carcinogen.
In these studies, coumarin was found to suppress 7, 12-dimethylbenz(a)
anthracene-induced carcinoma in rats (Feuer, Kellen & Kovacs, 1976).

2. Antibiotic activity

Although coumarin itself has a very low antibacterial
activity, other members, such as ammoresinol, ostruthin (46),
dicoumarol (47); novobiocin (44), coumermycin, chartreusin (48) and
athamantin (49), exhibited significant activity. Ostruthin and
ammoresinol were most active against a wide spectrum of bacteria, e.g.
Staphylococcus aureus Rosenbach, *Micrococcus luteus* (Schroeter) Cohn,
Bacillus megatherium de Bary (Hodak, Jakesova & Dadak, 1967).
A number of coumarins have been tested for antifungal activity; and
three most effective ones were psoralen (5), imperatorin (50) and
ostruthin (46) (Tandon & Rastogi, 1979).

$(H_3C)_2C=CHCH_2CH_2C=CHH_2C$

Ostruthin

(46)

Dicoumarol

(47)

Chartreusin
(48)

Athamantin
(49)

Imperatorin
(50)

3. Anticoagulant

The coumarin anticoagulants, e.g. dicoumarol, are employed in therapy to depress blood coagulation and the present thrombosis in diseases of coronary artery and related conditions. These compounds acted only *in vivo* by blocking the synthesis of four proteins in the prothrombin complex necessary for the blood coagulation process. The coumarin anticoagulants are absorbed relatively quickly, but their effect is slow and, therefore, acute human toxicity of these compounds was rare, although poisoning may occur in children ingestion large doses (Ingram, 1961).

During fermentation of hay, some microorganisms (Such as *Aspergillus fumigatus* Fres.) transform coumarin into dicoumarol. Such spoiling of hay can be dangerous to animals as dicoumarol is a powerful blood anticoagulant and can cause fatal haemorrhages in cattle which eat it (Manitto, 1981).

4. Antispasmodic action

The coumarin pteryxin from *Pteryxia terebinthina* Nutt., and suksdorfin-A from *Lomatium suksdorfii* S. Wats (Willette & Soine, 1962) were found to exhibit this activity (Tandon & Rastogi, 1979).

5. Vasodilatory action

A number of coumarins have been isolated from Umbelliferae plants which possessed vasodilatory activity. Other active coumarins, are visnadin, samidin and dihydrosamidin. Visnadin has also been used in therapy for the treatment of angina pectoris under the registered name "provisimine" (Tandon & Rastogi, 1979).

6. Effect on central nervous system

Coumarin itself exerts hypnotic action on frogs, rabbits and mice, but it has not been used therapeutically because of its hepatic toxicity (Tandon & Rastogi, 1979).

7. Oestrogenic activity

All the coumarins which have been shown to possess this property belong to the group of 3-phenyl coumarins which has been discussed adequately (Krishnaswamy, 1965). It is recognized that the *trans*-stilbene character in these compounds was responsible for

oestrogenic properties. Coumestrol (51), one of the most potent
compounds occurs in lindo clover and alfalfa (Tandon & Rastogi, 1979).
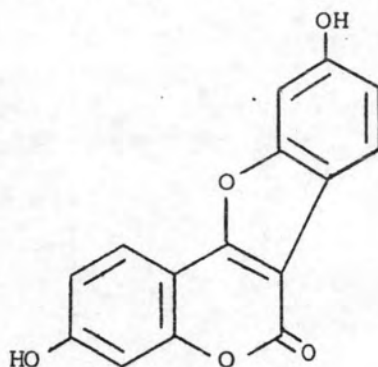
Coumestrol

(51)

8.   Molluscacidal activity

Some furanocoumarins possess molluscacidal activity.
Bergapten (6) and isopimpinellin (52) have shown activity similar
to those of some of the most potent synthetic compounds used for
snail control (Schonberg & Latif, 1954). The application of these
coumarins has been considered for the control of reproduction of the
snail which is a host animal for the blood fluke responsible for
human bilharziasis (Tandon & Rastogi, 1979).
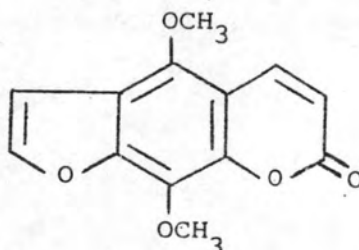
Isopimpinellin

(52)

9.   Miscellaneous activity

Certain plant extracts and juices have been known to
increase dermal photosensitivity. Application of these extracts to

the skin followed by exposure to sunlight cause erythema and
pigmentation. The substances responsible for this action were simple
furanocoumarin e.g. psoralen (5), bergapten (6), angelicin (7),
xanthotoxin (17), imperatorin (50), isopimpinellin (52), Xanthotoxol
(53), ostruthol, oxypeucidanin, etc. Warfarin (54) has been applied
as an effective rodenticide.
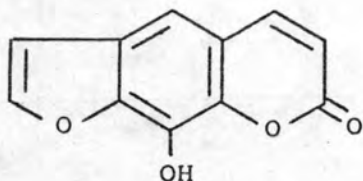
Xanthotoxol
(53)
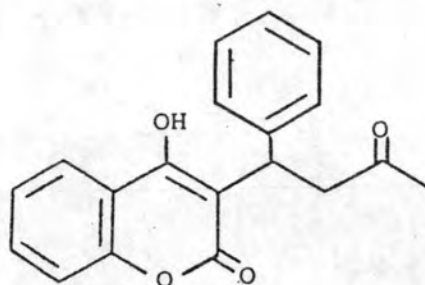
Warfarin (54)
(synthetic 4-hydroxycoumarin)

The coumarins of *Mammea americana* Linn. (Crombie, Games,
Haskins & Reed, 1972) have been reported to possess insecticidal
activity.

Xanthotoxin, a linear furanocoumarin, showed 100% mortality
to larvae of armyworm (Berenbaum, 1978).

Distribution of Simple Coumarins in Higher Plant

In subdivision Angiospermae, simple coumarins are
distributed in many groups of plants. Few simple coumarins are
distributed in Apetalae while more are distributed in Polypetalae
and Sympetalae. From the reports (Gibbs, 1974), illustrated that
simple coumarins are significant in family Umbelliferae and family
Rutaceae. Few simple coumarins distributed in the class Monocotyledonae.

The distribution of simple coumarins in higher plants are shown in Table 3.

Table 3. The Distribution of Simple Coumarins in Higher Plants.

Class Monocotyledonae

Order	Family	Genus
Glumiflorae	Gramineae	1
Spadiciflorae	Palmae	1
Liliiflorae	Iridaceae	1
Microspermae	Orchidaceae	1

Class Dicotyledonae

Group	Order	Family	Genus
Apetalae	Urticiflorae	Moraceae	1
	Centrospermae	Caryophyllaceae	1
Polypetalae	Ranales	Magnoliaceae	1
		Calycanthaceae	1
		Berberidaceae	1
		Ceratophyllaceae	1
	Guttiferales	Guttiferae	1

Table 3. Cont.

Group	Order	Family	Genus
Polypetalae	Tricoccae	Euphorbiaceae	1
	Geraniales	Zygophyllaceae	1
	Rutales	Rutaceae	14
		Meliaceae	1
	Rhamnales	Vitidaceae	1
	Rosales	Saxifragaceae	10
		Hydrangeaceae	1
		Cunoniaceae	1
		Pittosporaceae	1
		Rosaceae	2
		Leguminosae	4
	Myrtiflorae	Thymelaeaceae	2
	Umbelliflorae	Umbelliferae	19
Sympetalae	Ebenales	Ebenaceae	1
		Sapotaceae	1
	Oleales	Oleaceae	1

Table 3. Cont.

