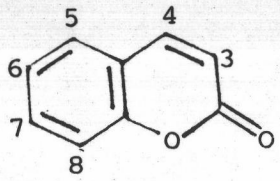
CHAPTER II

HISTORICAL

I. Classification of coumarins.

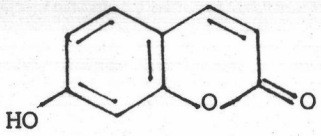
The term coumarin is applied, collectively, to a large group of naturally occurring compounds possessing a 2-H-1-benzopyran-2-one nucleus. Steck, W. and Mazurek, M.[2] have divided Coumarins into two types. One is 'normal' type, which coumarins have an oxygen function at C-7 and hydrogens at C-3 and C-4 (unsubstituted pyrone ring). This type of coumarin comprises the great majority of natural derivatives. The other is 'abnormal' type, which coumarins either lack the C-7 oxygen or possess pyrone ring substituents. Seshadri, T.R., and Vishwapaul[3] and Tandon, S., and Rastogi, R.P.[4] have classified coumarins into five heads as :-

1. Simple coumarins - This type of coumarin is possessed a 2-H-1-benzopyran-2-one nucleus (coumarin nucleus) and there are side chains substituted at the benzene ring. 7-oxygenated coumarins are the most common in this type, for example, umbelliferone, the simplest coumarin in this type, has hydroxy group substituted at the 7-position of coumarin nucleus. The coumarins in this type have been listed in table 1.



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

(coumarin nucleus)



umbelliferone

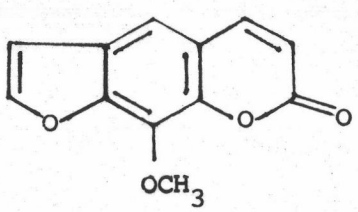
Table 1 The example of some common simple coumarins.

name	structure	source	Reference
demethylsuberosin	7-OH, 6-	*Ruta graveolens* L.,+	5,6
suberosin	7-CH_3, 6-	*Clausena indica* Oliv.,+	5,7
osthenol	7-OH, 8-	+	5
osthol	7-OCH_3, 8-	+	5
herniarin	7-OCH_3	+	5
umbelliferone	7-OH	+	5

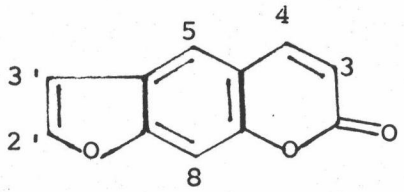
+ : These compounds can find commonly in Rutaceae and Umbelliferae

2. Furanocoumarins - This type of coumarin consisted the furan ring which is fused with the coumarin nucleus at the various position on benzene ring to form linear furanocoumarin or angular furanocoumarin. This type of coumarin can be classified, considering the fusion of furan, into six subtypes :-

2.1 Psoralene type (linear) - The furan ring is fused with the benzene ring at C-6 and C-7 positions as linear structure. The double bond occured at C-2', C-3'. The coumarin in this type is xanthotoxin, which has a methoxy substituent at C-8.

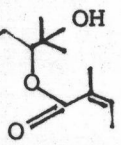
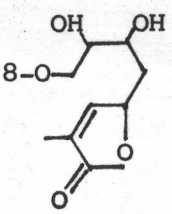


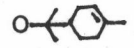
xanthotoxin

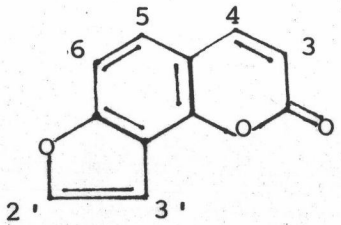


psoralene nucleus

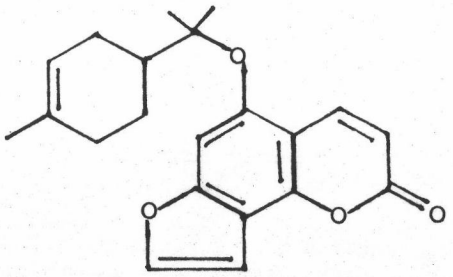
Table 2. The example of some psoralene type furanocoumarins.

name	structure	source	reference
chalepensin (xylotenin)	3 	*Ruta graveolens* L. *Ruta chalepensis* L.	3
halfordin	3,4,5-$(OCH_3)_3$	*Hafordia scleroxyla* F.Muell.	3
clausindin	3-CH(-CH_2-CH_3)-C(CH_3)=	*Clausena indica* Oliv.	8
byakangelicin	5-OCH_3,8-O- 	*Angelica ursina* (Papr.) Regel &Schmalh.	3
		Clausena indica Oliv.	7
xanthotoxol	8-OH	*Clausena indica* Oliv.	7
phellopterin	5-OCH_3,8-O- 	*Clausena indica* Oliv.	9
imperatorin	8-O- 	*Clausena indica* Oliv.	9
indicolactonediol	8-O- 	*Clausena indica* Oliv.	7

2.2 Angelicin type (angular) - The furan ring is fused with the benzene ring at C-7 and C-8. The C-2 ' and C-3 ' have one double bond. Some compounds have side chains substitution such as :- archangelin[3] has  as substituent at C-5 position. This compound was found in *Angelica archangelica* L.[3].

