CAHPTER II

HISTORICAL

1. The Occurrence of Chemical Compounds in *Clausena*

Clausena species are widely investigated, especially in the field of chemical studies. Many chemical compounds, isolated from this plant, have been reported into two groups, coumarins and carbazole alkaloids. The occurrence of chemical compounds in *Clausena* species are shown on Table 1.

Table 1 The Occurrence of Chemical Compounds in *Clausena* species *Clausena anisata* Hook. f.

Compound	Plant part	Type of compound	References
Atamisatin	stem	Carbazole alkaloid	10
Chalepin	stem, root	Furanocoumarin	10
Clausenitin	root	Carbazole alkaloid	10
Coumurrayin	root	Simple coumarin	10
Imperatorin	root	Furanocoumarin	10
Mupamine	root bark	Carbazole alkaloid	11
Osthol	root bark	Simple coumarin	12
Xanthoxyletin	root bark	Pyranocoumarin	12
3-(1,1-dimethylallyl)-xanthoxyletin	root bark	Pyranocoumarin	12

Table 1 (continued) *Clausena dentata* (Willd.) R.& S.

Compound	Plant part	Type of compound	References
Dentatin	root bark	Pyranocoumarin	13
Imperatorin	root bark	Pyranocoumarin	13
Nordentatin	root bark	Pyranocoumarin	13

Clausena excavata Burm. f.

Compound	Plant part	Type of compound	References
Clausarin	root bark	Pyranocoumarin	7
Clausenidin	root, stem bark	Pyranocoumarin	7,14
Clausenin	root, stem bark	Pyranocoumarin	7,14
Heptaphylline	root bark	Carbazole alkaloid	7
Nordentatin	root bark	Pyranocoumarin	7
Xanthoxyletin	root bark	Pyranocoumarin	7

Clausena heptaphylla Wight & Arn.

Compound	Plant part	Type of compound	References
Clausenidin	root	Pyranocoumarin	15
Clausenin	root	Pyranocoumarin	15
Dentatin	root	Pyranocoumarin	16
Girinimbine	root	Carbazole alkaloid	17
Heptaphylline	root	Carbazole alkaloid	17

Table 1 (continued) *Clausena heptaphylla* Wight & Arn.

Compound	Plant part	Type of compound	References
Heptazaline	root	Carbazole alkaloid	16
Murrayanine	root	Carbazole alkaloid	16

Clausena indica Oliv.

Compound	Plant part	Type of compound	References
Chalepin	root	Furanocoumarin	18
Chalepensin	root	Furanocoumarin	18
Clausindine	root	Furanocoumarin	19
Byakangelicin	aerial part	Furanocoumarin	20
Imperatorin	aerial part	Furanocoumarin	18
Indicolactonediol	aerial part	Furanocoumarin	20
Phellopterin	root	Furanocoumarin	18
Suberosin	aerial part	Simple coumarin	20
Xanthotoxol	aerial part	Furanocoumarin	20
6-Methoxyheptaphylline	root	Carbazole alkaloid	21
Indizoline	root	Carbazole alkaloid	22

Table 1 (continued) *Clausena lansium* Skeels

Compound	Plant part	Type of compound	References
Dehydroindicolactone	Leaves	Furanocoumarin	23
Heptaphylline	Leaves	Carbazole alkaloid	24
Lansine	Leaves	Carbazole alkaloid	24

Clausena pentaphylla DC.

Compound	Plant part	Type of compound	References
Clausarin	root	Pyranocoumarin	25
Clausenidin	root	Pyranocoumarin	25
Clausmarin A	aerial part	Pyranocoumarin	26
Clausmarin B	aerial part	Pyranocoumarin	26
Dentatin	root	Pyranocoumarin	25
Heptaphylline	root	Carbazole alkaloid	25

Clausena willdenowii W. & A.

Compound	Plant part	Type of compound	References
3(1,1-dimethylallyl) - xanthyletin	root, bark	Pyranocoumarin	27

2. Coumarins

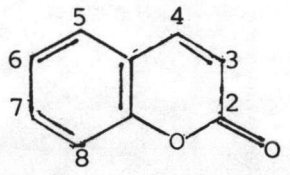
Coumarin, the parent compound possing 2H-1-benzopyran-2-one, which has the odour of fresh mown hay, occurs in over sixty plants and its derivatives occurs mainly in plants belonging to the families Umbelliferae, Rutaceae, Labiatae, Leguminosae and Orchidaceae. A few like novobiocin, alternariol and the aflatoxins are mould products. The only coumarins of animal origin known so far are the two benzo-coumarins of castoreum, the secretion of the scent gland of the beaver.[28]

2.1 Classification of Coumarins

No completely satisfactory classification of the coumarins is possible at present. Steck, W. and Mazurek, M.[29] have divided coumarins into two types. One is 'normal' type, which coumarins have an oxygen function at C-7 and hydrogens at C-3 and C-4 (unsubstituted pyrone ring). The other is 'abnormal' type, which coumarins either lack the C-7 oxygen or posses pyrone ring substituents. However, Seshadri and Vishwapaul,[30], have classified coumarins into five groups as follow:-

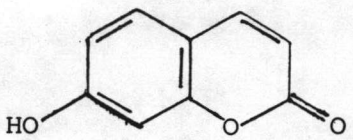
1) Simple coumarins

This type of coumarin have coumarin nucleus, which there are side chains substituted at the benzene ring. Umbelliferone, for exsample, the simplest coumarin of this type, has hydroxy group substituted at the 7-position of coumarin nucleus. 7-oxygenated coumarins are the most common in this type. There are widely distributed of simple coumarins in Rutaceae and Umbelliferae[32] and those are shown on Table 2.



2-H-1-benzopyran-2-one nucleus

(coumarin nucleus)



Umbelliferone

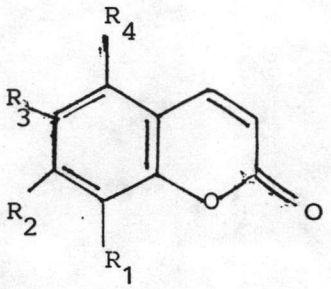
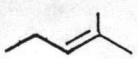
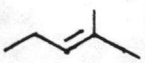
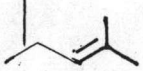
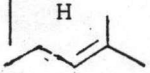


Table 2 The example of some simple coumarins

Compound	substituents				Source
	R_1	R_2	R_3	R_4	
Coumurrayin		OMe	H	OMe	*Clausena anisata* (Willd.) Oilv.[10]
Osthol		OMe	H	H	*Clausena anisata* (Willd.) Oliv.[12]
Suberosin	H	CH_3		H	*Clausena indica* Oilv.[20]
Osthenol		OH	H	H	*
Umbelliferone	H	OH	H	H	*
Peucedanol	H	OH		H	*

*These compounds can find commonly in Rutaceae.[33]

2) Furanocoumarin

These furan ring is fused with the coumarin nucleus at the various position on benzene ring to form linear or angular, are classified 'Furanocoumarin', which ring fused in a linear fashion are more common than in one of the several possible angular modes.[34] Considering the fusion of furan ring, furanocoumarin can be classified into six subtypes :-

a) Psoralene type (linear)

b) Angelicin type (angular)

c) Dihydrofuranocoumarin [4,3]

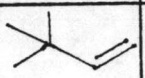
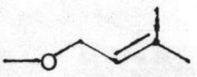
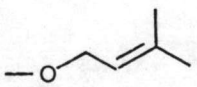
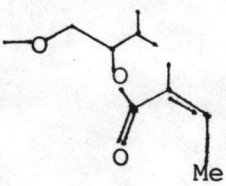
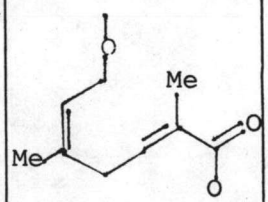
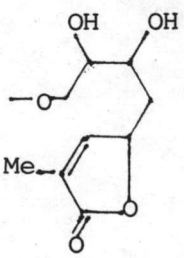
d) Dihydrofuranocoumarin [5,6]

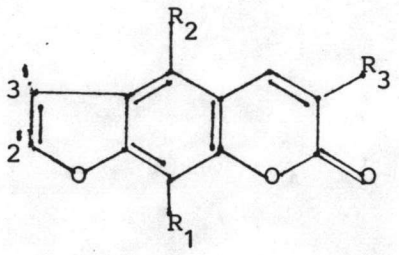
e) Dihydrofuranocoumarin [7,6]

f) Dihydrofuranocoumarin [8,7]

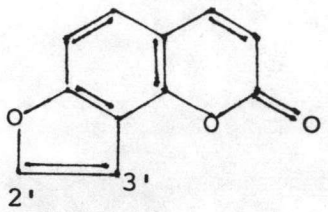
The difference of psoralene type and angelicin type are the position of fusion of furan ring on benzene ring of coumarin nucleus. Psoralene type, the furan ring is fused with the benzene ring at C-6 and C-7 positions as linear structure. But angelicin type, the furan ring is fused with the benzene ring at C-7 and C-8. Both psoralene type and angelicin type have similar structure of furan ring, and the position at C-2' and C-3' have double bond. Table 3 show the coumarin in psoralene type.

Table 3 The example of some Psoralene type

Compound	substituents			Source
	R_1	R_2	R_3	
Chalepensin	H	H		*Clausena indica* Oliv.[18]
Xanthotoxol	OH	H	H	*Clausena indica* Oliv.[20]
Imperatorin		H	H	*Clausena anisata* (Willd)[10] *Clausena dentata* (Willd)R.& S.[13] *Clausena indica* Oliv.[17]
Clausindine	H	H		*Clausena indica* Oliv.[19]
Phellopterin		OMe	H	*Clausena indica* Oliv.[18]
Byakangelicin		OMe	H	*Clausena indica* Oliv.[20]
Dehydroindico-lactone		H	H	*Clausena lanium* Skeels[23]
Indicolactone-diol		H	H	*Clausena indica* Oliv.[20]

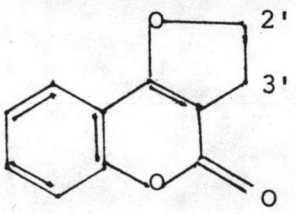


Psoralene nucleus

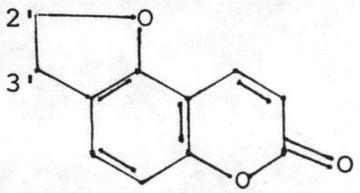


Angelicin nucleus

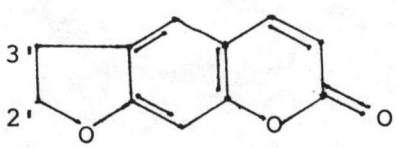
The position of coumarin nucleus, which is fused with the furan ring, can be fused the various types. For example, if the position at C-4 and C-3 of coumarin nucleus are fused with the furan ring, it is classified to 'Dihydrofuranocoumarin [4,3]. The real characteristic of Dihydrofuranocoumarin type is no the double bond at C-2' and C-3' of the furan ring. Dihydrofuranocoumarin [7,6], the furan ring is fused with the benzene ring at C-6 and C-7 position as same as the psoralene type, but the double bond at C-2' and C-3' position of furan ring is saturated. An example of 6,7-Dihydrofurano-coumarin is show in Table 4.