Group	Order	Family	Genus
Sympetalae	Contortae	Loganiaceae	1
		Apocynaceae	3
		Asclepiadaceae	1
	Convolvulales	Convolvulaceae	2
	Tubiflorae	Labiatae	2
		Solanaceae	5
		Scrophulariaceae	1
		Bignoniaceae	2
		Acanthaceae	2
	Rubiales	Caprifoliaceae	4
	Campanulales	Goodeniaceae	1
	Asterales	Compositae	6

(Gibbs, 1974)

Coumarins in the Compositae

In 1964 only coumarin itself [from 12 species belonging to
the genera *Ageratum, Eupatorium, Liatris, Trilisa* (Eupatorieae),
Chrysanthemum, Matricaria (Anthemideae) and *Rudbeckia* (Heliantheae)],
umbelliferone, herniarin (55), esculetin, scopoletin, scoparone (56),
and ayapin (57) and some of their glucosides could be reported as
constituents of a number of species representing several tribes.
Some further new coumarins and coumarin-producing taxa may serve to
illustrate the more or less general tendency in Compositae to produce
appreciable amounts of coumarins (Hegnauer, 1977). Haskins, Gorz &
Leffel (1972) investigated the origin of coumarin in cured leaf of
Carphephorus odoratissimus (J. F. Gmel.) Herbert; fresh leaves contain
large amounts of the glucosides of *trans-*and *cis-o*-Hydroxycinnamic
acid; during curing coumarin arises by glucoside hydrolysis and
spontaneous lactonization of the aglucone.
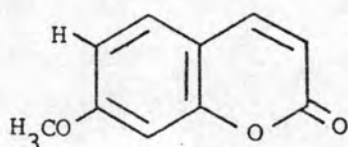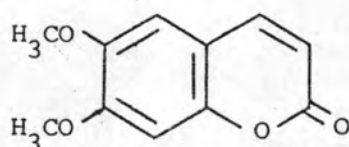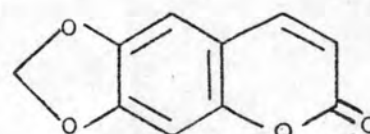
Herniarin	Scoparone	Ayapin
(55)	(56)	(57)

Coumarins Isolated from Species of *Ageratum*

The only coumarin reported to be distributed in genus *Ageratum*
is simple coumarin which is found in *A. brachystephanum* Regel (Wehmer,
1929), *A. conyzoides* Linn. (Wehmer, 1929), *A. corymbosum* Zucc,
(Quijano, Calderón, Gómez, Soria & Rios, 1980) and *A. strictum* Hemsl.
(Quijano, Calderón, Gómez & Rios, 1980).

Introduction to Flavonoids

The flavonoids are a group of some 750 naturally occurring plant constituents whose structures are derived in one way or another from the aromatic nucleus of flavan or 2-phenyl benzopyran (58). The simplest member is flavone (59), which occurs naturally as the farina of leaves and stems of many *Primula* species, but this is untypical of the series, since the vast majority have phenolic hydroxyls attached at the 5, 7, 3′, - and 4′ positions. Flavonoids are usually divided into classes depending on the oxidation level of the central pyran ring, the two most important classes being the flavonols or 3-hydroxylflavones [e.g. quercetin (60)] and the anthocyanidins [e.g. cyanidin (61)].
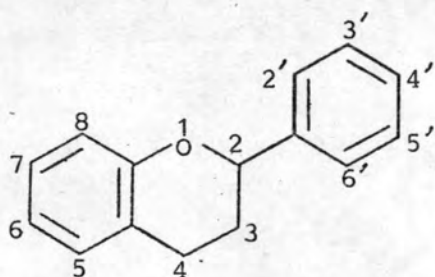
Flavan
(58)
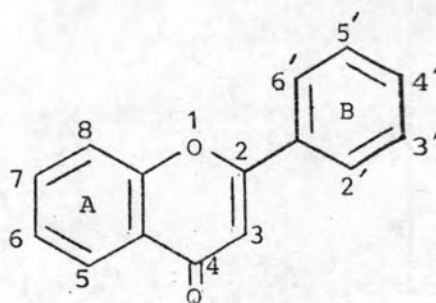
Flavone
(59)
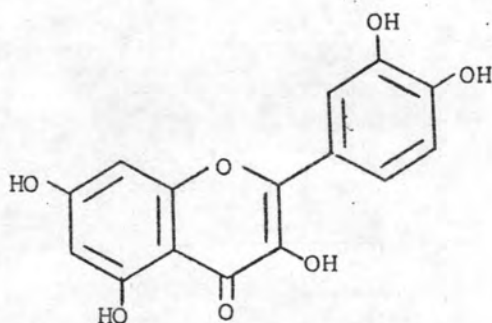
Quercetin
(60)
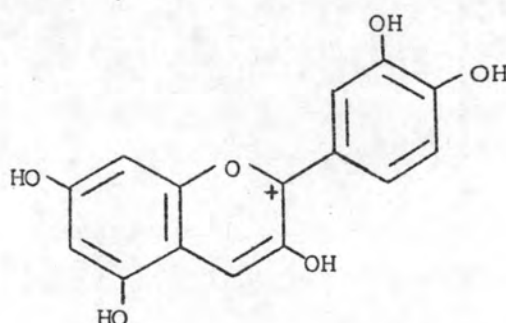
Cyanidin
(61)
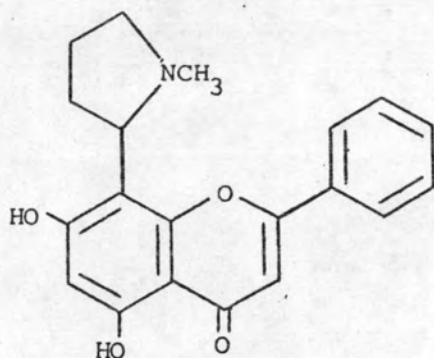
Ficine
(62)
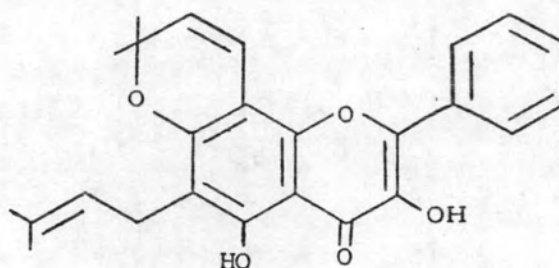
Sericetin
(63)

Flavonoids are a relatively uniform group structurally, though occasionally more complex derivatives are found, such as alkaloidal substituted flavone, ficine (62) and terpenoid substituted flavone, sericetin (63). Flavonoids are related biosynthetically to the aromatic amino acids, phenylalanine (64) and tyrosine (65), *via* the corresponding cinnamic acid, and the basic C_{15} skeleton is probably derived in plants from the coupling of a C_6-C_3 unit produced by the shikimic acid pathway and the C_2 acetate units (Harborne, 1973).
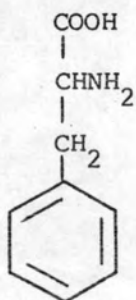
Phenylalanine
(64)
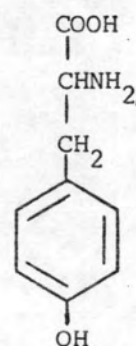
Tyrosine
(65)

Free flavonoids are rarely found in plants. They occur more commonly as glycosides by combination with a range of mono- and oligosaccharides including glucose, rhamnose, xylose, galactose and gentiobiose. Such glycosides can occur in any part of the plant; flowers, leaves, shoots or roots. The glycosides of the anthocyanidins, which are termed anthocyanins, are responsible for most red and blue colours in plants whereas the flavonols, flavones and their glycosides tend to be yellowish in colour. Colourless flavonoids also occur. These pigments, particularly the anthocyanins are conspicuous and relatively easy to isolate and identify and have been used as markers in genetic and taxonomic studies (Street and Cockburn, 1972). Flavonoids dissolve in alkalis, giving yellow solutions which on the addition of acid become colourless (Trease and Evans, 1983).

Classification of Flavonoid Compounds

According to Harborne (1973) flavonoids are divided into two groups

1. The common flavonoids

    1.1 Anthocyanidins
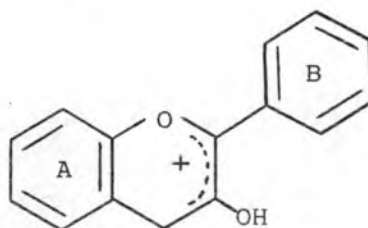
Anthocyanidin (cyanidin)

The number of B-ring hydroxyls present is correlated with colour properties; thus pelargonidin (66), with one hydroxyl is scarlet, cyanidin (67), with two is crimson, and delphinidin (69), with three is mauve. These three anthocyanidins, together with the

three simple derived methyl ether, paeonidin (68), petunidin (70), and
malvidin (71), are very widely distributed in plants, being particularly
abundant in coloured flowers and fruits, while pelargonidin and
delphinidin occur with much frequency in cyanic flowers, they are
uncommon in pigmented leaves (e.g. *Begonia, Coleus*) which nearly always
contain cyanidin (Harborne, 1973).
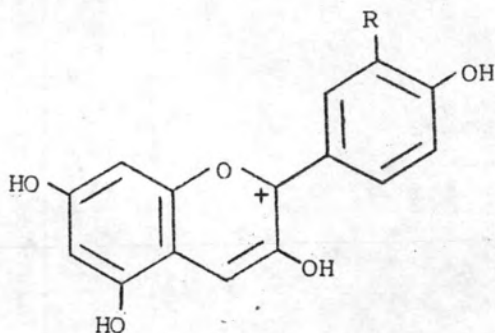
Pelargonidin, R = H

(66)

Cyanidin , R = OH

(67)