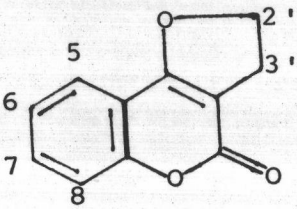


angelicin type

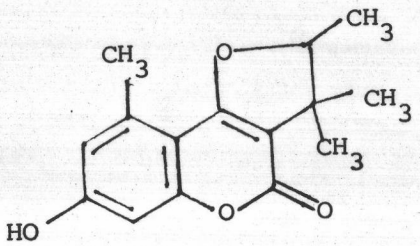


archangelin

2.3 Dihydrofuranocoumarin [4,3] - The furan ring is fused with the benzene ring at C-3 and C-4 positions. The C-2 ' and C-3 ' have no double bond, but there are substituents in the side chains, for example :- glaupalol has hydroxy group at C-7, methyl group at C-5 and C-2 ', and two methyl groups at C-3 '. This compound was found in *Glaucidium palmatum*[3].

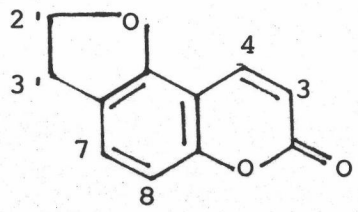


dihydrofuranocoumarin [4,3]

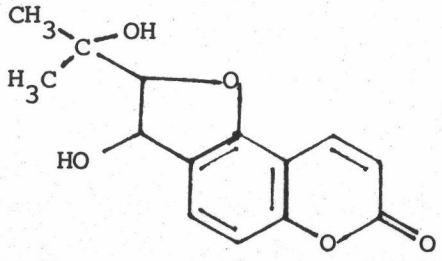


glaupalol

2.4 Dihydrofuranocoumarin [5,6] - The furan ring is fused with the benzene ring at C-5 and C-6 positions. The C-2 ' and C-3 ' have no double bond. For example :- xanthoarnol has hydroxy group at C-3 ' and $-C(OH)Me_2$ at C-2 '. This compound has been reported to be found in *Xanthoxylum arnottianum* Maxim.[4].

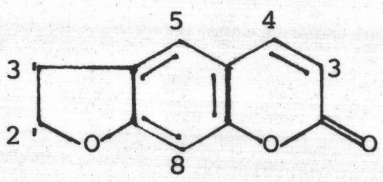


dihydrofuranocoumarin [5,6]

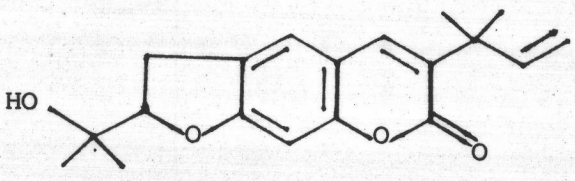


xanthoarnol

2.5 Dihydrofuranocoumarin [7,6] - The furan ring is fused with the benzene ring at C-6 and C-7 position as same as in psoralene type, but there are no double bond at the C-2 ' and C-3 '. The sample of compound in this type is chalepin (heliettin). This compound has 3,3-dimethylallyl group at C-3 and ←OH at C-2 '. The compound was found in *Ruta chalepensis* L. and in *Helietta longifoliata* Britt.[3].

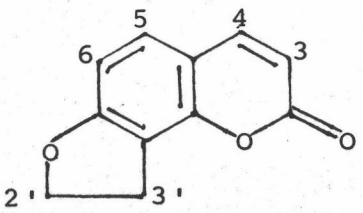


dihydrofuranocoumarin [7,6]

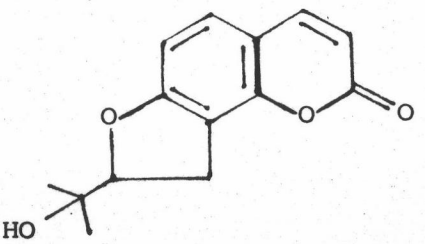


chlepin (heliettin)

2.6 Dihydrofuranocoumarin [7,8] - The furan ring is fused with the benzene ring at C-7 and C-8 positions as same as in angelicin type, but there are no double bond at the C-2 ' and C-3 '. Columbianetin is the example of coumarin in this type.