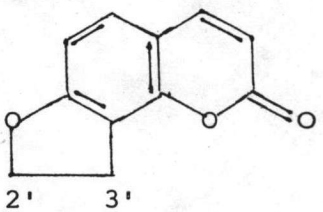
Dihydrofuranocoumarin [4,3]



Dihydrofuranocoumarin [6,5]



Dihydrofuranocoumarin [7,6]



Dihydrofuranocoumarin [8,7]

Furanocoumarin, like their simple relatives often have isoprenoid residues attached either to oxygen or to carbon. Indeed, the isopropyldihydrofuran ring can well be regarded as arising from cyclization of isoprenoid residues onto neighbouring hydroxyl groups in appropriately substituted coumarins.(34)

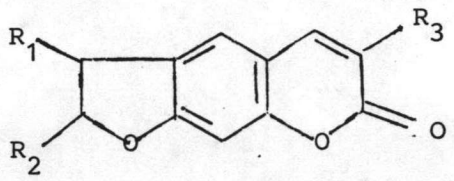


Table 4 The example of some 6,7-Dihydrofuranocoumarin

Compound	substituents			Souce
	R_1	R_2	R_3	
Chalepin	HO—	H		*Clausena anisata* (Willd.)Oliv.(10) *Clausena indica* Oliv.(18)
Rutamarin	AcO—	H		*
Xanthoarnol	HO—	OH	H	*

*These Compounds can find commonly in Rutaceae.(32)

3) Pyranocoumarins

The pyran ring is fused with the coumarin nucleus at various position on benzene ring to form linear or angular pyranocoumarin. They can be classified into five subtypes :-

a) Xanthyletin type (linear)

b) Xanthyletin type (angular)

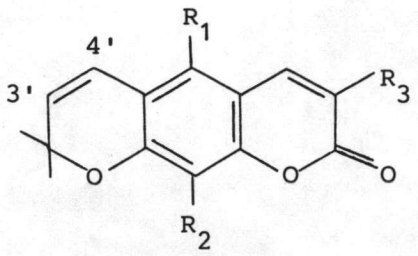
c) Seselin type (angular)

d) Dihydroxanthyletin type (linear)

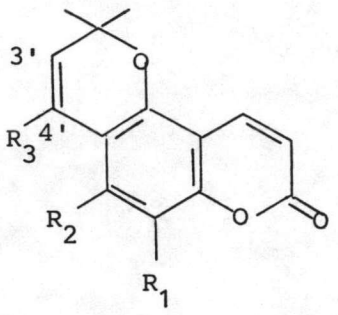
e) Dihydroseselin type (angular)

The various position on benzene ring of the coumarin nucleus can be fused with the pyran ring, are Xanthyletin (linear), Xanthyletin (angular) and Seselin (angular). The linear form, the pyran ring is fused at the position the C-6 and C-7 of the coumarin nucleus, while the angular form, the pyran ring is fused with the C-5 and C-6 of the coumarin nucleus. Both of them are classified into 'Xanthyletin', as well as the C-7 and C-8 fusion is classified into 'Seselin' type. The general characteristic of these type, are the C-3' and C-4' of the pyran ring has one double bond, and the C-2' is substituted with two methyl groups.

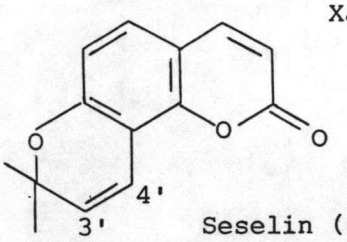
Table 5 and Table 6 show some coumarin in Xanthyletin (linear) and Xanthyletin (angular) respectively.



Xanthyletin (linear)



Xanthyletin (angular)



Seselin (angular)

Table 5 The example of some Xanthyletin (linear)

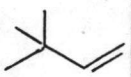
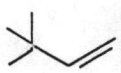
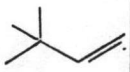
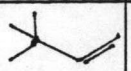
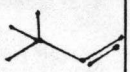
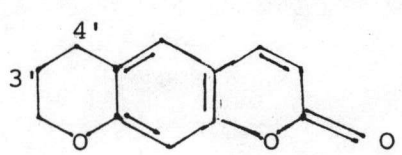
Compound	substituents			Source
	R_1	R_2	R_3	
Xanthoxyletin	OMe	H	H	*Clausena anisata* Willd.(12) *Clausena excavata* Burm.f.(17) *Clausena harmandiana* Pierre(9)
Clausarin	OH			*Clausena excavata* Burm.f.(7) *Clausena pentaphylla* DC.(25)
3-(1,1-dimethyl-allyl)-Xanthyletin	H	H		*Clausena anisata* (Willd.) Oliv.(12) *Clausena willdenowii* W. & A.(27)

Table 6 The example of some Xanthyletin (angular)

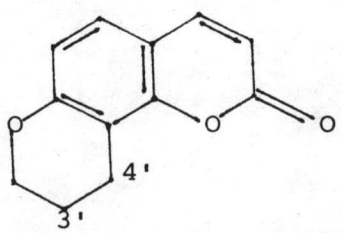
Compound	Substituents			Source
	R_1	R_2	R_3	
Dentatin		OMe	H	*Clausena harmandiana* Pierre (9) *Clausena dentata* (Willd.) R.& S. (13) *Clausena heptaphylla* Wight & Arn. (18)
Nordentatin		OH	H	*Clausena dentata* (Willd.) R.& S. (13) *Clausena excavata* Burm. f. (7)
Alloxanthoxyletin	H	OMe	H	*

*These compounds can find commonly in Rutaceae (32)

The dihydroxanthylein type and dihydroseselin type are as same as Xanthyletin type (linear) and seselin type (angular), but the position C-3' and C-4' has no double bond. The example of dihydroxanthyletin type coumarins are shown on Table 7.

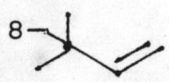
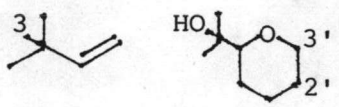


Dihydroxanthyletin (linear)



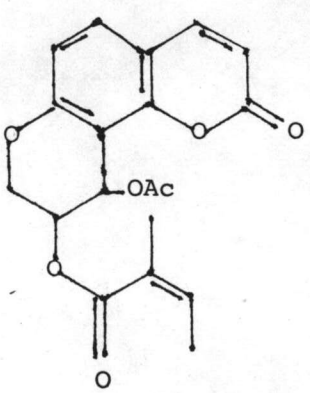
Dihydroseselin (angular)

Table 7 The example of some dihydroxanthyletin (linear)

Compounds	Structure	Source
Clausenin	5-OH, 4'=O	*Clausena excavata* Burm. f.[7] *Clausena heptaphylla* Wight & Arn.[18]
Clausenidin	5-OH, 4'=O 	*Clausena excavata* Burm. f.[14] *Clausena heptaphylla* Wight & Arn.[18] *Clausena pentaphylla* DC.[25]
Clausemarin A,B		*Clausena pentaphylla* DC.[26]
Decursinol	3'-OH	*

*These compounds can find commonly in Rutaceae[32]

For dihydroseselin (angular) type, coumarin which can be found in *Pteryxia terebinthina* var. *californica*, was isopteryxin.[12]

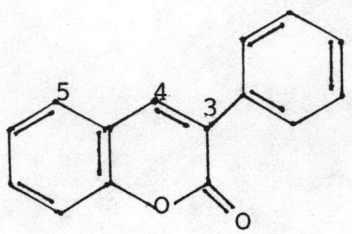


Isopteryxin

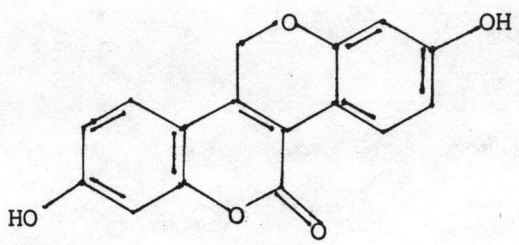
4) Phenyl coumarins

There is phenyl substituted at C-3 or C-4 of coumarin nucleus. This type is divided into seven subtypes :-

a) 3-Phenylcoumarins



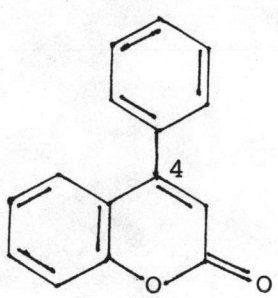
3-phenylcoumarin nucleus



Coumestrol

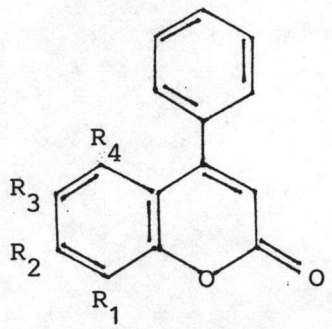
Coumestrol, which was found in ladino clover and alfalfa[28], is the example of coumarin in this type.

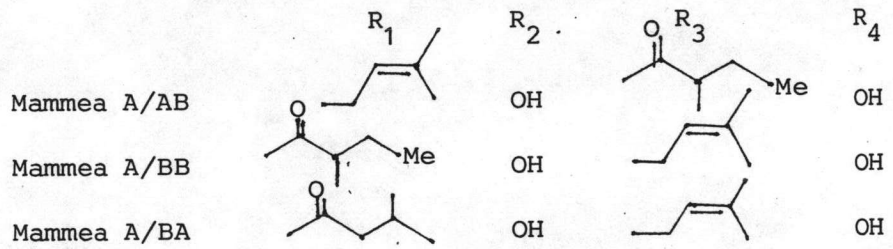
b) 4-Phenylcoumarins



4-Phenylcoumarin nucleus

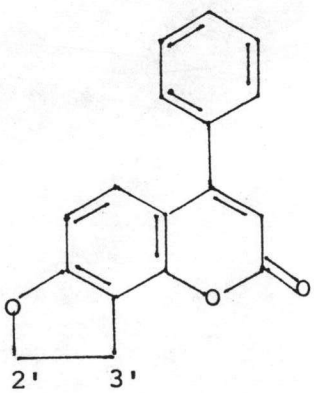
For example Mammea A/AB, Mammea A/BB and Mammea A/BA, which can be found in *Mammea americana* L.,[30] are coumarins in this type.



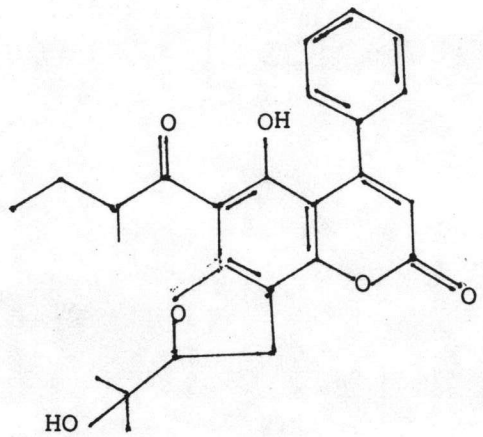


c) 4-Phenyldihydroangelicin

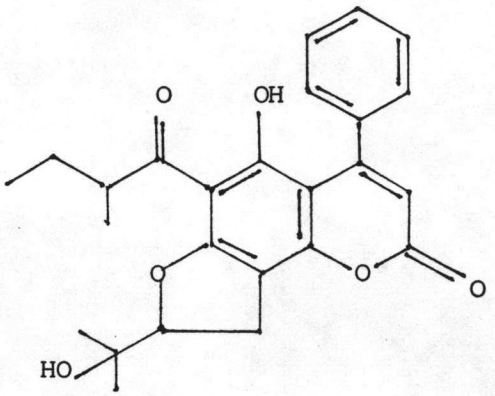
There is a phenyl ring substituted at C-4 of the dihydro-furanocoumarin [7,8] nucleus



Mammea americana L. contiuned phenylmammea A,B[30] which were compounds of this type.