Paeonidin , R = OCH_3

(68)
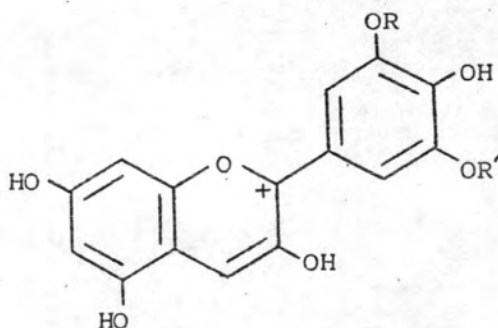
Delphinidin, R = R' = H

(69)

Petunidin , R = CH_3, R' = H

(70)

Malvidin , R = R' = CH_3

(71)

1.2 Flavonols

The flavonols corresponding to the three main anthocyanidin types are kaempferol (72), quercetin (73), and myricetin (74). While these flavonols occur widely in flowers, often accompanying the anthocyanidins, formulae (66-71), they are virtually colourless at the pH of cell sap and do not themselves contribute significantly to flower colour, apart from acting as co-pigments to the anthocyanidins or providing "body" in white flowers. Besides occurring in flowers, flavonols occur with great frequency in leaves. For example, a leaf survey of over 1,000 species showed that 48% contained kaempferol, 56% quercetin, and 10% myricetin (Swain & Bate-Smith, 1962).
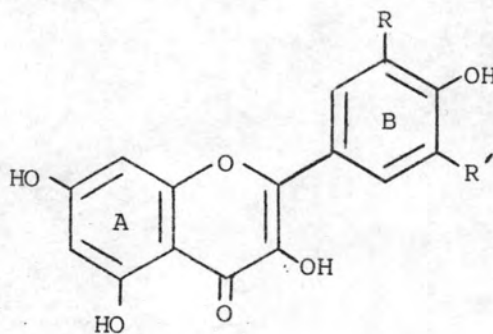
Kaempferol, R = R' = H

(72)

Quercetin , R = OH, R' = H

(73)

Myricetin , R = R' = OH

(74)

1.3 Flavones

The flavones and the anthocyanins are sap-soluble
and have γ-pyrone ring. The flavones and their colour relations are
often yellow (Latin flavus, yellow). They are widely distributed in
nature but are more common in higher plants and in young tissues,
where they occur in the cell sap (Trease and Evans, 1983). Flavones
are usually found in plants in place of flavonols, and while flavonols
are most abundant in woody angiosperms, flavones occur characteristi-
cally in the more herbaceous plant families (e.g. Umbelliferae,
Labiatae, Compositae, and so on). The change over from woody to
herbaceous habit in higher plants also produces another change in
flavonoid pattern, namely the disappearance of the trihydroxylated
flavonol myricetin. This is the probable reason why the flavone
corresponding to myricetin, i.e., tricetin (75), is very rare
(Harborne, 1973). Indeed, although long sought for, it has only
recently been detected in plants, having been found in leaves of the
legume, *Lathyrus pratensis* Linn. and of two gymnosperms (Lamer, 1968)
The flavone luteolin (76) is reported to be the oldest known European
dyestuff (Bently, 1960). The flavones occur both in the free state
and as glycosides, most are O-glycosides but a considerable number of
flavonoid C-glycosides are known. The glycosides are generally soluble
in water and in alcohol, but insoluble in other organic solvents, the
genins are only sparingly soluble in water but are soluble in ether.
Many flavones are phenolic or methoxy derivatives and form sap-soluble
glycosides. The intensity of their yellow colour increases with the
number of hydroxyl groups and with increase of pH (Trease and Evans,
1983). Most hydroxy flavones occur as glycosides, those in which only
methoxy or methylenedioxy substituents are present cannot, of course,

be glycosylated and occur as the free compounds in the plant tissues (Geissman and Crout, 1969).
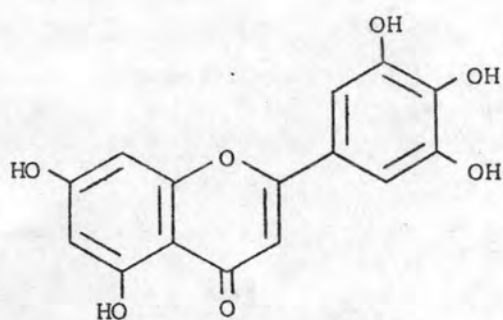
Tricetin

(75)
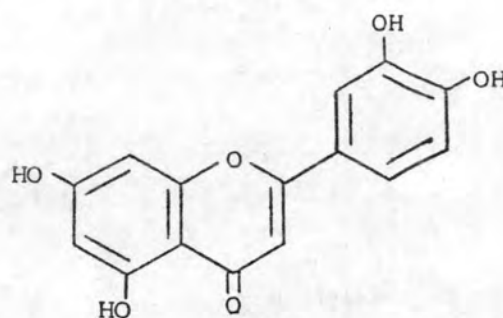
Luteolin

(76)

Flavones occur in leaves both as O-glycosides and as C-glycosides, the latter are a distinctive class of glycoside in which the sugar is attached by a carbon-carbon link to the flavone in ether the 6- or 8- position (or both). Evidence is a accumulating that C-glycosylflavones are biosynthetically distinct from O-glycosides (McClure, 1970). Certainly from the point of view of their distribution, they are different from the more common O-glycosides. Their occurrence (sometimes as the only flavonoid type present) in mosses, liverworts, and even green algae suggest that they are a "primitives" class. Their distribution in the angiosperms tends to be scattered, but again there are indications that they may be primitive "relicts". Thus, they are present much more frequently in woody than in herbaceous plants and appear to occupy an intermediate position in the evolutionary series: flavonol $\longrightarrow$ C-glycosylflavone $\longrightarrow$ flavone (as O-glycoside). There are, of course, some exceptional occurrences of C-glycosylflavones in more highly advanced groups (e.g., in the grasses or Gramineae) and it is in this incompleteness of correlation that systematic influence can be seen to operate (Harborne, 1972).

2. Other classes of flavonoids

2.1 Chalcones

Chalcones are not strictly speaking flavonoid, since they have an open-chain structure. However, they are usually classified with them because on acid treatment they isomerize to flavanones, and furthermore they are generally considered to be the immediate precursors of common flavonoids. Chalcones are of relatively infrequent occurrence in the plant kingdom. Butein (77), a typical chalcones, occurs in the free state in heartwood of *Acacia* and as 4'-glucoside. The chalcone corresponding to luteolin, i.e., 2', 4', 6', 3, 4-pentahydroxychalcone, is not known in the free state, since it spontaneously isomerizes to the corresponding flavanone, but it does occur naturally as the 2'-glucoside, isosalipurposide (78), in willow bark and in yellow carnations (Harborne, 1973). Chalcone numbering is different from the system used for flavonoid types that possess heterocyclic rings. In chalcones the ring A, always written to the left, is given the primed numbers, while the B-ring carbon are given the unprimed numbers. The positions are shown numbered in structure. Bridge carbon are marked relative to the carbonyl function (Bohm, 1982).
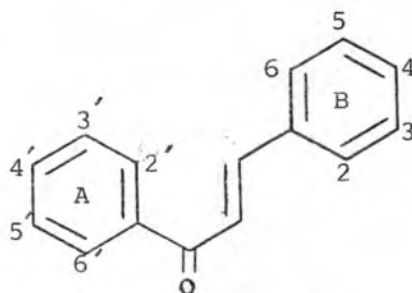
Chalcone nucleus
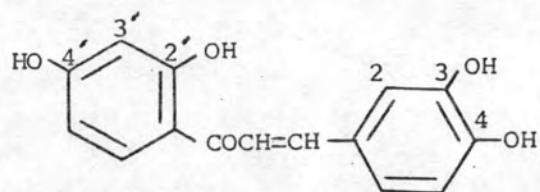
Butein

(77)
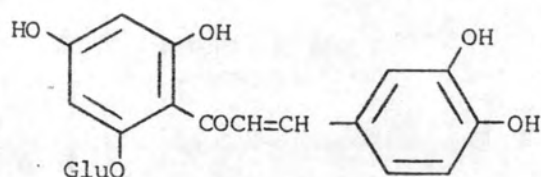
Isosalipurposide

(78)

2.2 Aurones

Aurones are formed from chalcones by aerial or enzymic oxidation and are deeper yellow in colour, hence the name from the Greek auros, "golden". Sulphuretin (79), for example, occurs in the yellow flowers of *Cosmos sulphureus* Cav. and of *Coreopsis* species and the related aureusidin in present as the 6-glucoside, aureusin (80), in yellow blooms of snapdragon, *Antirrhinum majus* Linn., and of toadflax, *Linaria vulgaris* Mill.