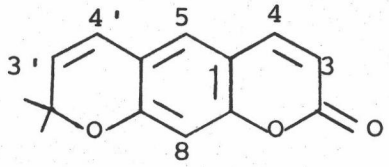
dihydrofuranocoumarin [7,8]



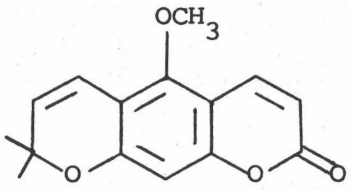
columbianetin

3. Pyranocoumarins - The pyran ring is fused with the coumarin nucleus at various position on benzene ring to form linear pyranocoumarin or angular pyranocoumarin. This type of coumarin can divide to five subtypes :-

3.1 Xanthyletin type (linear) - The pyran ring is fused with the benzene ring at the C-6 and C-7 positions. The hydrogen at C-2 ' is substituted with two methyl groups, and between C-3 ' and C-4 ' has one double bond. The sample of this compound is xanthoxyletin, which was found in *Clausena anisata* Willd.[13] and in *Clausena excavata* Burm.f.[14]. The coumarins in this type were showed in Table 3.



xanthyletin type (linear)

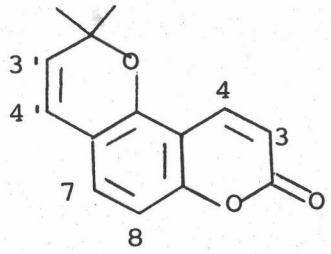


xanthoxyletin

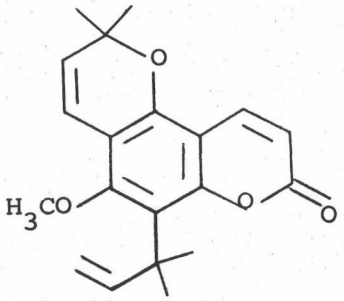
Table 3 Xanthyletin linear pyranocoumarins type.

name	structure	source	reference
xanthoxyletin	5-OCH_3	*Clausena anisata* Willd.	13
		Clausena excavata Burm.f.	14
3(1,1-dimethyl-allyl)-xanthyletin	3 	*Clausena anisata* Willd.	13
		Clausena willdennovii W. & A.	16
clausarin	3  , 5-OH, 8 	*Clausena excavata* Burm.f.	14
		Clausena pentaphylla (Roxb.)DC.	18

3.2 Xanthyletin type (angular) - This type of compound is as same as xanthyletin linear type but the pyran ring fused position is C-5 and C-6 instead of C-6 and C-7. Dentatin is the coumarin represent in this type. It was found in *Clausena dentata* (Willd.) R.& S.[20], *Clausena heptaphylla* Wt.& Arn.[22] and *Clausena pentaphylla* (Roxb.)DC.[18]. Table 4 was the coumarins which were classified into this type.



xanthyletin type (angular)

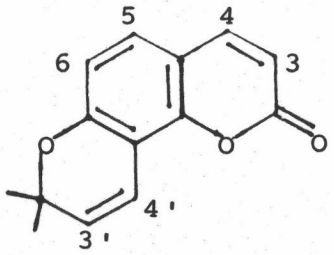


dentatin

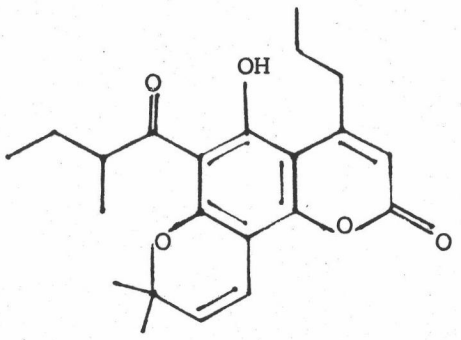
Table 4 Xanthyletin angular pyranocoumarins type.

name	structure	source	reference
dentatin	7-OCH_3, 8- 	*Clausena dentata* (Willd.) R. & S.	20
		Clausena heptaphylla Wt. & Arn.	22
		Clausena pentaphylla (Roxb.) DC.	18
nordentatin	7-OH, 8- 	*Clausena dentata* (Willd.) R. & S.	20
		Clausena excavata Burm.f.	14

3.3 Seselin type (angular) - The coumarin pyran ring is fused with the benzene ring at C-7 and C-8 positions. The C-2' substituted with two methyl groups and between C-3' and C-4' has one double bond. The sample of this compound is MAB 6 which was found in *Mammea africana* G.[3].

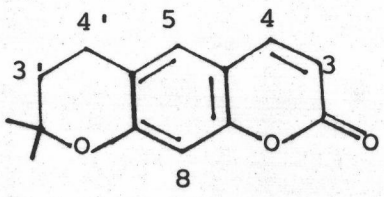


seselin type (angular)

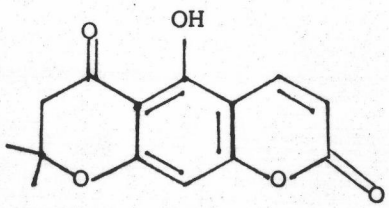


MAB 6

3.4 Dihydroxanthyletin type (linear) - The structure of this compound is as same as xanthyletin type (linear) but at C-3' and C-4' has no double bond. The sample of this compound is clausenin which was found in *Clausena heptaphylla* Wt. & Arn.[23]. Other coumarin in this type was collected and showed in table 5.