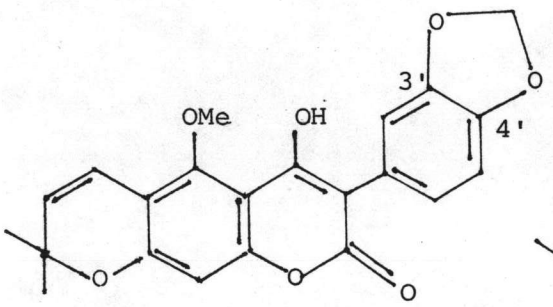


Phenylmammea A



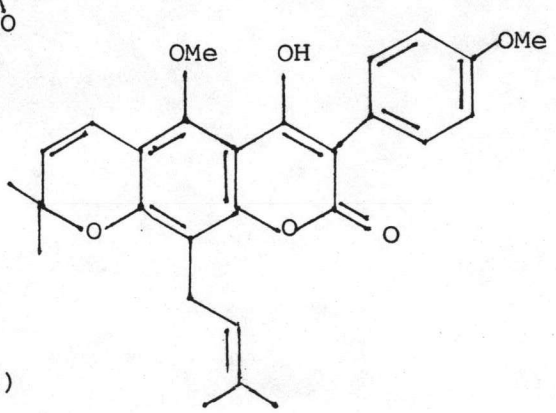
Phenylmammea B

d) 3-Phenylxanthyletin



Robustin

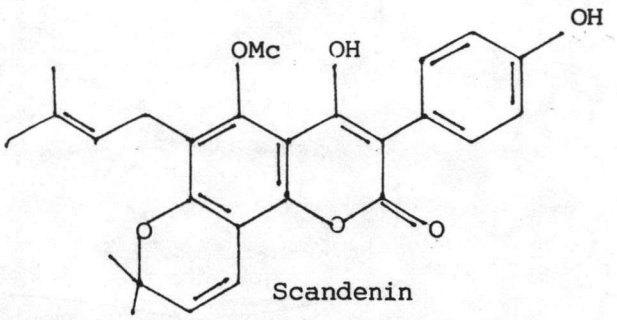
(*Derris robusta* Roxb.)[30]



Lonchocarpenin

(*Derris scandens* Roxb.)[30]

e) 3-Phenylseselin



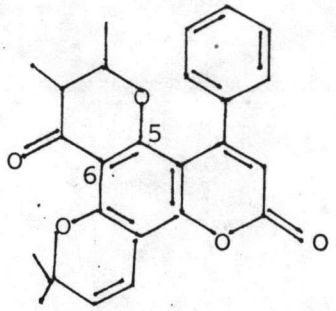
Scandenin

(*Derris scandens* Roxb.)[30]

An example of the compound in this type is scandenin.

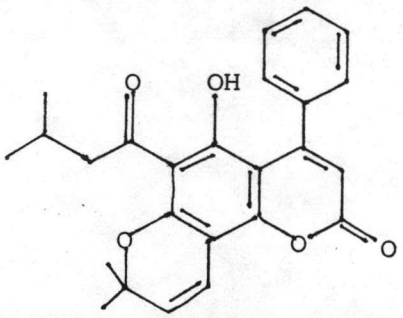
f) 4-Phenylseselin (angular)

Tomentolide A and Mammeigin are the example.



Tomentolide A

(*Calophyllum apetalum* Willd.)[30]

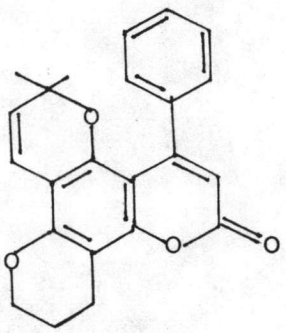


Mammeigin

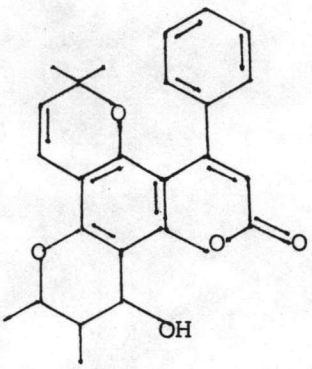
(*Mesua americana* L.)[30]

g) Tripyran derivatives

Two pyran ring and one phenyl ring attacked in the structure of coumarin nucleus.



Tripyran derivatives

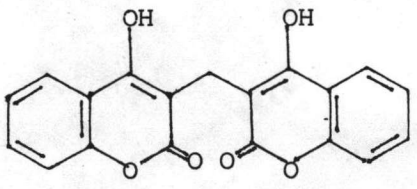


Inophyllum A

(*Calophyllum inophyllum* L.)[31]

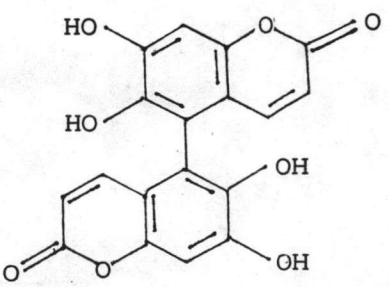
5) Bicoumarins

Two coumarin nucleus unit are bond with or without methylene group.



Dicoumarol

(*Melilotus officinalis* L.)[31]



Euphorbetin

(*Euphorbia lathyris* L.)[31]

2.2 Biosynthesis of coumarins

The coumarins are typical metabolites of higher plants, and have only seldom been isolated from microorganisms. The benzo-2-pyrone nucleus of the simple coumarins derives from the phenylacrylic skeleton of cinnamic acids via *ortho*-hydroxylation, *trans-cis* isomerisation of the side chain double bond, and lactonisation.[35] Early studies on the biosynthetic reaction leading to the simple coumarins in plants demonstrated derivation from the shikimic acid pathway via cinnamic acid and either *ortho*- or *para*-hydroxycinnamic acid. Radiotracer experiments later indicated that the furan chiefly in the Rutaceae and Umbelliferae, is constructed on the coumarin nucleus following attachment of a mevalonate-derived side-chain to Umbelliferone, and cyclization to dihydrofuranocoumarin intermediates. These findings have been substantiated and extended by the isolation and characterization of several of the enzymes participating in the biosynthetic pathway.[36]

The common precursors of the benzopyrone nucleus was showed in early biosynthetic pathways, are shikimic acid, phenylalanine and *trans*-cinnamic acid.[36] Hydroxylation of the *o*-position of the particular cinnamic acid in question takes place first and the resultant *o*-coumaric acid derivative is subsequently glucosylated. It is then rearranged in a spontaneous light-dependent reaction to the corresponding coumaric acid glucoside, which is structure derived from *cis*-cinnamic acid. By enzymetic elimination of glucose, free coumarinic acid is formed which cyclizes spontaneously to coumarin.[31] All known rutaceous coumarin are oxygenated at C-7 indicating that

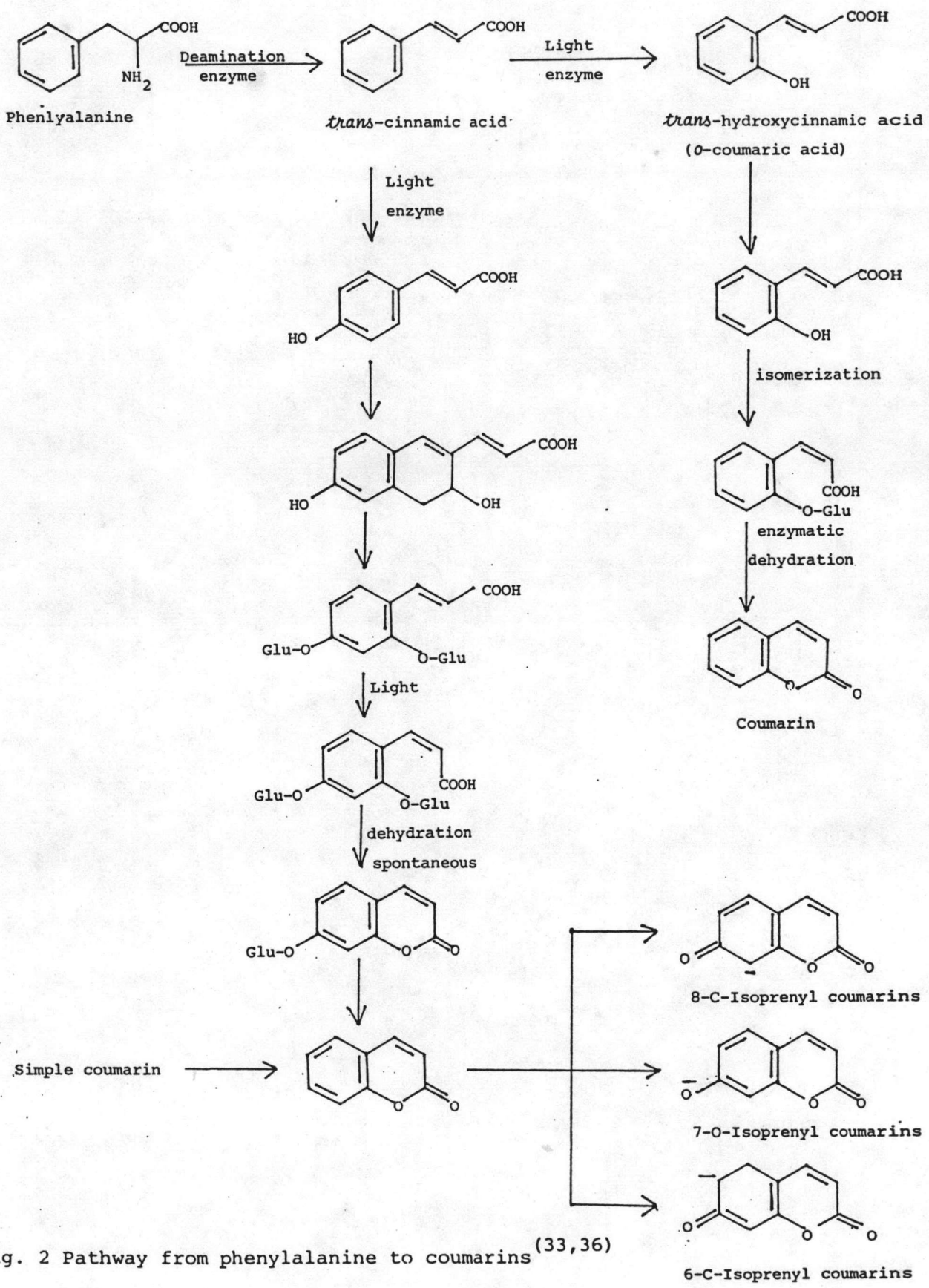
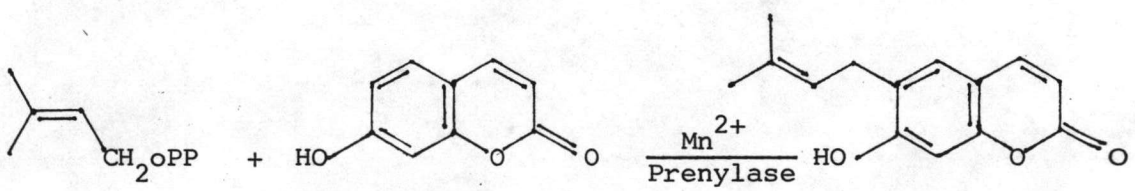