Both chalcones and aurones are detected by the fact that yellow flowers containing them, when fumed with the alkaline vapour of a lighted cigarette, become orange or red as a result (Harborne, 1973).
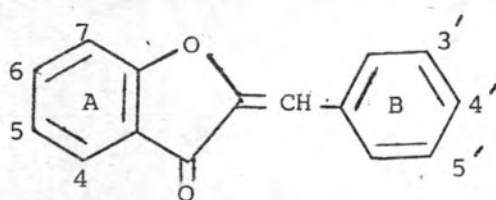
Aurone nucleus
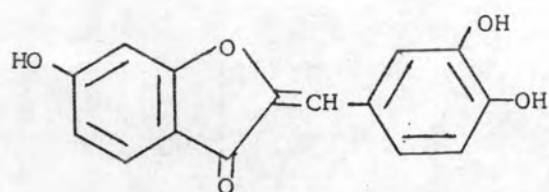
Sulphuretin
(79)
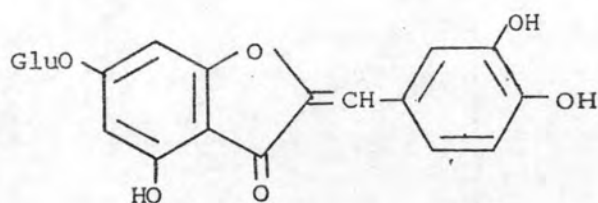
Aureusin
(80)

Aurones are hydroxylated 2-benzylidenecoumaranones, the parent compound being the "normal" numbering system applies to this group of compounds, position on the A-ring are identified by unprimed numbers and the B-ring position by primed numbers. Note that in aurones position 4 corresponds biosynthetically to position 5 of other hetero-cyclic flavonoids. Included in this section are two 'auronol' derivatives, distinguished by the presence of a methoxyl group in place of the carbonyl function at position 3 (Bohm, 1982)

2.3 Flavanones

These colourless substances are simple reduction products of flavones, and like chalcone are possible precursors of the more highly oxidized flavonoids in plants. The two flavanones eriodictyol and naringenin correspond in structure of luteolin and apigenin and occur fairly frequently in plants. From the economic point of view, the most important flavanone is naringin (81), the 7-neohesperidoside of naringenin, a substance nearly as bitter on a molar basis as quinine and the major soluble bitter principles of many citrus fruits. Bitterness is due to a combination of the

flavanone structure with unusual disaccharide neohesperidose
(rhamnosyl α 1→2 glucose), the related 7-rutinoside, remarkably
enough, is tasteless. Other changes in structure, namely ring opening
and reduction, produce compounds which are sweet (Harborne, 1973),
and the dihydrochalcones neohesperidoside (82), is reported to be
2,000 times as sweet as sucrose (Krbechek, Inglett, Holik, Dowling,
Wagner & Riter, 1968).
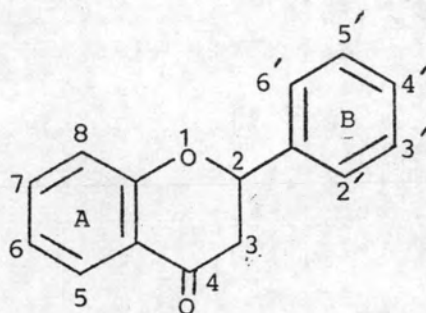
Flavanone nucleus
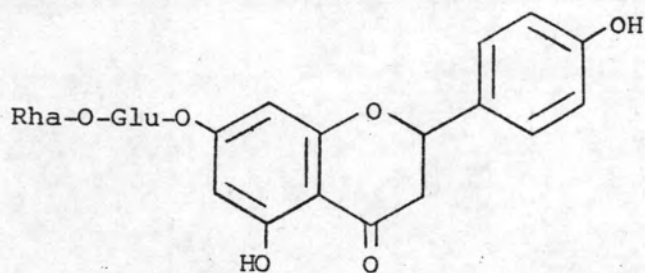
Naringin

(81)
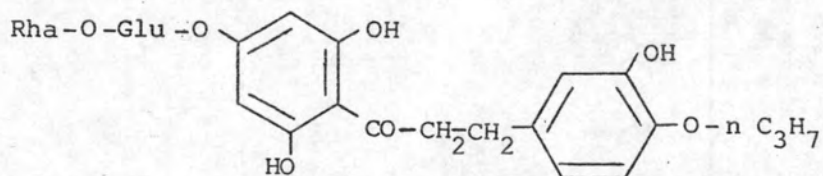
Neohesperidoside

(82)

2.4 Dihydroflavonols

These colourless substances are simple reduction products of flavonols, and like chalcones are possible precursors of the more highly oxidized flavonoids in plants. Dihydroflavonols have no particularly interesting taste properties, but are fairly widespread in nature. Dihydroquercetin (83), for example, is a well known heartwood constituent of many trees, particularly gymnosperms, and has more recently been found in flowers e.g. *Petunia* and leaves e.g. *Rhododendron*, in glycosidic form (Harborne, 1973).
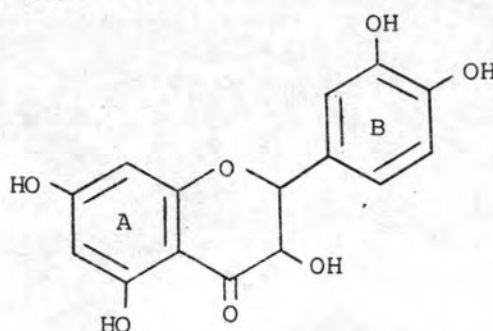
Dihydroquercetin

(83)

2.5 Isoflavones

Isoflavones e.g. genistein (84), of the leaves of Dyer's broom, *Genista tinctoria* Linn., are isomeric with flavones and are derived biosynthetically from the same C_{15} precursor by aryl migration. They are, again, colourless substances, though they are capable of forming colours with metals and were once used as mordant dyes. Structural variation in this series is quite considerable, and over sixty different isoflavones have been described in nature. They occur quite characteristically in one subfamily of the Leguminosae, the Papilionaceae, and are rarely found elsewhere. Genistein (84), for example, besides occurring in *Genista*, is also found in *Lupinus*,

Sophora, Glycine, Trifolium, and *Ulex* species. It occurs in these
plants in glycosidic form as the 7- or 4´ glucoside or as the 7- or
4´-rutinoside.

More complex phenols having the isoflavan skeleton
are known. Examples are rotenoids, insecticide present in *Derris*
root, such as rotenone (85), and the coumarinochromans, such as
pisatin (86), the phytoalexin in *Pisum sativum Linn*. These complex
isoflavonoids, like the simple isoflavones which are weak oestrogens,
are physiologically active (Harborne, 1973).
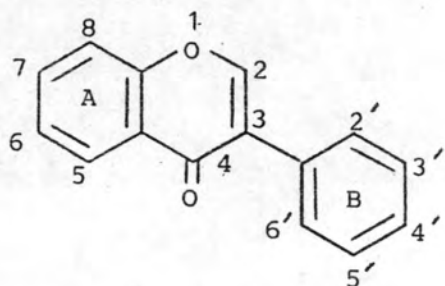
Isoflavone nucleus
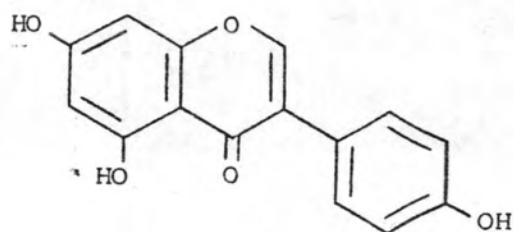
Genistein

(84)
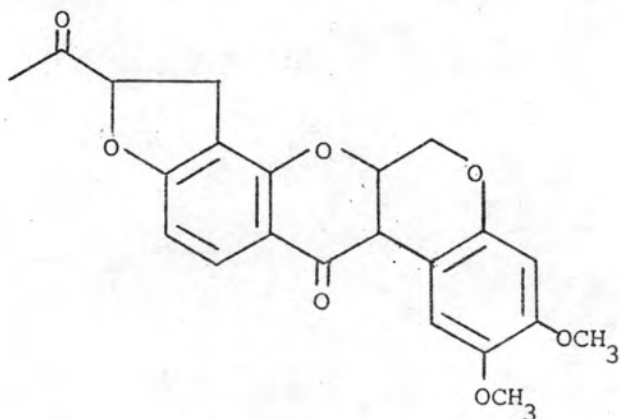
Rotenone

(85)
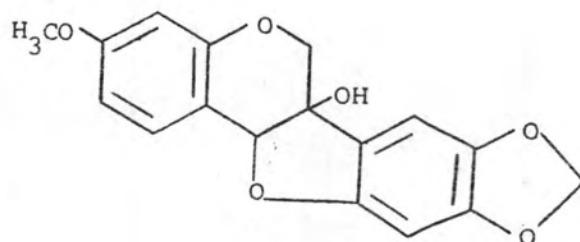
Pisatin

(86)