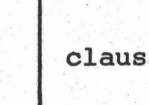


dihydroxanthyletin type (linear)

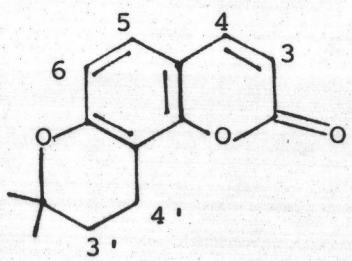


clausenin

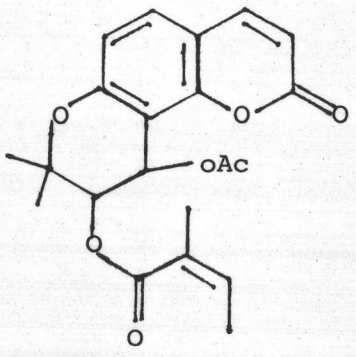
Table 5 Dihydroxanthyletin type (linear).

name	structure	source	reference
clausenin	4'=O , 5-OH	*Clausena heptaphylla* Wt. & Arn.	23
clausenidin	4'=O , 5-OH, 8-	*Clausena excavata* Burm.f.	14
		Clausena heptaphylla Wt. & Arn.	23
		Clausena pentaphylla (Roxb.)DC.	18
clausamarins A,B	3- 	*Clausena pentaphylla* (Roxb.)DC.	19

3.5 Dihydroseselin type (angular) - The structure of this compound is as same as seselin type (angular) but between C-3' and C-4' has no double bond. The sample of this compound is isopteryxin which was found in *Pterryxia terebinthina* var. Californica (coult. & Rose) Mathias[13].



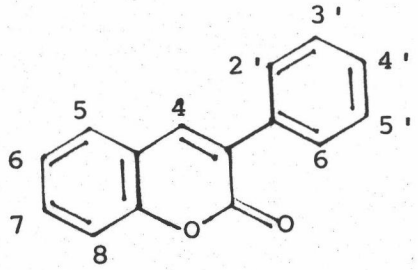
dihydroseselin type (angular)



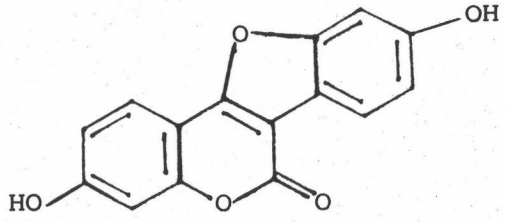
isopteryxin

4. Phenyl coumarins - There is phenyl substituted at C-3 or C-4 in the structure of coumarin nucleus. This type is divided into seven subtype as :-

4.1 3-Phenylcoumarins - There is phenyl substituted at C-3 of the simple coumarin nucleus. Coumestrol,which was found in ladino clover and alfalfa[1],is the example of coumarin in this type.

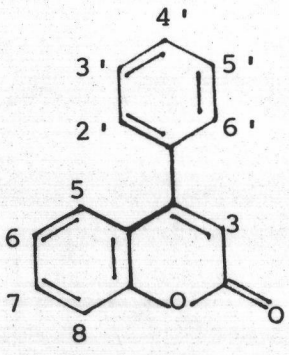


3-phenylcoumarin nucleus

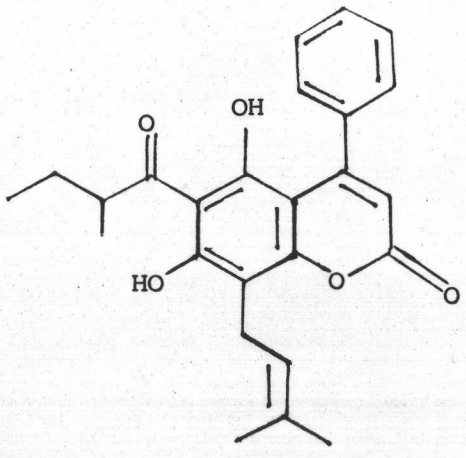


coumestrol

4.2 4-Phenylcoumarins - There is a phenyl ring substituted at C-4 of the simple coumarin nucleus. The compound of this type, Mammea A/AB, which was found in *Mammea americana* L.[3], has phenyl substituent at C-4.