Fig. 2 Pathway from phenylalanine to coumarins[33,36]

the *trans* and *cis* p-coumaric acids are the usual precursors.[33] (Fig. 2)

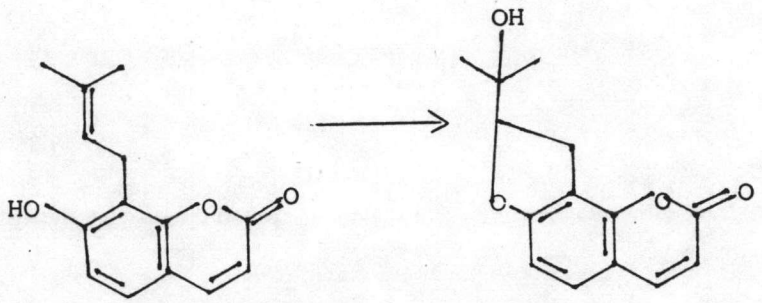
The major feature in the diversification of simple coumarins in both Rutaceae and Umbelliferae is the widespread incorporation of prenyl unit. Prenylation has been demonstrated to occur at the umbelliferone stage which will permit the electrophilic attack of carbonium ion at either C-6 or C-8 to yield C-prenyl coumarins or on the phenoxide to give *O*-prenyl compounds. Perhaps the role of the prenylating enzyme is to lacalize the charge on the anion and thereby direct the attack of the prenyl unit.[33] In *Ruta graveolens* L., the addition of the dimethylallyl unit at C-6 appears to be specifically controlled by enzyme dimethylallylphosphate (prenyl) transferase, which has a requirement for Mn^{2+} to form demethylsuberosin. (Fig. 3)



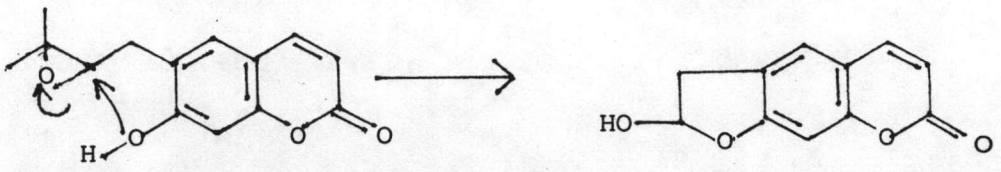
Umbelliferone demethylsuberosin

Fig. 3 Formation of demethylsuberosin by a prenylase from *Ruta graveolens* L.[36]

In more complex compounds, from tracer experiment have demonstrated that 7-demethylsuberosin (Fig. 4) is a precursor of linear furanocoumarins (marmesin) and osthenol is of the angular furanocoumarins (columbianetin).



Osthenol Columbianetin



Demethylsuberosin marmesin

Fig. 4 Hypothetical cyclization reaction to marmesin formation

The mechanism, in which a carbonium ion is qenerated at C-4' followed by a 1,3-elimination (isopropyl side chain) form marmesin and columbianetin as shown in Fig.5. The 3 carbon side chain being converted to acetone in the process and the double bond introduced to form the furan ring.

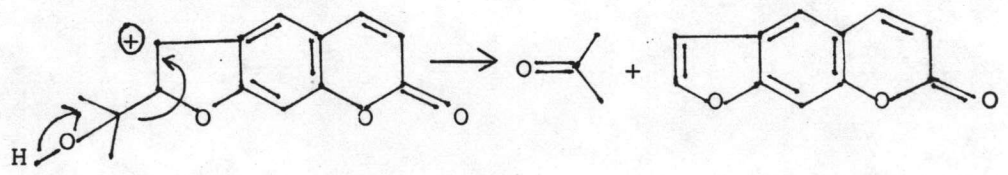


Fig. 5 Mechanism proposed by Birch et al. for the conversion of marmesin to psoralen.[36]

These observation were confirmed by carrying out the same feeding experiments with cell cultures of *Ruta graveolens* L. In

addition, it was found that both demethylsuberosin and marmesin were excellently incorporated into four coumarins with degraded isoprenoid side-chains, i.e. psoralen, xanthotoxin, bergapten and isopimpinellin. Also, psoralen was found to be a good precursor of the above three methoxylated coumarins.[31] (Fig. 6)

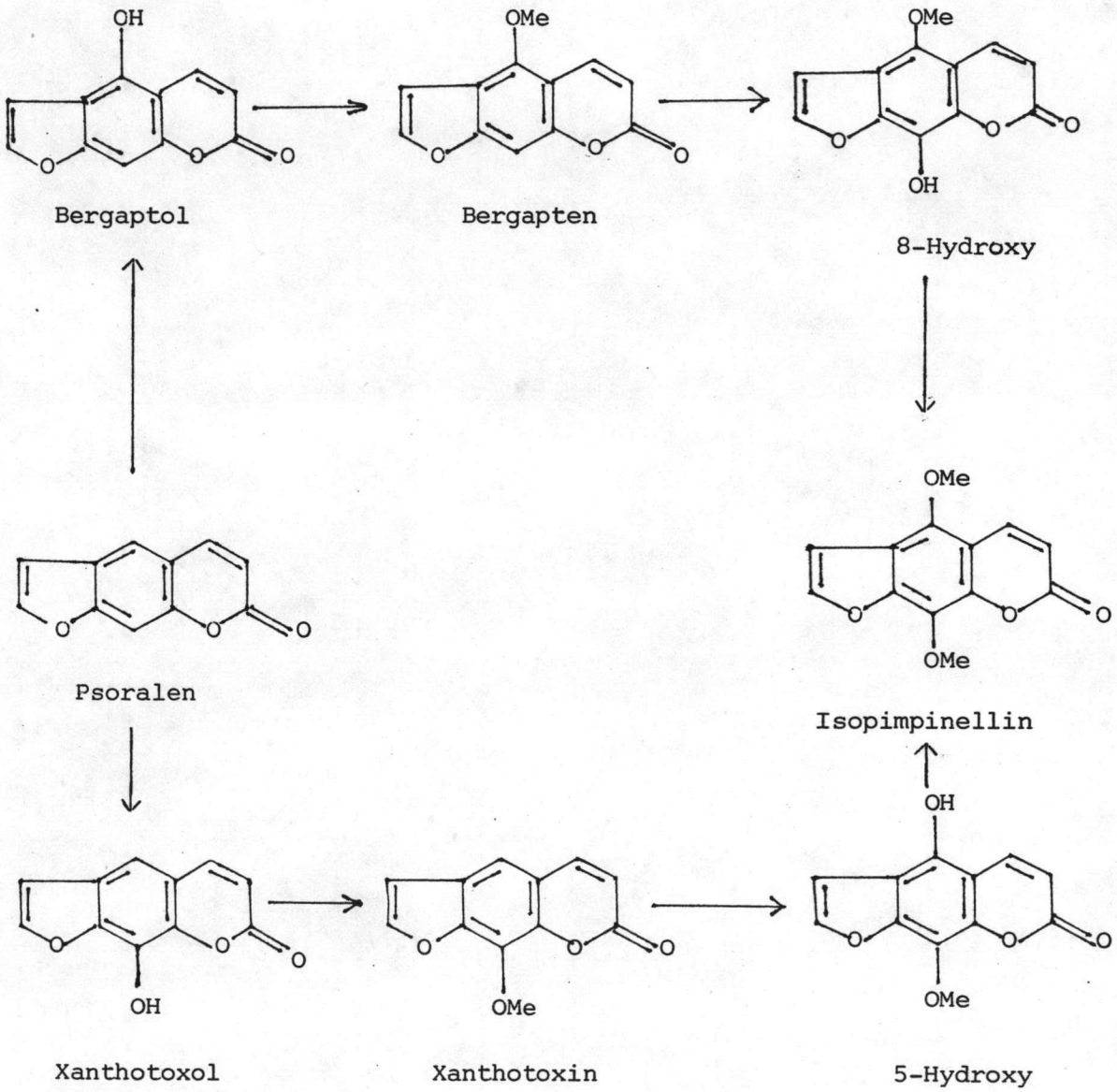


Fig. 6 Biosynthetic routes to Isopimpinellin in *Ruta* [36]

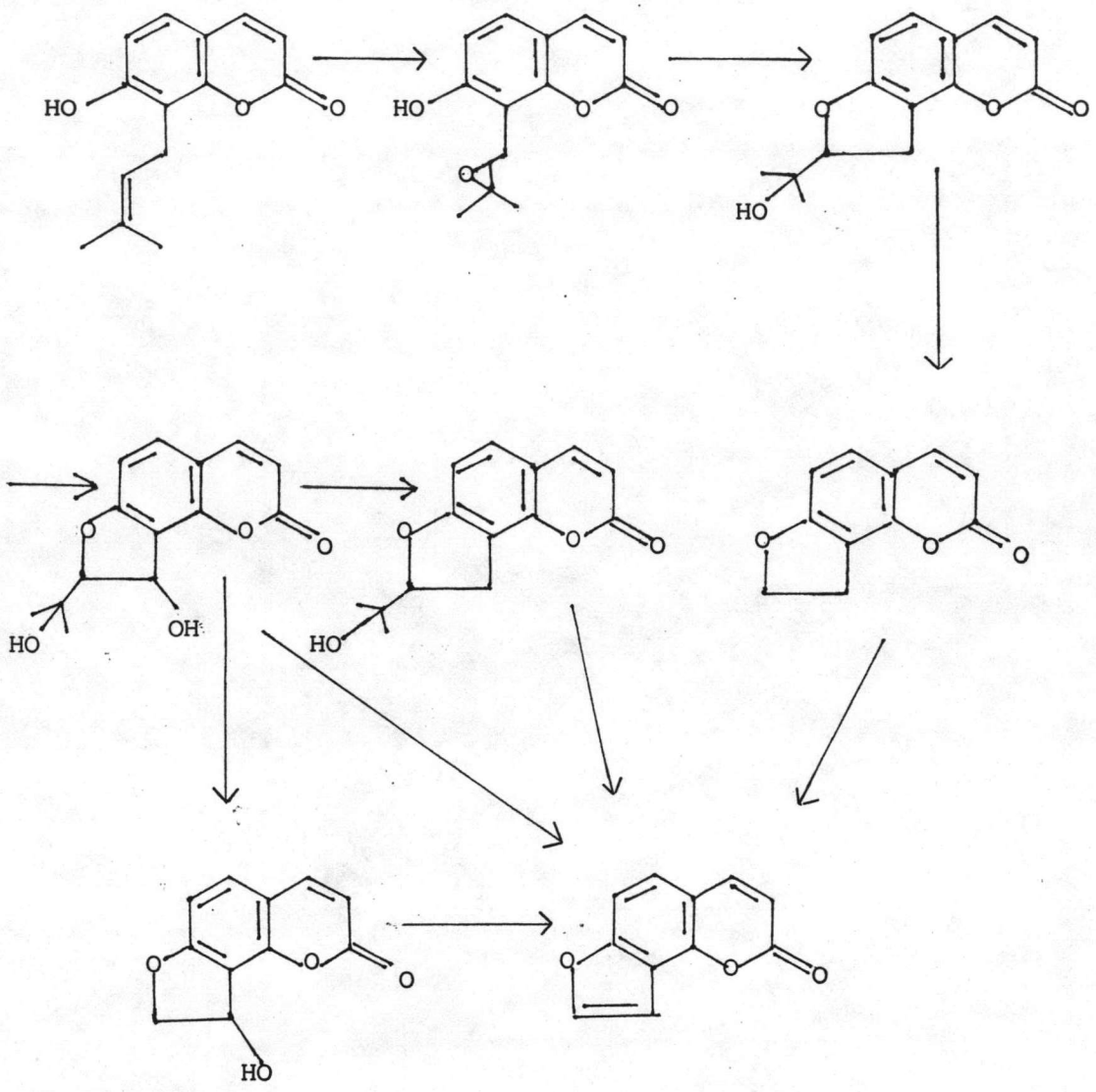


Fig. 7 Possible ways of formation of the furan ring in furanocoumarins.[37]