2.6 Biflavonyls

These compounds are flavonoid dimers, presumably formed *in vivo* by oxidative coupling from the monomeric flavones. Nearly all known substances are dimers of apigenin, they fall into three classes depending on whether their linkage is through a carbon-carbon bond between the 8- and 3'-positions (amentoflavone), 8- and 8'-positions (cupressoflavone) or through a carbon-oxygen bond between the 8- and 4'-positions (hinokiflavone). Most known biflavonyls are mono-, di-, or trimethyl ethers of amentoflavone (87). They have a striking distribution pattern in nature in that they are almost completely confined to the gymnosperms, they are very widespread here, apart from their singular absence from the Pinaceae. In the angiosperms, they have been positively reported in only a very few plants, notably in *Casuarina stricta* Miq. and *Viburnum prunifolium* Linn. In addition, biflavonyls have been detected in such lower plants as *Selaginella tamariscina* (Beauv.) Spring, *Psilotum triquetrum* Sw. Other related flavonoid dimers have been reported in plants in recent years. These include the biflavanones found in *Garcinia* (Guttiferae) and the catechin-flavan-3, 4 diol dimers which are the simplest type of condensed tannin in nature (Harborne, 1973).
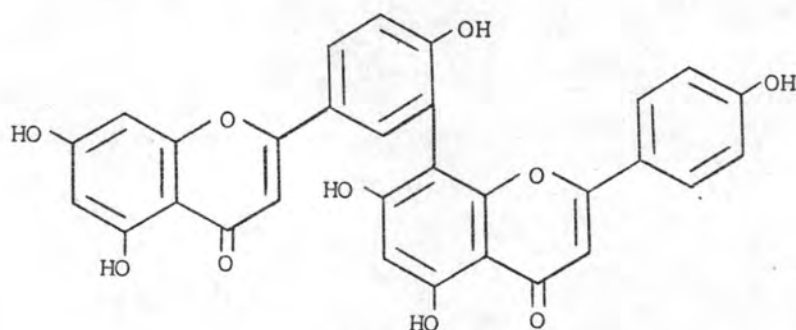
Amentoflavone

(87)

2.7 Leucoanthocyanidins

The terminology of leucoanthocyanidin was reserved
for the monomeric proanthocyanidins such as the phenolic flavan-3, 4-
diols (Haslam, 1982). Tissue containing leucoanthocyanidins, when
heated with 2N hydrochloric acid, give rise to anthocyanidins. These
unlike the parent substances are red in acid solution and may be
extracted with amyl alcohol (Gibb, 1974).
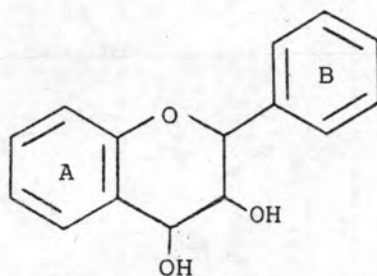
Leucoanthocyanidin nucleus

Biosynthesis of flavonoids

By feeding C^{14}- labelled acetate, phenylalanine, cinnamic acid
or shikimic acid to tobacco, buckwheat, and red cabbage, Neîsh,
Grisebach, and others have demonstrated quite conclusively that the
C_{15} skeleton of flavonoids is derived from two separate pathways,
from acetate and from shikimic acid (Figure 4). The A-ring arises by
the head-to-tail condensation of two malonyl Co-A units and acetyl
Co-A. The B-ring and the C_3 unit come from a C_6-C_3 precursor, which
may be cinnamic acid itself (Harborne, 1973). The interconversion of
flavonoid compounds are shown in Figure 5, page 51.
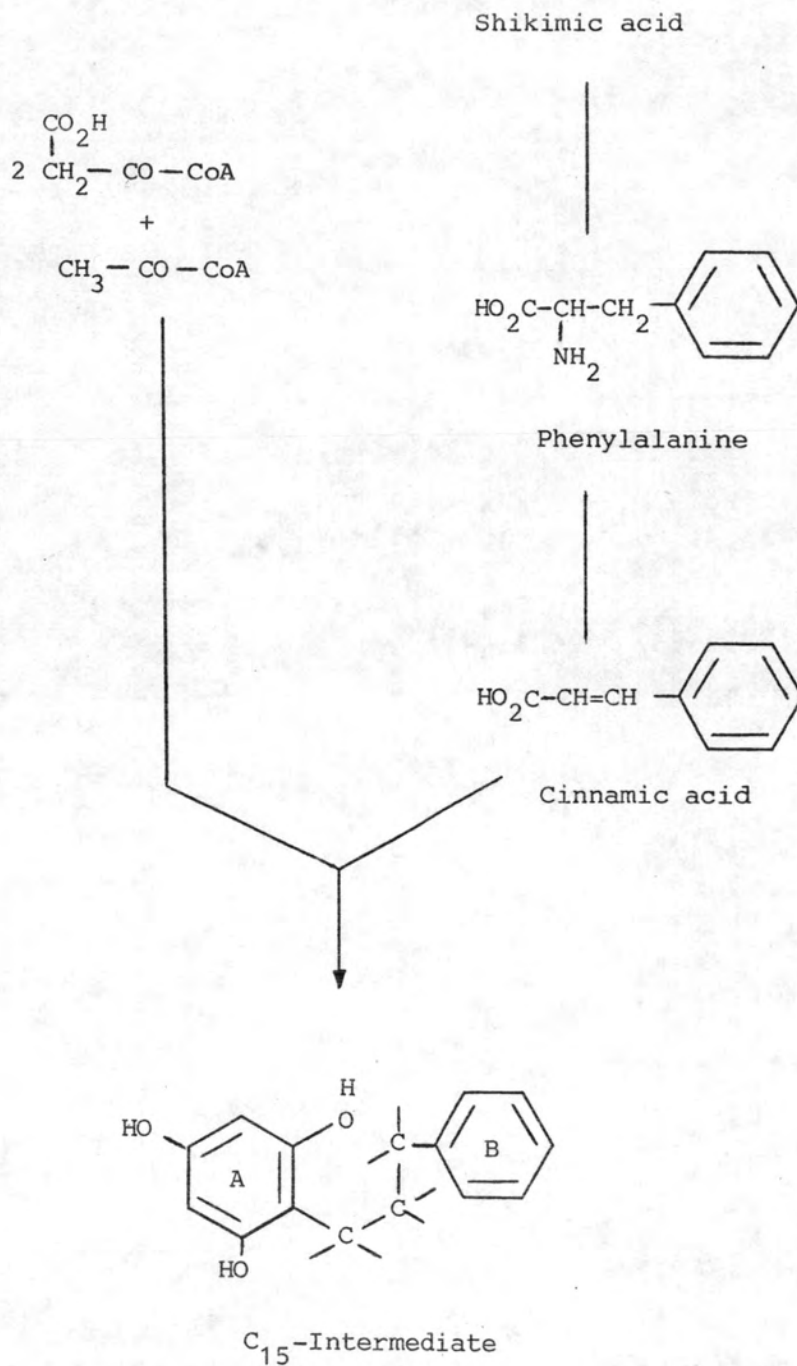
Figure 4   Biosynthetic pathways to flavonoids (Harborne, 1973).

Leucoanthocyanin

↑

Polymerization

Flavanone   Isoflavone   Flavan-3,4-diol

⇅   aryl migration ↗   ↑

Isomerization   + 2H

C_{15}Intermediate ⟶ Chalcone ⟶$+ OH$ Flavanonol ⟶ [Flavenol]