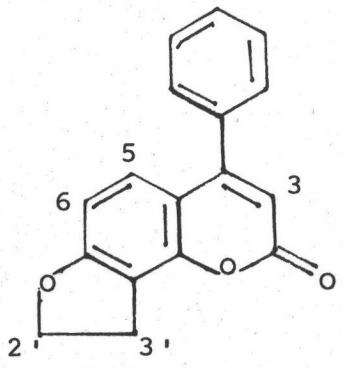


4-phenylcoumarin nucleus

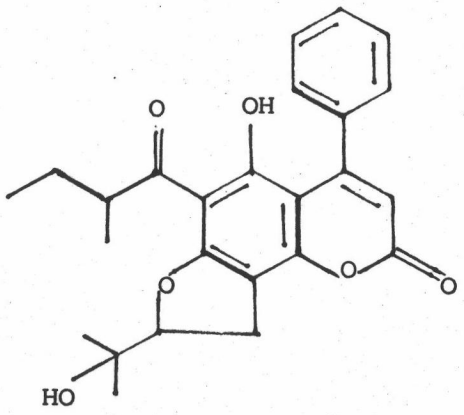


mammea A/AB

4.3 4-Phenyldihydroangelicin - There is a phenyl ring substituted at C-4 of the dihydrofuranocoumarin [7,8] nucleus. The sample of this type is phenylmammea A, which was found in *Mammea americana* L.[3].

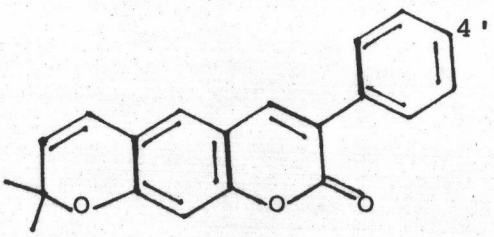


4-phenyldihydroangelicin type

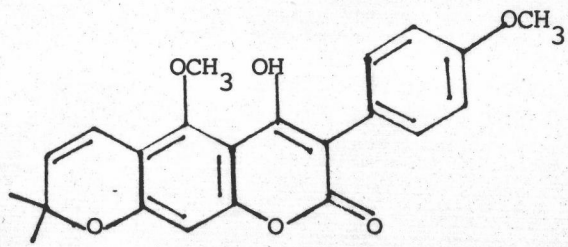


phenylmammea A

4.4 3-Phenylxanthyletin - The coumarin nucleus has a phenyl substituent ring at C-3 of the xanthyletin linear type nucleus. The sample of this type is robustic acid which was found in *Derris robusta* (Roxb.) Benth.[3].

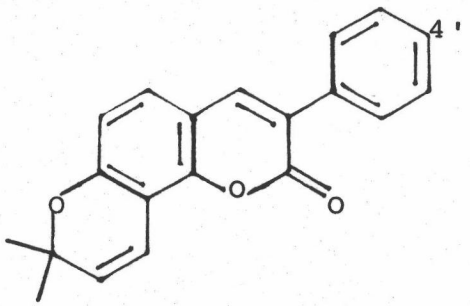


3-phenylxanthyletin type

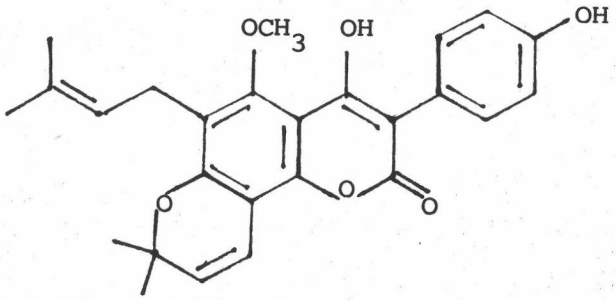


robustic acid

4.5 3-Phenylseselin- There is a phenyl ring substituted at C-3 of the seselin angular type nucleus. *Derris scandens* (Roxb.) Benth. is the plant which has been shown to contain scadenin which is coumarin in the this type.[3]

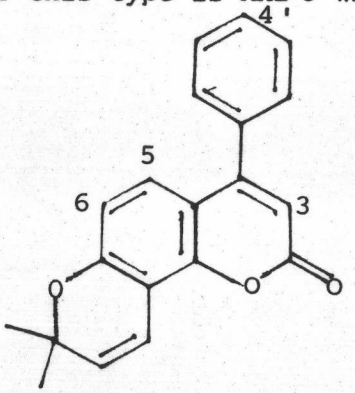


3-phenylseselin type

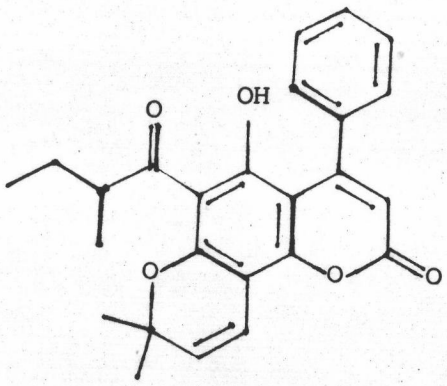


scandenin

4.6 4-Phenylseselin (angular) - There is a phenyl ring substituted at C-4 of the seselin angular type nucleus. The sample of this type is MAB 5 which was found in *Mammea americana* L.[3].