Although no detailed investigation into the formation of pyranocoumarins have yet been reported, the observation that demethylsuberosin is heavily incorporated into 3',4'-dihydroxanthyletin, suggests a pathway analogous to that for furanocoumarins is in operation (Fig.7). It has been noted that, as anticipated, the configuration of the C-prenyl epoxide intermediate is retained during the formation of pyranocouma-

rins. (Fig. 8) The Xanthyletin angular pyranocoumarins are probably the product of cyclization of a C-6 prenyl unit and a free C-5 hydroxy substituent.(33)

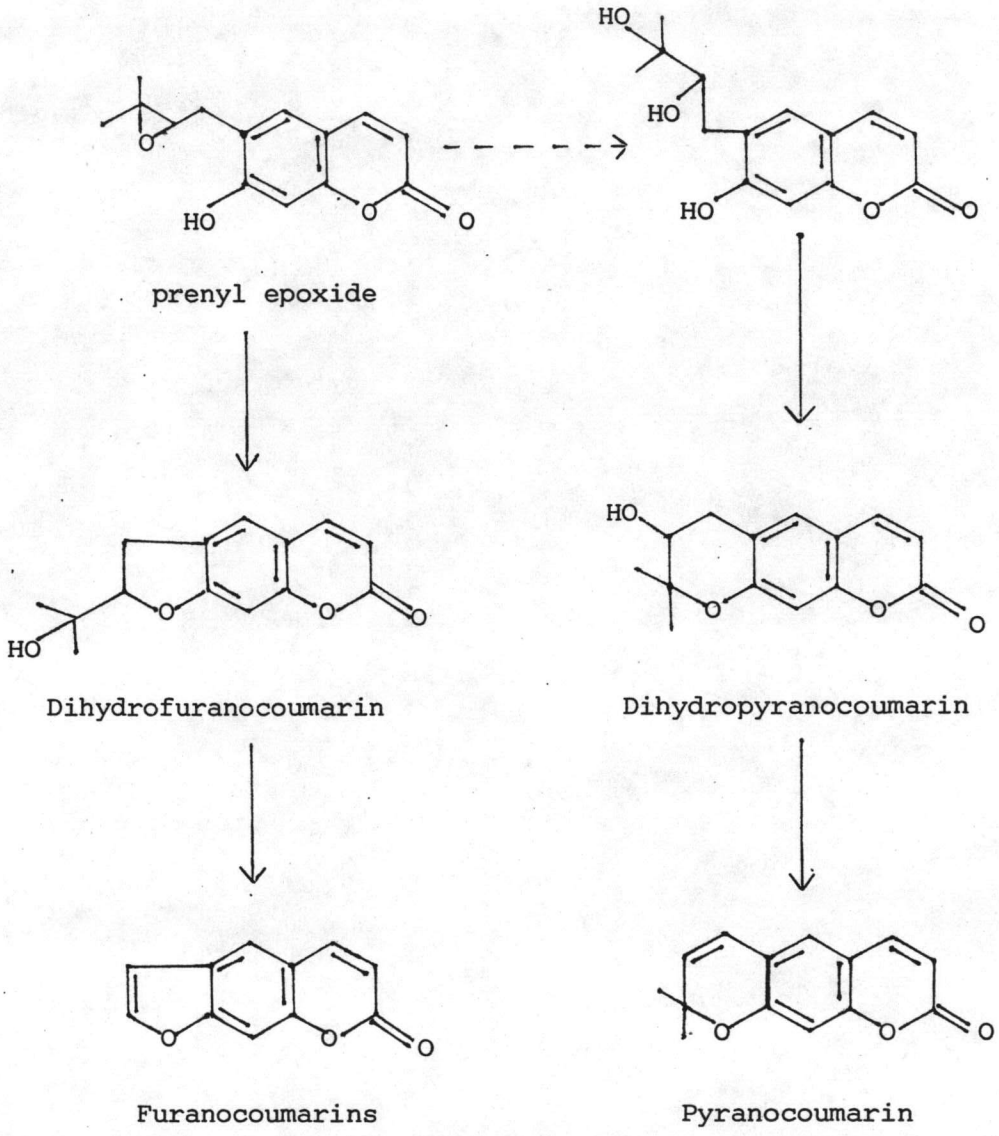
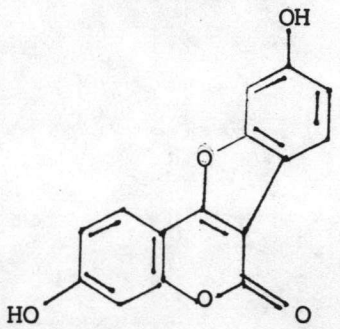


Fig. 8 Formation of linear furanocoumarins and pyranocoumarins from 6-C-isoprenyl coumarin precursor.(33)

The coumarins with an aryl group at C-3 or C-4 or, much more rarely, with a non-prenylic alkyl group in C-4 are biogenetically very different from the above systems. In the 3-aryl coumarins, of

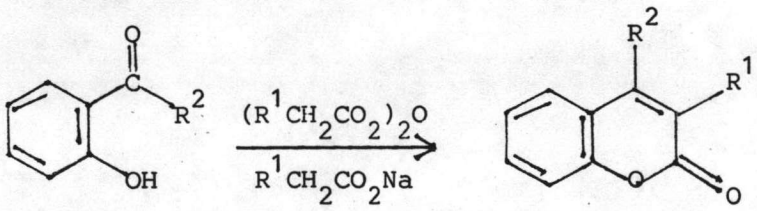
which coumestrol is a typical example. The C-3 benzene ring and the three carbon atoms of the pyrone ring derive from the same molecule of phenylalanine, whilet the benzene ring of the coumarin nucleusis formed by the condensation of three acetate units.(35)



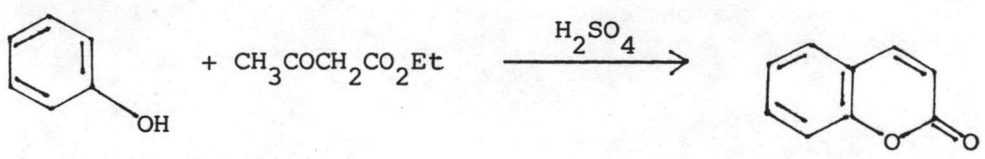
Coumestrol

2.3 Synthesis of Coumarins

Many reactions have been used for systhesis of coumarins. Some of them are Perkin reaction, the condensation of an α-acylphenol with an anhydride in the presence of the corresponding sodium salt can lead to coumarins.(38)

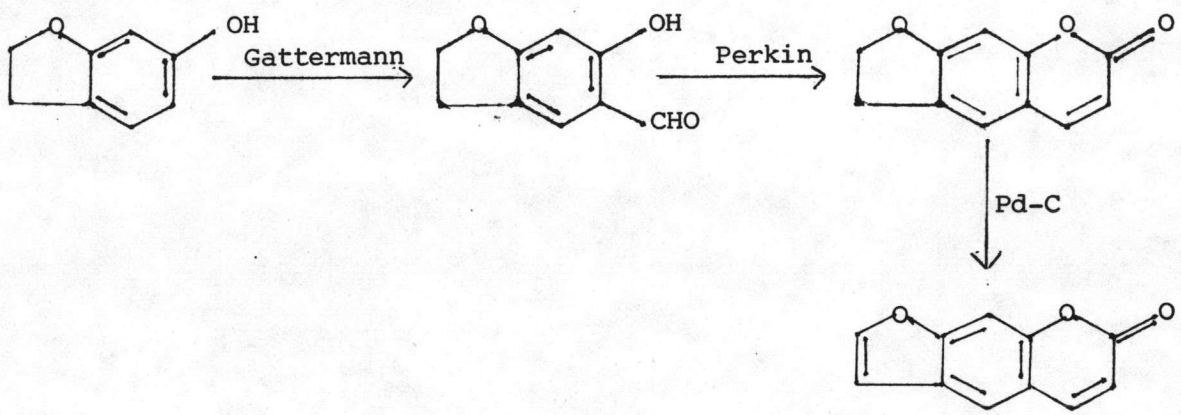


and Pechmann reaction, by condensing phenols and β-keto ester in the presence of H_2SO_4, coumarins can be obtained.(38)

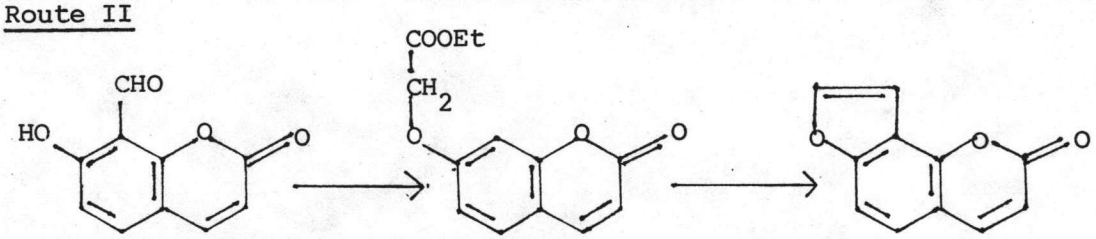


The systhesis of some furanocoumarins can be performed some reaction as show below.(39)