↓ - 2H   ↓ - 2H   ↓

Flavone   Flavonol   Anthocyanidin
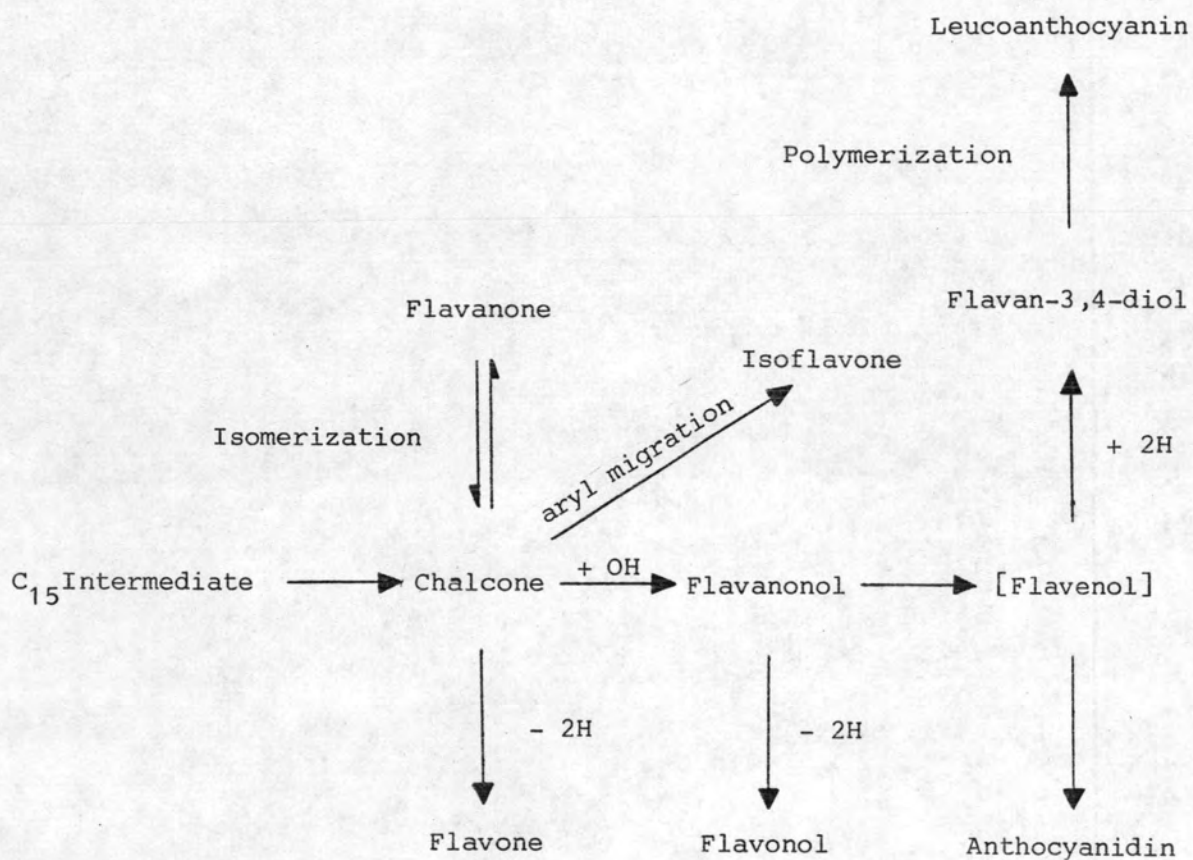
Figure 5   Interconversions of flavonoid compounds

(Harborne, 1973)

Biosynthesis of Flavones

Flavones can be formed by the oxidation of flavanone by flavanone oxidase (Figure 6, pathway A). Another possible route to flavones is indicated in Figure 7, in which the oxidation takes place at the chalcone level (Figure 6, pathway B) *via* a hypothetical chalcone epoxide. Dibenzoylmethanes of the type envisaged in Figure 7 are natural products and can easily be converted chemically into flavones. Chalcone epoxides are easily converted into dibenzoylmethanes photochemically (Goodwin and Mercer, 1983).

Chalcone

Flavanone

B

A
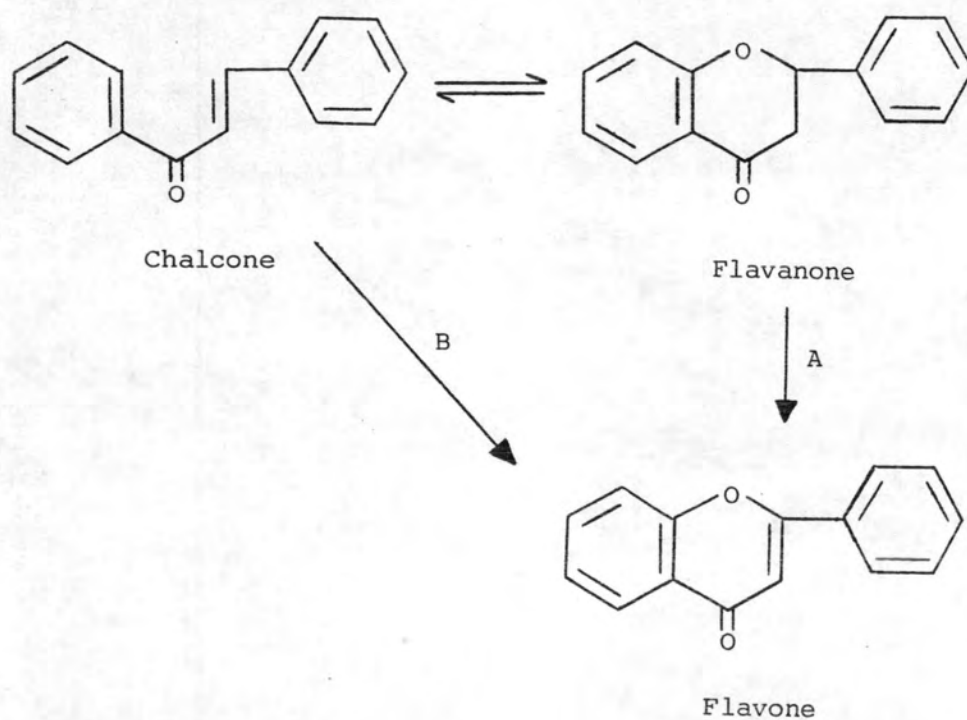
Flavone

Figure 6   General pattern of flavone biosynthesis from chalcone
            (Goodwin and Mercer, 1983)

Chalcone

Chalcone epoxide

Flavone

Dibenzoylmethane
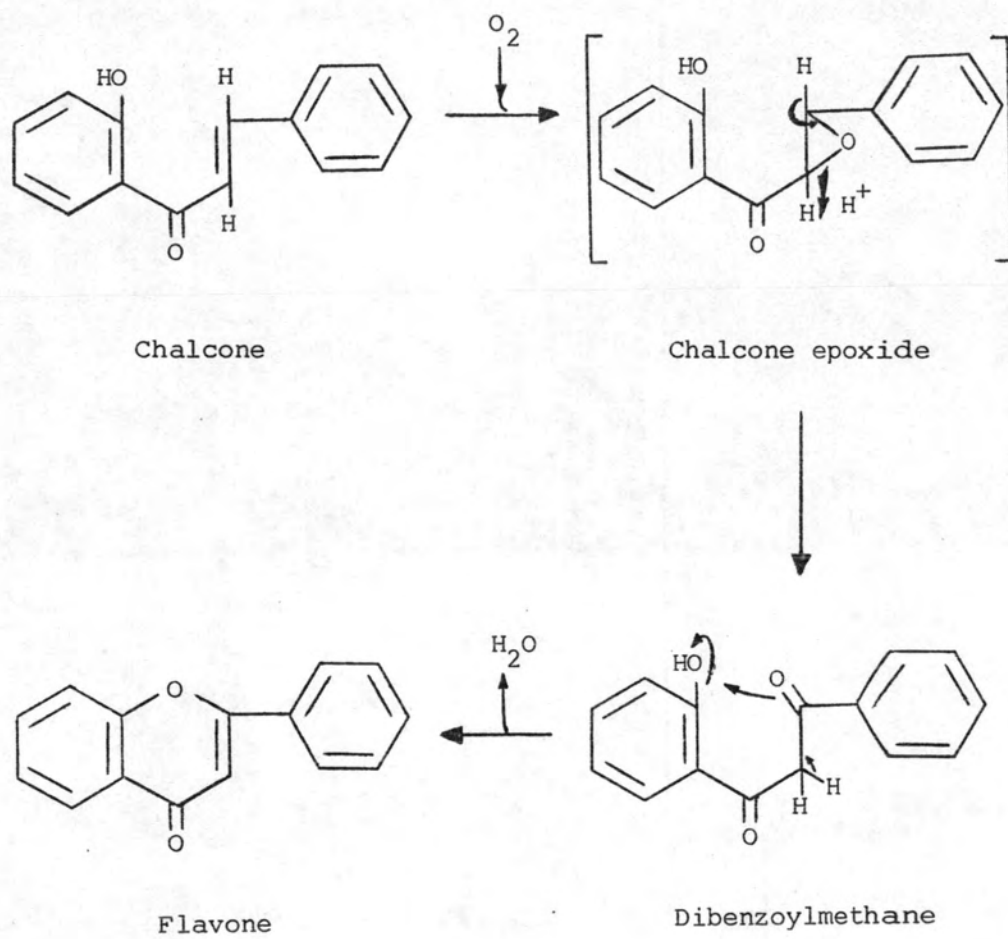
Figure 7 Possible route for the conversion of a chalcone into a flavone

(Goodwin and Mercer, 1983)

Functions of Flavonoids

The function of flavonoid pigments in flower petals is clearly known to attract pollination vectors (bees, birds, butterflies, and moths) to the plants. Colour is thus, with scent and nectar, one of the main attracting forces by which plants ensure the fertilization of the flower, subsequent seed set and hence survival. This function has been realized since the time of Charles Darwin (1876) and has been re-emphasized again in modern ecological studies of pollination mechanisms in higher plants (Faegri and Van der Pijl, 1966).