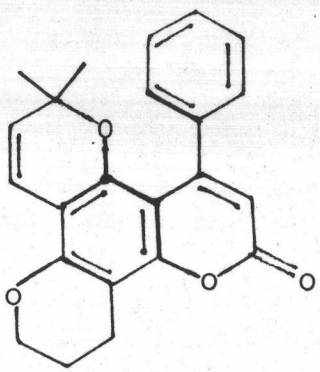


4-phenylseselin type



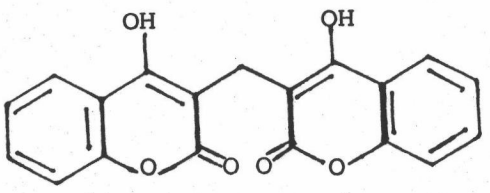
MAB 5

4.7 Tripyran derivatives - There is two pyran rings and one phenyl rings in the structure of coumarin nucleus. This type of structure can be found in Calophyllum inophyllum.[4]



tripyran derivatives

5. Bicoumarins - There are two coumarins nucleus in the structure. Dicoumarol is important in this type, it is found in spoiled sweet clover[1] and is function as anticoagulants.



dicoumarol

II. Structure determination of coumarins.

Coumarin structure can be determined by spectroscopic method. Some special character of coumarin from the spectrometers are summarized as see below.

UV spectrophotometer - Several investigators have measured ultraviolet (UV) absorption spectra of coumarins and attemped to correlate them with structure. The double bond between 3- and 4-positions is important, because when it is reduced, the characteristic absorptions are lost[3]. Substitution on coumarin nucleus produces a marked effect; consequently, the UV data provide useful information. Hydroxy group at the 6-position produces a much larger shift[30]. Coumarin itself in alcohlic solution has λ_{max} at 275, 284 and 310 nm (log. ε 4.30, 3.96 and 3.72) but 6-hydroxycoumarin has λ_{max} at 349 nm (log. ε 3.63). Free -OH on the benzene ring causes bathochromic shift for the longer wavelength maximum in the presence of KOH[31]. Umbelliferone has λ_{max} at 300, 305 and 325 nm (log. ε 3.0, 3.95 and 4.15) in neutral and λ_{max} at 372 (log. ε 4.2) in basic solution. 5-Hydroxycoumarin causes hypsochromic shift. 5,7-Dihydroxycoumarin has a large bathochromic shift at λ_{max} 282, 326 and 372 nm (log. ε 3.43, 4.09 and 3.36) while 6,7-dihydroxycoumarin differ little from 6-hydroxycoumarin at λ_{max} 295 and 350 nm (log ε 3.70 and 4.07).

Comparative UV absorption study of furanocoumarins by Lee and Soine[32] has revealed that linear and angular furanocoumarins show distinctly different spectra. The linear furanocoumarin has

λ_{max} at 242-245 nm and above 260 nm, but angular furanocoumarin has no this characteristic. C_5 or C_8 monosubstituted linear furanocoumarins have λ_{max} at about 260 nm and λ_{min} at 276 nm, whereas C_5 and C_8 disubstituted ones have characteristic λ_{max} at 273 and 286 nm. The C_5 monosubstituted compounds having λ_{max} at about 268 and 308 nm and λ_{min} at 254 nm can be readily differentiated from C_8 substituted compounds, which have λ_{max} at 301 nm. The nature of substituents of OCH_3 has little influence, since almost identical spectra are obtained with them.

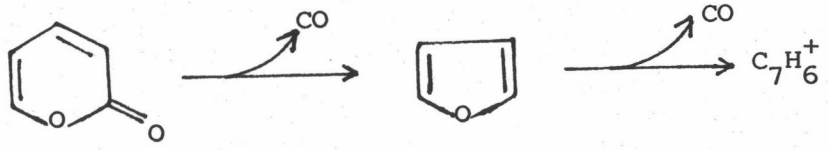
IR spectrophotometer - Infrared analysis has also found to be used in the structure characterization of coumarins. The particular value of such analysis, apart from enabling the detection of substituent groups, lies in its ability to assign a lactone function to the coumarin. Characteristic bands for the α-pyrone moiety are found, as a rule, in the region 1715-1745 cm^{-1} together with those of the conjugated [3,4] double bond at 1625-1640 cm^{-1}. Aromatic absorption bands are found in the usual regions[3].