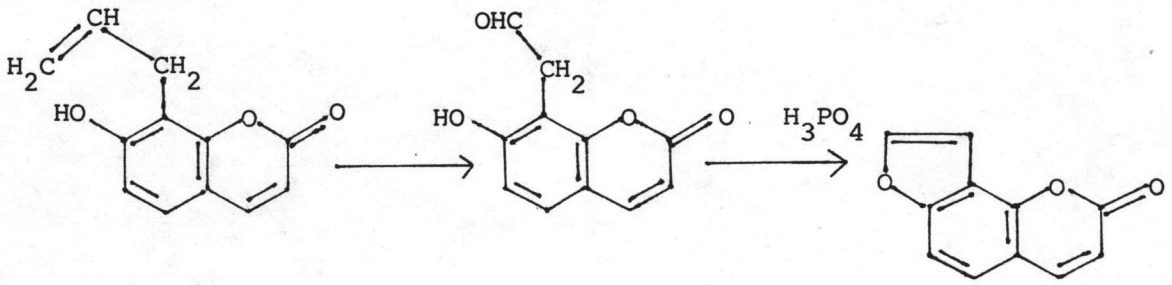
Route I



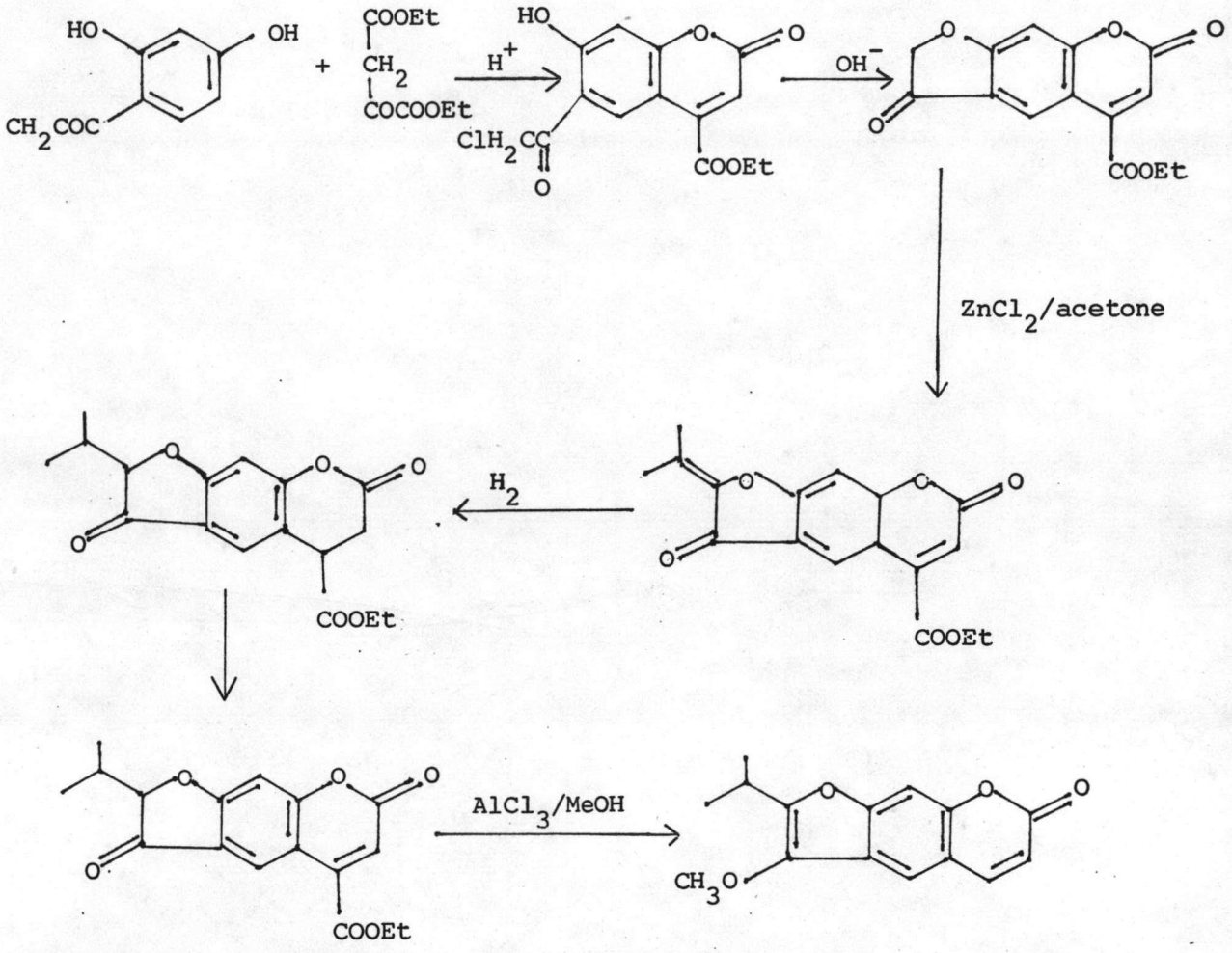
Route II



Route III



Route IV



2.4 Pharmacological Activity

There have been reported on the pharmacological activity of coumarin.[31] The coumarin, pteryxin from *Pteryxia terebinthina*, and suksdorfin-A from *Lomatium suksdorfii* were found to exhibit antispasmodic action.

A number of coumarins which have been isolated from Umbelliferae plants, possessed vasodilatory activity. These studies were stimulated by the finding that khellin found in *Amni visnaga*, showed strong activity in clinical trials. Forther investigations led to the isolation of several other active coumarins, e.g. visnadin,

samidin and dihydrosamidin. Visnadin has also been used in therapy for the treatment of angina pectoris under the registered name 'provisimine'.

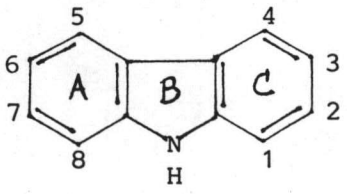
Coumarin itself exerts hypnotic action on frogs, rabbits and mice, but it has not been used therapeutically because of its hepatic toxicity.

3. Carbazole Alkaloids

Introduction

Carbazole alkaloids represent a new and interesting variant in the large number of existing indole alkaloids which in the past have yielded several important drugs.[40] They are subgroup of indole alkaloids, which basic structure are dibenzo-pyrole nucleus. They were first reported of carbazoles alkaloids isolated from the stem bark of *Marraya koenigii* Spreng,[41] and this is the richest source of phytocarbazole so far reported. The plant contains this alkaloid belongs to the family Rutaceae of the order Rutales, and is in the subtribe Clauseneae of the subfamily Aurantiodae. Subsequently the alkaloids were isolated from the genera *Glycosmis* and *Clausena*, belonging to the same subtribe of the family Rutaceae. So far, the genus *Glycosmis* has been found to elaborate simple carbazoles with a C_{13}-skeleton, while in the genus *Clausena* with C_{13} and C_{18} skeletons, and in *Murraya* with C_{13}, C_{18} and C_{23} skeletons.[42]

Chemistry of Carbazole Alkaloids



Carbazole nucleus (dibenzo-pyrrole)

Three basic types of carbazole alkaloids have been recognized, and all are based on the parent carbazole nucleus, but they differ in the number of carbon atoms attached to nucleus. The groups are better analyzed however in terms of the number of isoprene units liked to an indole nucleus. Thus the first group has an indole unit plus one isoprene unit (C_{13}), the second group has an indole unit plus two isoprene units (C_{18}), and the third group has an indole unit plus three isoprene units (C_{23}).[43] Table 8,9 and 10 showed the compounds and sources of three groups of carbazole alkaloids which have been reported.

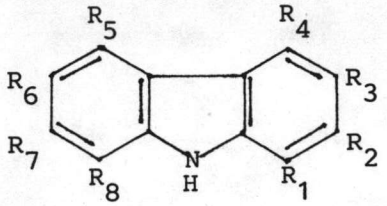


Table 8 A Member of the C_{13}-Skeleton Group

Compound	Substituents	Source
Murrayanine	$R_2 = R_4 = R_5 = R_6 = R_7 = R_8 = H$	*Clausena heptaphylla* Wight.[44]
	R_1 = OMe, R_3 = CHO	*Murraya koenigii* Spreng.[45]
Lansine	$R_1 = R_4 = R_5 = R_7 = R_8 = H$ R_2 = OH, R_3 = CHO, R_6 = OMe	*Clausena lansium* Skeels.[24]
Glycozolidine	$R_1 = R_2 = R_4 = R_6 = R_8 = H$ R_3 = Me, $R_5 = R_7$ = OMe	*Glycomis pentaphylla* (Retz.) DC.[46]
Glycozoline	$R_1 = R_2 = R_4 = R_5 = R_7 = R_8 = H$ R_3 = Me, R_6 = OMe	*Glycomis pentaphylla* (Retz.) DC.[47,48]
Mukoeic acid	$R_2 = R_4 = R_5 = R_6 = R_7 = R_8 = H$ R_1 = OMe, $R_3 = CO_2H$	*Murraya koenigii* Spreng.[49]

Table 8 (continued)

Compound	Substituents	Source
Mukonine	$R_2 = R_4 = R_5 = R_6 = R_7 = R_8 = H$ $R_1 = OMe$, $R_3 = CO_2Me$	*Murraya koenigii* Spreng.[42]
3-Methylcarbazole	$R_1 = R_2 = R_4 = R_6 = R_7 = R_8 = H$ $R_3 = Me$	*Clausena heptaphylla* Wt. & Arn.[50]
Mukonidine	$R_1 = R_4 = R_5 = R_6 = R_7 = R_8 = H$ $R_2 = OH$, $R_3 = CO_2Me$	*Murraya koenigii* Spreng.[42]

Table 9 A Member of the C_{18}-Skeleton Group

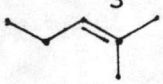
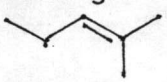
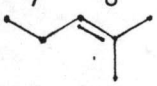
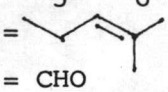
Compound	Substituents	Source
Atanisatin	$R_1 = R_4 = R_5 = R_6 = R_7 = H$ $R_2 = OMe$, $R_3 = CHO$ $R_8 =$ 	*Clausena anisata* Hook. f.[10]
Clausanitin	$R_1 = R_4 = R_5 = R_6 = R_7 = H$ $R_2 = OH$, $R_3 = CHO$ $R_8 =$ 	*Clausena anisata* Hook. f.[10]
Indizoline	$R_4 = R_5 = R_6 = R_7 = R_8 = H$ $R_1 = OMe$, $R_2 =$  $R_3 = CHO$	*Clausena indica* Oliv.[22]
Heptaphylline	$R_4 = R_5 = R_6 = R_7 = R_8 = H$ $R_1 =$  $R_2 = OH$ $R_3 = CHO$	*Clausena excavata* Burm.f.[17] *Clausena heptaphylla* Wight.[17] *Clausena lansium* Skeels[24] *Clausena pentaphylla* DC.[25]

Table 9 (continued)

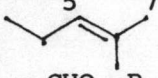
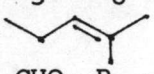
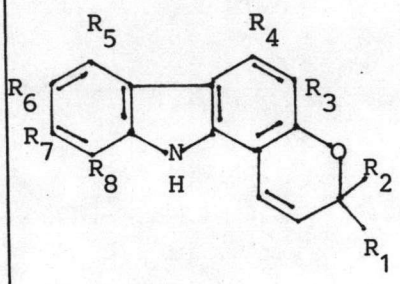
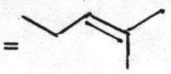
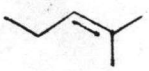
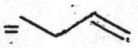
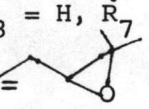
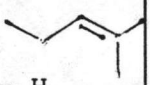
Compound	Substituents	Source
6-Methoxy-hepta-phylline	$R_4 = R_5 = R_7 = R_8 = H$  $R_2 = OH$ $R_3 = CHO$, $R_6 = OMe$	*Clausena indica* Oliv.[21]
Heptazoline	$R_4 = R_5 = R_6 = R_7 = H$  $R_2 = OH$ $R_3 = CHO$, $R_8 = OH$	*Clausena heptaphylla* Wight.[50,51]
		