The purpose of flavonoids in leaves and other organs is more obscure and no single overiding function has emerged, as it has for leaf carotenoid pigments, now recognized as being accessory photosynthetic pigments of chlorophyll. While the same set of carotenoids occurs in all higher plant leaves, this is not true of flavonoids. A wide range of different flavonoids accumulates in leaves, suggesting a multiplicity of functions for them, which may well vary from family to family.

In recent years, presumptive evidence has been obtained for a function of leaf flavonoids in disease resistance in some plants, in controlling plant growth in others, and in repelling or attracting insect predators.

These evidence have been based mainly on *in vitro* experiments and further studies are required to established and obligatory role for the flavonoids concerned. One consistent factor emerging from *in vitro* studies of flavonoids is the importance of the phenolic hydroxylation pattern and the fact that small changes in this pattern

can produce large changes in flavonoid properties. The flavonoid molecule, because of this, provides a very versatile basis for a wide range of differing function (Harborne, 1972).

Relation between Structure and Function of Flavonoids

The reason why plants accumulate flavonoid constituents in their leaves and flowers, often in high concentrations, is not altogether clear even today. However, because flavonoids are not closely involved in primary metabolism it is probably that their major role is a biological one, in relation to insects and animals which pollinate or feed on plants. Some flavonoids, certainly provide the plant with one of a number of means (colour) of attracting insects such as butterflies and bees to them for purpose of pollination; and others may well provide a means (bitter taste) of repelling caterpillars from feeding on their leaves. From the evolutionary point of view, plants may develop the ability to synthesize a particular flower colour by different routes (e.g. yellow pigmentation may be carotenoid or flavonoid), and similarly bitterness may be given by a range of totally unrelated secondary constituents (i.e. terpenoid derived cucurbitacins, alkaloids such as quinine, or flavanones such as naringin). Such factors would explain the apparently sporadic distribution of some flavonoids (e.g. flavanones) and the more constant occurrence of others (e.g. anthocyanins) (Harborne, 1973).

Harborne (1973) demonstrated that the structural features of flavonoids known to be of importance in function are:

(1) presence of an extended conjugated resonating system, often with a carbonyl chromophore.

(2) presence of aromatic hydroxyl group, and

(3) molecular shape.

The first feature means that many flavonoids are coloured and are important pigments in plants. The presence of aromatic hydroxyl groups and the fact that flavonoids can be broadly divided into two groups depending on whether the B-ring is substituted with a 4'-hydroxyl or a 3', 4'-dihydroxyl means that flavonoids are capable of alternately inhibiting or stimulating certain enzyme system. Third, molecular shape is important, particularly in the case of isoflavonoids, because their physiological activity can be accounted for, by their similarity in structure to animal hormones (Harborne, 1973).

Physiological Activities of Flavones

Flavones are known to prevent the contraction of rabbit intestine and other muscles (Mentzer, Ferrando, Bost, Pillon & Garnier, 1953). Small quantities of flavones may act as cardiac stimulant, but in larger doses systolic stoppage occurs (Fukuda, 1932). However, they are not normally considered to be toxic, especially when ingested, and if injected into the blood stream they are either eliminated intact as conjugates with sulphuric or glucuronic acids, on else degraded to simpler phenols and then excreted (Haley & Bassin, 1952). Quercetin (73), for example, is degraded by rats to m-hydroxyphenylacetic acid, homovanillic acid, vanillic acid and 3, 4-dihydroxyphenylacetic acid, all these products coming from ring B. Although the mechanism of the effect is obscure, some flavones, such as quercetin (73), appear to strengthen weak capillary blood vessels and are, therefore, of interest in the search for means of counteracting the evils of exposure to radioactivity (Krewson & Couch, 1952). Highly hydroxylated flavones

act as diuretics (Koike, 1931). Flavones behave like auxins in stimulating the germination of wheat seeds (Di Maggio, 1954).

Distribution and Chemotaxonomy of Flavonoids

As a result of general plant surveys for anthocyanins and flavonols and of more detailed flavonoid studies of some 200 or more plant species, Harborne (1973) assembled the distribution of flavonoids in plants. Four main generalizations can be made, which are summarized in the following paragraphs:

"(1) General complexity in pattern increases with evolutionary advancement. The morphologically primitive groups such as mosses, horsetails, and ferns have simple flavonoids, the more highly specialized angiosperms have much more complex flavonoid constituents. As to be expected, the monocotyledons and the dicotyledons, the two major angiosperm division and both highly advanced, show no significant differences in their flavonoid patterns.

(2) The changeover from woody to herbaceous habit in the angiosperms has caused a corresponding change in flavonoid pattern, chiefly in the leaves. The woody pattern is characterized by high concentrations of flavonol, presence of myricetin and leucoanthocyanidins, and frequent occurrence of cyanidin. By contrast, the herbaceous pattern is shown by infrequency of flavonol, almost complete absence of myricetin and leucoanthocyanidins, presence of flavones, and frequent occurrence of delphinidin or pelargonidin (as flower pigments).

(3) Anthocyanin type is correlated with natural selection for flower colour. Thus, selection for blueness in the Boraginaceae, a family which has mainly bee-pollinated flowers, has led to delphinidin being the principal petal pigment, whereas selection for red in the bird-pollinated Gesneriaceae and Bignoniaceae has produced plants with pelargonidin and apigenidin as flower pigments. Further proof of this point is that in wind-pollinated families such as the grasses, Gramineae, and the sedges, Cyperaceae, there is no selection for inflorescence colour; for example, cyanidin, the biosynthetically simplest anthocyanidin, is uniformly present in the Gramineae.

(4) Certain classes of flavonoids are restricted to single plant groups. Example are the biflavonyls, found only in abundance in the Gymnospermae, and the isoflavones, which are characteristic of the Leguminosae. Exceptions exist, however, which prevent such characters being of use for purposes of classification."

Distribution of Flavones in Higher Plant

Gibbs (1974) wrote a book described chemosystematics of flavones in several places as follows:

1. Flavones itself, and one or more simple hydroxy-derivatives occur as farina or meal on the surface of *Primula* and a few closely related members of Primulaceae.

2. Methyl-flavones are quite uncommon, but eucalyptin, 6-methyl-5-hydroxy-4', 7-dimethoxy-flavone, and sideroxylin all from *Eucalyptus spp.*

3. Flavone-C-glycosides are much commoner, and widely spread.

4. Many of the flavones are widely distributed in many plant groups of Subdivision Angiospermae. Since detailed investigations yield new flavones and new distributions in almost every case.

Flavones in the Compositae

In composites, the common flavones apigenin and luteolin are widely distributed; they are represented by several of their glucosides (Hegnauer, 1964). At the flavone level, trihydroxylation of the B-ring occurs, tricin 5-glucoside (88) being one of the main flavones of
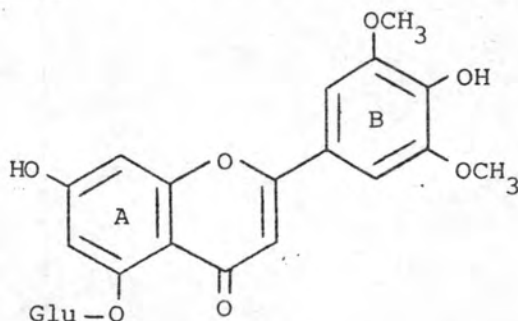
Tricin 5- glucoside

(88)

Cirsium arvense Linn. (Wallace, 1974) occurs. Some rarer flavones also occur in many composites and account for a somewhat distinctive flavone chemistry for the family. These features are:

1. Frequent occurrence of 6-hydroxylated flavones such as scutellarein (6-hydroxyapigenin) (89) and 6-hydroxyluteolin (90) (Hegnauer, 1977).