Lee and Soine[32] studied furanocoumarins. They observed a triplet at 3025-3130 cm^{-1} which was CH stretching of substituted furan, benzene and α-pyrone rings, and aromatic C=C stretching in the region of 1535-1623 cm^{-1}. A characteristic furan ring peak at 870-885 cm^{-1} was due to the out-of-plane deformation vibration of C-H bands. The position of the C=O stretching band of the α-pyrone was apparently determined by the inductive effect of the substituents at the 5- and 8- positions of linear furanocoumarins. When an methoxy or isoprenyl ether group is

attached at C_5 and the C_8 is not substituted, the C=O band shifts to a frequency higher than 1720 cm^{-1}, but if it is at the C-8 position, a lower frequency (lower than 1720^{cm-1}) is obtained.

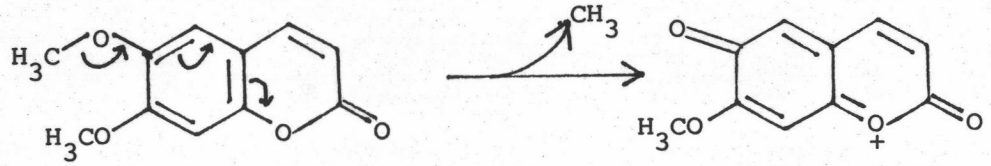
Bukreeva and Pigulevskii[33] have found that furanocoumarins with substituents at C-5 and C-8 positions have bands at 1624-7, 1607-14. 1588-95 and 1546-57 cm^{-1}, the strongest band at 1588-95 cm^{-1}. Derivatives with substituents at the C-5 position only have bands at 1616-24, 1601-8 and 1577-81 cm^{-1}, the strongest band at 1616-24 cm^{-1}. Derivatives with substituents at C-8 only have bands at 1621-5, 1583-5, 1559-61 and 1545 cm^{-1}, the strongest absorption being at 1583-5 cm^{-1}. Bands of medium intensity at 1500-15cm^{-1} found in the IR spectra of some angular furanocoumarins and simple coumarins can serve to differentiate them from linear furanocoumarins. Very strong absorption at 740-60 cm^{-1} in furanocoumarins was assigned to the C-H in-plane deformation vibration; in other coumarins, the band is weak or absent.

Mass spectrometer - The use of mass spectrometry in studies on coumarins was earlier limited to the determination of the exact molecular weight of compounds in scarce supply. The characteristic and well known fragmentation of coumarin under electron impact is the ready loss of carbon monoxide from pyrone ring to from an ion having benzofuran structure, followed by the a further loss of the remaining oxygen atom again as CO to give an ion, $C_7H_6^+$, of uncertain structure[4].



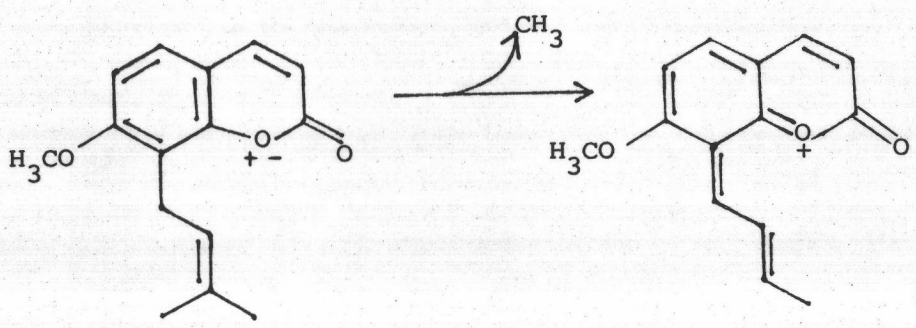
pyrone ring furan ring

Methoxycoumarins readily eliminate CO and methyl radical either simultaneously or one after the other, depending upon the structure[34]. The loss of that methyl radical is favoured, which yields an ion probably stabilized by a quinonoid structure. The loss of the methoxy group fission of the phenyl-oxygen bond does not readily occur. C-prenylated coumarins loss methyl radical



methoxycoumarin quinonoid structure

from the prenyl substituent rather than from the methoxy group, as the former gives rise to highly conjugated ion, which is not possible if the methoxymethyl group is lost[3].

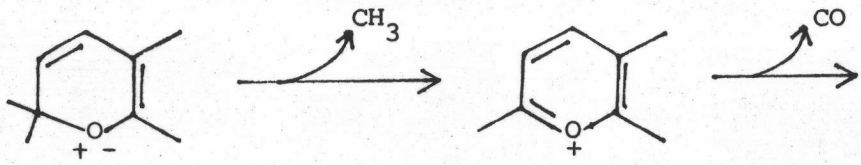


C-prenylated coumarin

In coumarins having a five-membered or longer chain allyl ether function, fragmentation of the aliphatic residue with hydrogen rearrangement is very facile[3].

C-7-Methoxycoumarins show a strong molecular ion peak, then a loss of CO, followed by methyl radical and carbon-monoxide[4].