Mupamine	$R_1 = R_2 = R_3 = Me$ $R_4 = R_5 = R_6 = R_7 = H$ $R_8 = OMe$	*Clausena anisata* Hook. f.[12]
Girinimbine	$R_1 = R_2 = R_3 = Me$ $R_4 = R_5 = R_6 = R_7 = R_8 = H$	*Clausena heptaphylla* Wight.[17] *Murraya koenigii* Spreng.[52]
koenimbine	$R_1 = R_2 = R_3 = Me$, $R_6 = OMe$ $R_4 = R_5 = R_7 = R_8 = H$	*Murraya koenigii* Spreng.[53]
Heptazolidine	$R_1 = R_2 = Me$, $R_3 = OMe$ $R_4 = R_7 = R_8 = R_5 = H$, $R_6 = Me$	*Clausena heptaphylla* Wight.[54]
Murrayacine	$R_1 = R_2 = Me$, $R_6 = CHO$ $R_3 = R_4 = R_5 = R_7 = R_8 = H$	*Murraya koenigii* Spreng.[55,56] *Clausena heptaphylla* Wight.[57]
Koenigine	$R_1 = R_2 = R_3 = Me$, $R_6 = OMe$ $R_7 = OH$, $R_4 = R_5 = R_8 = H$	*Murraya koenigii* Spreng.[58]

Table 9 (continued)

Compound	Substituents	Source
Koenidine	$R_1 = R_2 = R_3 = Me$ $R_4 = R_5 = R_8 = H$ $R_6 = OMe = R_7$	*Murraya koenigii* Spreng.[58]
Koenine	$R_1 = R_2 = R_3 = Me$, $R_6 = OH$ $R_4 = R_5 = R_7 = R_8 = H$	*Murraya koenigii* Spreng.[58]

Table 10 A Member of the C_{23}-Skeleton Group

Compound	Substitents	Source
Mahanimbine	$R_2 = R_3 = Me$, R_1 =  $R_4 = R_5 = R_6 = R_7 = R_8 = H$	*Murraya koenigii* Spreng.[59,60]
Mahanimbicine	$R_2 = R_6 = Me$, R_1 =  $R_3 = R_4 = R_5 = R_7 = R_8 = H$	*Murraya koenigii* Spreng.[61]
Mahanine	$R_2 = R_3 = Me$, R_1 =  $R_4 = R_5 = R_6 = R_8 = H$, $R_7 = OH$	*Murraya koenigii* Spreng.[58]
Mahanimbinine	$R_2 = R_3 = Me$, R_1 =  $R_4 = R_5 = R_6 = R_7 = R_8 = H$	*Murraya koenigii* Spreng.[62]
Murrayacinine	$R_2 = Me$, $R_3 = CHO$, R_1 =  $R_4 = R_5 = R_6 = R_7 = R_8 = H$	*Murraya koenigii* Spreng.[65]

Biosynthesis

Although most of the building units in the biosynthesis of indole alkaloids are now known with certainly, The understanding of the biosynthesis of carbazole alkaloids is extremely meagre.[40] Chakraborty[64] has considered that anthranilic acid (1) could be a precursor of these alkaloids via the diphenylamine derivatives (2). The latter, on decarboxylation and cyclization, could give rise to these alkaloids in nature (Fig. 9). According to Chakraborty, and Das;[65] the extra methyl (or its equivalent) originations by C-methylation at the 3- or 6-position prior to or after the formation of the carbazole nucleus.[40]

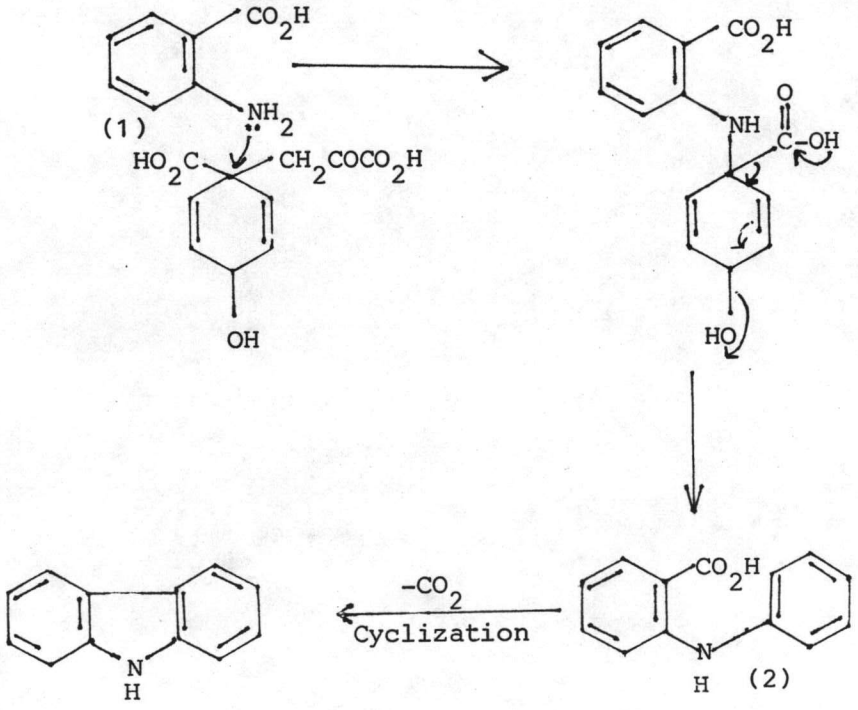
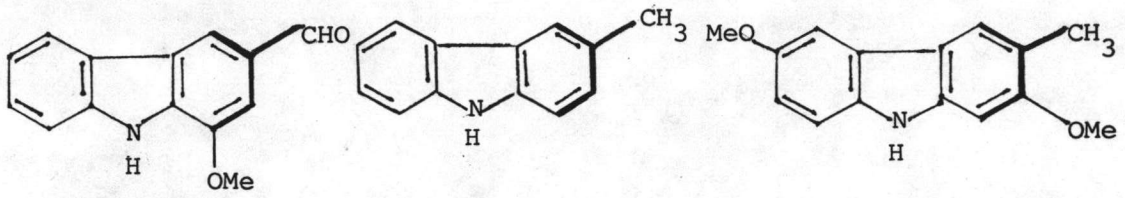


Fig. 9 Pathway from anthranilic acid to carbazole nucleus

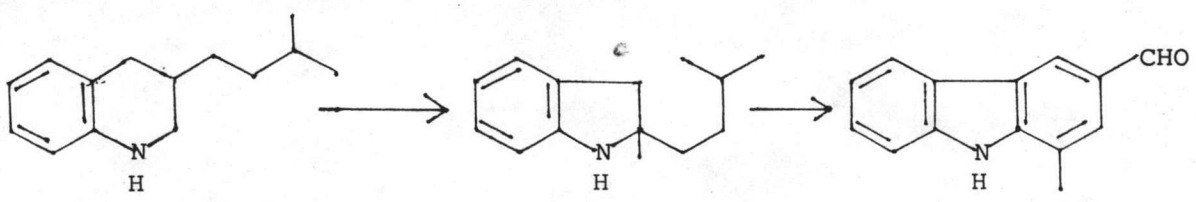
Because mevalonic acid derived units participate in the biogenesis of indole bases and anthraquinones, Chakraborty[42] suggested that the five carbon atoms linked by bold lines in (3), (4) and (5) are derived from mevalonate.



(3) (4) (5)

Kureel *et al.*[41] suggested that ring C in the carbazole alkaloids is of mevalonic acid origin and that the extra methyl group is a part of this unit. As the carbazole is a symmetrical molecule, the 3- and 6- positions are in fact identical, and it is the further substitution in the molecule which really determines the orientation. They further considered that the indole ring was derived from allylquinolines. The latter on ring contraction and further modification, could give rise to carbazole alkaloids.[40]

Independently, Erdtman has also considered the view that carbazole alkaloids are of mevalonid origin and are biosynthesized from a 3- prenylated quinoline (6) via 2-prenylated indole (7) precursors.[40]



(6) (7)

Narashimhan[61] considers that tryptophan is the substrate to which the C_5-fragment is add after which a series of reactions eliminate the tryptophan side chain. The C_5-unit initially attacks the 3-position of the heterocyclic system, subsequently cyclisation, loss of the serine residue in the presence of pyridoxal coenzyme, gives a dihydrocarbazole which on dehydrogenation yields a 3-methylcarbazole.[42] (Fig. 10)

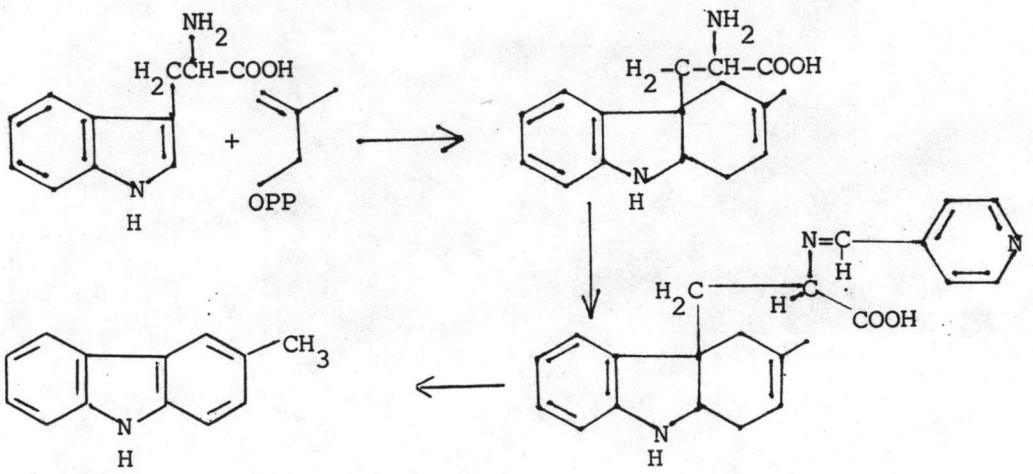
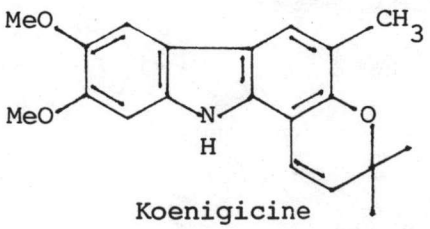


Fig. 10 Mechanism of carbazole alkaloids derived from tryptophan

Kapil and Popli[43] showed that the additional carbon atom on the carbazole ring of koenigicine was not derived from [^{14}C-methyl] methionthine, but that as expected that carbon atoms of the methoxy groups were labeled. The isotopes label of mevalonic acids were shown to be a good precursors of koenigicine biosynthesis, together



Koenigicine

with the evidence from isolated, Kapil propose that 3-methylcarbazole is the key biosynthetic intermediate in the formation of the carbazole alkaloids and that this compound may then be the object of hydroxylation and prenylation reaction (Fig. 11). None of the simple prenylation biogenetic precursors are known, and there is no information as to the mechanism of formation of 3-methyl carbazole.