Scutellarein (89) 6-Hydroxyluteolin (90)
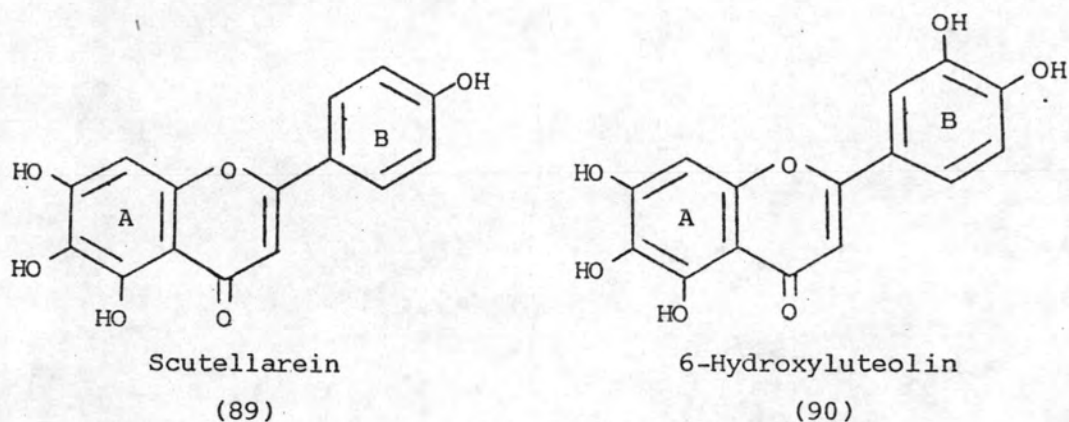
2. Methylated flavones occur free or as glycosides. Several of these compounds have cytotoxic properties (Kupchan, Sigel, Hemingway, Knox & Udayamurthy, 1969).

Example of methylated flavones are:

(91)
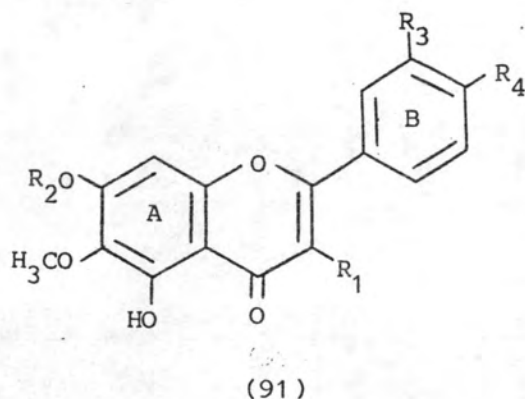
Artemitin $(R_1 = R_3 = R_4 = OCH_3, R_2 = CH_3)$

Jaceidin $(R_1 = R_4 = OCH_3, R_2 = H, R_3 = OH)$

Centaureidin $(R_1 = R_3 = OCH_3, R_2 = H, R_3 = OH)$

Eupatilin $(R_1 = R_2 = H, R_3 = R_4 = OCH_3)$

Eupatorin $(R_1 = H, R_2 = CH_3, R_3 = OH, R_4 = OCH_3)$

Eupafolin $(R_1 = R_2 = H, R_3 = R_4 = OH)$

Tetramethoxyflavone $(R_1 = H, R_2 = CH_3, R_3 = R_4 = OCH_3)$

Hispidulin $(R_1 = R_2 = R_3 = H, R_4 = OH)$

The Eupatorieae are rich in polymethoxylated flavones and flavonols and their glycosides, particularly apigenin and quercetin derivatives. Several flavonoids show cytotoxic activity (Kupchan *et al.*, 1969). *Eupatorium cuneifolium* Willd. afforded two cytotoxic flavones. Hispidulin also present in *E. rotundifolium* Linn. and eupatilin present in *Liatris punctata* Hook. etc. (Wagner, Iyengar & Herz, 1973).

Flavones Isolated from Species of *Ageratum*

The flavones reported to be distributed in genus *Ageratum* are summarized in Table 4

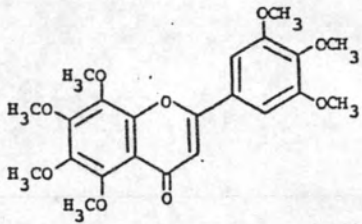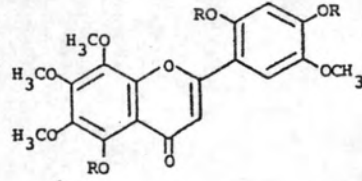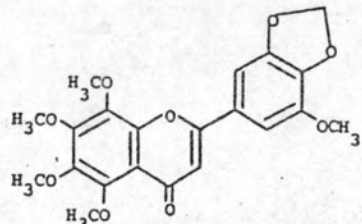Table 4 Flavones Isolated from Species of *Ageratum*

Plant	Flavone	Reference
Ageratum conyzoides Linn. (stem and leaf)	5′-Methoxynobiletin (Gardenin-A methyl ether)	Adesogan & Okunade, 1979
A. corymbosum Zucc. (aerial part)	1. Agecorynin-C 2. Agecorynin-D 1. R=CH₃ 2. R=H 3. Eupalestin (5′-Methoxylucidin dimethyl ether) 4. 5′-Methoxynobiletin	Quijano *et al.*, 1980

Table 4 Cont.

Plant	Flavone	Reference
A. houstonianum Mill. (aerial parts)	1. Agecorynin-C 2. Eupalestin 3. Agehoustin-A 4. Agehoustin-B 5. Agehoustin-C 6. Agehoustin-D	Quijano *et al.*, 1982 Quijano, Calderón, Gómez, Escolor & Rios, 1985

3. $R_1=R_2=R_3= OCH_3$

4. $R_1=H$, $R_2=R_3= OCH_3$

5. $R_1=R_3=OCH_3$, $R_2= OH$

6. $R_1=OCH_3$, $R_2=R_3= OH$

	7. Lucidin dimethyl ether	Quijano *et al.*,1982

The Occurrence of Chemical Compounds in *Ageratum conyzoides* Linn.

        Ageratum conyzoides Linn. are widely investigated, especially in the field of chemical studies. Many compounds, isolated from this plant, have been reported. The occurrence of chemical compounds in *A. conyzoides* Linn. are shown in Table 5.

Table 5. The Occurrence of Chemical Compounds in *Ageratum conyzoides* Linn.

Compound	Organic part	Type of compound	Reference
Ageratochromene	essential oil	oxygen heterocycle	Kasturi, Thomas & Abraham, 1973.
2, 2-Dimethyl-6-7 dimethoxy ageratochromene	essential oil	oxygen heterocycle	Sharma, Gary, Girgyne & Jain, 1980.
7-Demethoxy ageratochromene	essential oil	oxygen heterocycle	Pham Truong Thi Tho & Nguyen Van Dan, 1977.
2, 2-Dmethyl-7-methoxy ageratochromene	essential oil	oxygen heterocycle	Sharma *et al.*, 1980.
6,6',7-7-Tetramethoxy-2 2',2',2,-tetramethyl-3',4'-dehydro-3',4-(s)-bichroman	essential oil	oxygen heterocycle	Kasturi and Manithomas, 1967.
γ-Cadinene	essential oil	sesquiterpene	Sharma *et al.*, 1980.
Caffeic acid	leaf	lignan	Ramachandran, Kotiyal & Subramanian, 1977.
7-Methoxy-2,2-dimethyl-chromene	essential oil	oxygen heterocycle	Kasturi & Manithomas, 1967.

Table 5. Cont.

Compound	Organic part	Type of compound	Reference
Conyzorigun	leaf	chromene	Adesogan & Okunade, 1978.
Coumarin	essential oil	coumarin	Wehmer, 1929.
n-Dotriacontane	entire plant	alkane	Horng, Lin & Chen, 1976.
Essential oil	aerial parts	essential oil	Schimmel & Co, 1915.
Eugenol	essential oil	lignan	Joly, 1937.
Eugenol methyl ether	essential oil	lignan	Joly, 1937.
Friedelin	entire plant	triterpene	Hui & Lee, 1971.
Fumaric acid	leaf	alkene	Ramachandran *et at.*, 1977.
n-Hentriacontane	entire plant	alkane	Horng *et al.*, 1976.
n-Heptacosane	entire plant	alkane	Horng *et al.*, 1976.
Kaempferol	leaf	flavonoid	Ramachandran *et al.*, 1977.
Kaempferol-3,7-di-O-β-D-glucoside	aerial parts	flavonoid	Gill, Mionskowski, Janczewska & Kapsa, 1978
6-Demethoxyagerato-chromene	entire plant	oxygen heterocycle	Pham Truong Thi Tho & Nguyen Van Dan, 1976
Kaempferol-3-O-α-L-rhamnoside	aerial parts	flavonoid	Gill *et al.*, 1978.
Quercetin	leaf	flavonoid	Gill *et al.*, 1978.
Quercitrin	aerial parts	flavonoid	Gill *et al.*, 1978.

Table 5. Cont.

Compound	Organic part	Type of compound	Reference
β-Sitosterol	entire plant	steroid	Hui & Lee, 1971.
α-Spinasterol	entire plant	steroid	Horng *et al.*, 1976.
Stigmast-7-en-3-β-ol	leaf	steroid	Ramachandran *et al.*, 1977.
Stigmasterol	entire plant	steroid	Horng *et al.*, 1976.
Tritriacontene	leaf	alkene	Adesogan & Okunade, 1978.
5-Methoxynobiletin	stem, leaf	flavonoid	Adesogan & Okunade, 1978.