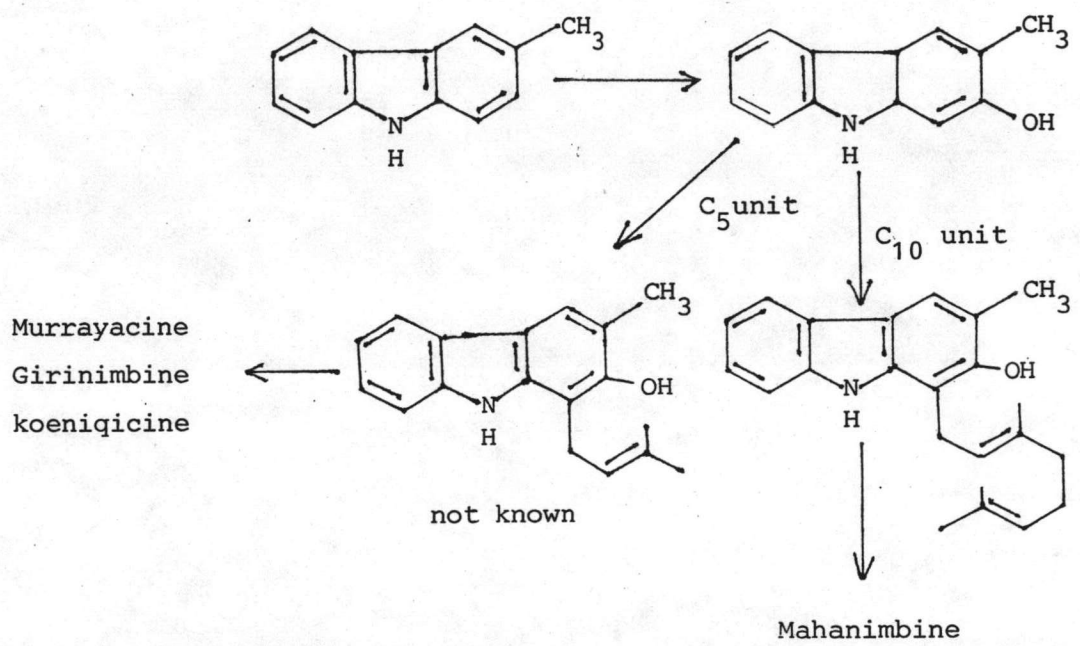
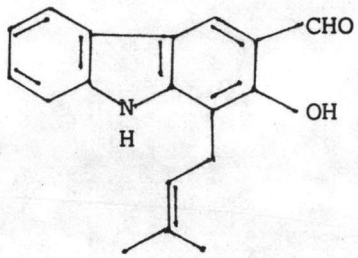
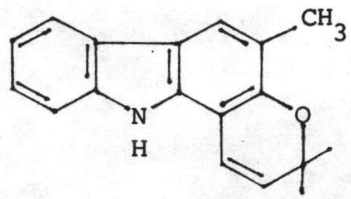


Fig. 11 Biosynthesis of carbazole alkaloids.(43)

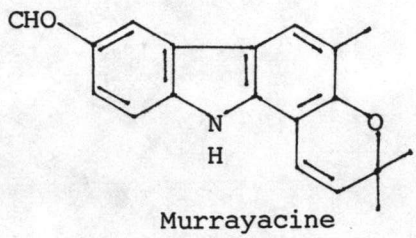
Heptaphylline, Girinimbine, Murrayacine and their congener are similar to typical plant phenolic with a modified mevalonic acid unit. In fact the occurrence of Heptaphylline and Girinimbine in *Clausena heptaphylla* Wight. may be considered circumstantial evidence in favour of the origin of the pyran ring from the prenylated congener.(42)



Heptaphylline

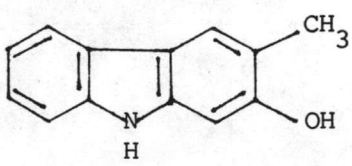


Girinimbine

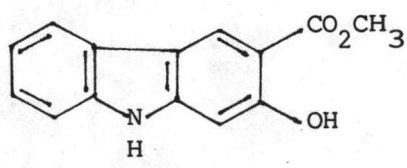


Murrayacine

The formation of pyranocarbazoles, 2-hydroxy-3-methyl carbazoles plays a prominent role. The recent isolated of 2-hydroxy-3-carbomethoxy-carbazole (mukonidine) provides strong circumstantial evidence for the above idea. Probable, the methyl group of 2-hydroxy-3-methylcarbazoles has been oxidized to the carbomethoxy group in Mukonidine.(67)



2-Hydroxy-3-methylcarbazole



Mukonidine

The postulated biosynthesis pathway of the formation of carbazole alkaloid was show on Fig. 12

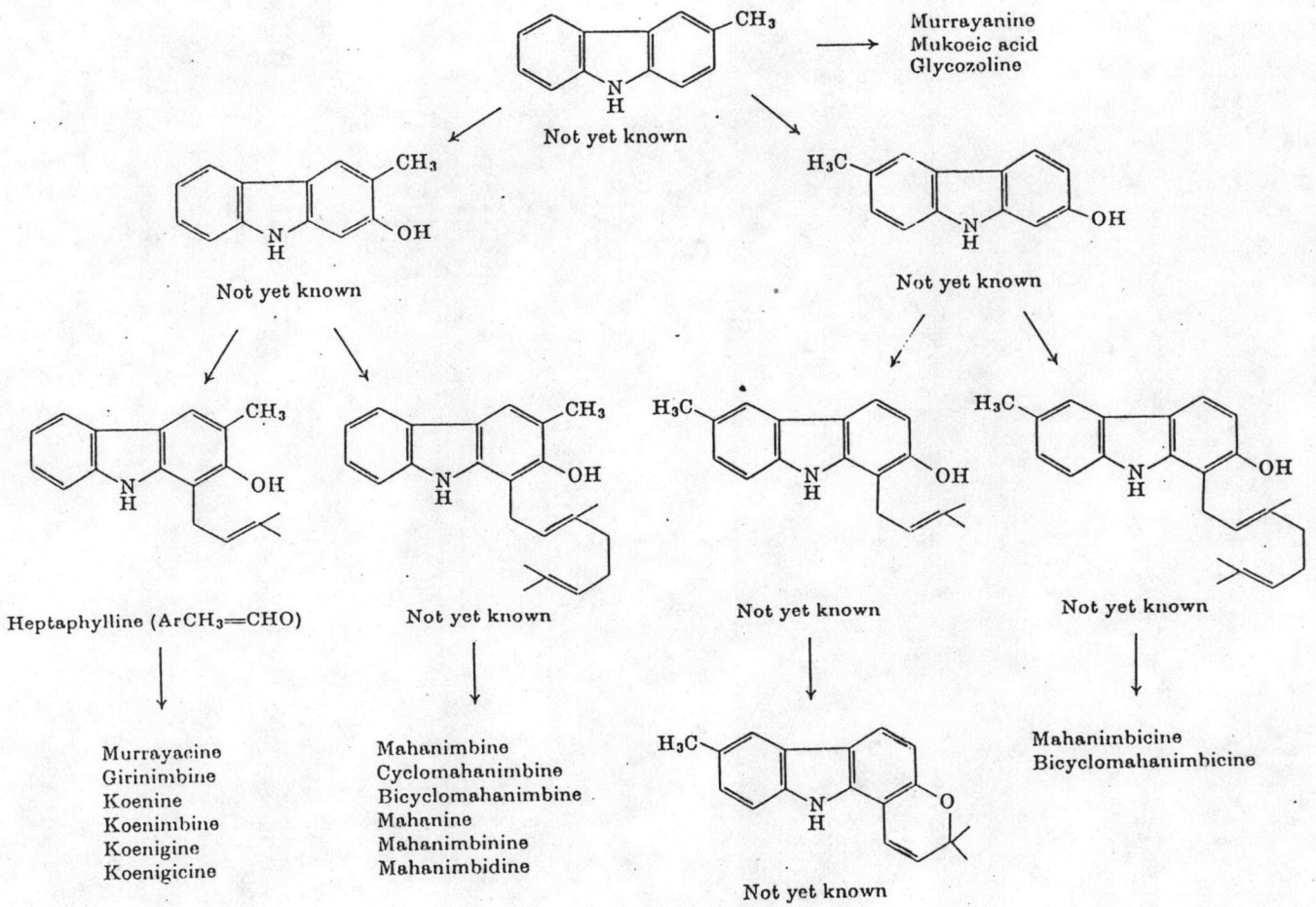


Fig. 12 Postulated pathway of formation of carbazole alkaloids[42]

Biochemical Properties of Carbazole Alkaloids

The antibiotic properties of some carbazole alkaloids have been fasted by Chakraborty, Das, and Bose,[68,69] by the usual agar cup assay method using Sabouraud's medium against *Microsporum gypseum, Trichophyton rubrum, Epidermophyton flaccusum, Candida albicans, Candida tropicalis, Staphyllococcus aureus* and *Escherichia coli*. Glycozoline, Demethylated glycozoline, 1-methyl-6-hydroxy-carbazole, 2-methyl-6-hydroxy carbazole, Glycozolidine, 1-hydroxy-carbazole, 2,6-dihydroxy-3-methyl carbazole, Murrayanine, Girinimbine, Mahanimbine and Heptazoline were examinded against those microorganism. All of them have feeble antibiotic action at 100 µg/ml but the most significant is that of 6-hydroxy-3-methylcarbazole. It is active against *Trichophyton rubrum* (10 µg/ml), *Epidermophyton floccusum* and *Microsporum gypseum*. Girinimbine was active against *Nocardia gymsum* and showed activity against *Nocardia asteroides* at a concentration of 30 µg/ml.

4. The Chemotaxonomic significance of carbazole alkaloids and coumarins in Plant

The carbazole alkaloids occures in several related genera, it appears that they are limited to the family Rutaceae, subtribe Clauseneae, subfamily Aurantiodea. They have also been found in *Glycosmis* and *Murraya*.[42]

Table 11 Distribution of Carbazole Alkaloids[42]

(Fam. Rutaceae; subfam. Aurantoidea; subtribe Clauseneae)

Genus	C_{13}-Alkaloids	C_{18}-Alkaloids	C_{23}-Alkaloids
Glycosmis	Glycozoline Glycozolidine	-	
Clausena	3-Methylcarbazole Murrayanine Lansine	Indizoline Heptazoline Heptaphylline 6-Methoxyheptaphylline Heptazolidine Girinimbine Murrayacine	
Murraya	Murrayanine Mukoeic acid Mukonine Mukonidine	Girinimbine Koenimbine Koenigine Koenidine Koenine Murrayacine	Mahanimbine *dl*-Mahanimbine Mahanine Mahanimbinine Murrayazoline Mahanimbidine (Currayangin) Murrayazolinine Cyclomahanimbine Murrayazolidine Bicyclomahanimbine Mahanimbicine Iso mahanimbine Bicyclomahanimbicine Murrayacinine

Table 11 show the genus *Glycosmis* has been found to elaborate simple carbazoles with a C_{13}-skeleton, *Clausena*, C_{13} and C_{18} skeleton and *Murraya*, C_{13}, C_{18} and C_{23} skeletons.[42] There were no reports of carbazole alkaloid isolated form other family and there genera.

Coumarins have been found to be distributed extensively in varied types of flora and in all parts of the plant. They have also been reported from microorganism and animals. The simple hydroxylated and methoxylated coumarins in free state or as glycosides occur widely in different plant families, but as the structural complexity of the compound increases, they seem to be restricted more and more to familial occurrence.[31] However, most of them are isolated from plants, especially Umbelliferae, Rutaceae and Leguminosae. Within the Rutaceae it would appear that there is no significant variation in the overall distribution of coumarin types between the three major subfamily, Rutioideae, Toddalioideae, and Aurantiodeae. In the Aurantiodeae, substitution at C-3 usually with a 1,1-dimethylallyl group, occurs in only a small number of genera and appears to be particularly significant in *Clausena* (with pyranocoumarin).[33]

Table 1, 8, 9, 10 show that the chemical investigation from the several species of the *Clausena* was indicated that carbazole alkaloids and coumarins have chemotaxonomic significant to the *Clausena* species.