A common feature of the fragmentation of 2,2-dimethylchromenopyrones consists of a process, which involes the loss of a methyl radical to form a stable benzopyrylium ion. It is followed by further loss of oxygen as CO[3].



2,2-dimethylchromenopyrone benzopyrylium ion

NMR spectroscopy - The ^{1}H-nuclear magnetic resonance spectra of more than one hundred natural coumarins were obtained and analysed to provide qualitative spectra-structure correlations based on chemical shifts and coupling constants. These data were in turn used to construct a program whereby the spectra of unfamiliar or novel coumarins could be processed systematically to deduce the probable structure of the unknown[2].

Ring proton analysis in coumarins

All coumarins unsubstituted in the pyrone ring exhibit doublets ($J \sim 9.5$ Hz) at δ 6.1-6.4 and δ 7.5-8.3 arising, respectively, from the *cis* protons H-3 and H-4 of this ring. This feature may be

taken as a firm indication of the presence of a coumarin nucleus. (It also allows clear and immediate distinction from chromones, which sometimes co-occur with coumarins in plant material; for chromones give entirely different characteristic signals of their own.) In natural coumarins, one can usually assume the presence of oxygen at C-7. If C-5 also bears an oxygen function, the H-4 resonance will be found at δ 7.9-8.2 in $CDCl_3$; δ 8.1-8.3 in DMSO-d_6 (35). In such a case, the proton at C-6 and C-8 will be meta-coupled doublets (J ∿ 2 Hz) if both are presence, or singlets if only one is presents. There is no general way to distinguish outright between H-6 and H-8 singlets, although, as explained below, the position of the proton can often be deduced by location of the substituent at the other position.

In coumarins lacking an oxygen function at C-5, the H-4 doublet falls at δ 7.5-7.9 in $CDCl_3$; 7.8-8.1 in DMSO-d_6. Three possibilities then exist for the arrangement of further benzene ring protons:

1. H-6 and H-8 may both be present, a pair of H-5/H-6 doublets (J ∿ 9 Hz) is found between the pyrone doublets, and the H-6 signal is meta-coupled to a nearby H-8 signal. The H-6 signals of coumarins are always upfield from the H-5 signals

2. H-8 may be absent, a very common situation. The H-5/H-6 double system is then very clear-cut, the H-5 signal being found close to 7.33 and H-6 signal near 6.80.

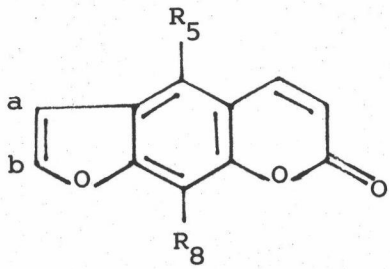
3. H-6 may be lacking, so that H-5 and H-8 are seen as singlets, with H-5 downfield from H-8.

Ring substituents in coumarins

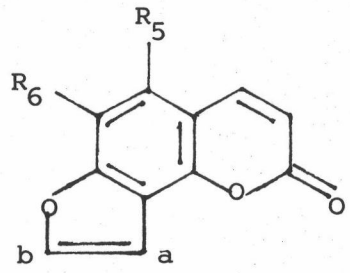
Aromatic methoxy groups resonate at δ 3.8-4.4 except in a few very instances; the methoxyls of furanocoumarins are the only ones which tend to occupy the 4.0-4.4 part of this range. The other common type of ether involves the 3-methyl-2-butenyl ("prenyl") group or its oxygenated congeners. The parents group itself is virtually unmistakable, giving a methylene doublet (J = 7 Hz) at δ 4.3-5.0, a coupled olefinic triplet at δ 5.1-5.7 and two nonequivalent methyl resonances at δ 1.6-1.9, one or both of which appear as a doublet coupled allylically (J ∿ 1 Hz) to the olefinic proton. (The group and its relatives are also typical ring alkyl substituents, which are easily distingished from ethers by the higher field of the methylene signal, δ 3.3-3.8)[2].

Furano- and pyranocoumarins

In a large percentage of natural coumarins, a third ring is fused with the benzopyrone system, forming a new oxygen heterocycle involving the C-7 oxygen atom. Such rings result from cyclizations of five-carbon chains and may be five-membered (furanocoumarins and dihydrofuranocoumarins) or six membered (pyranocoumarins and dihydropyranocoumarins) depending on the point of cyclization. The formation of furanocoumarins additionally requires loss of a three-carbon fragment.



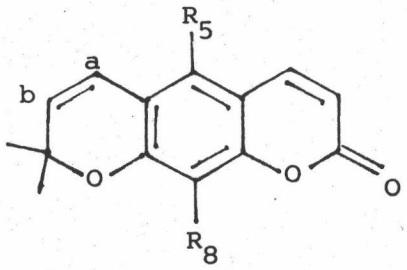
linear furanocoumarins



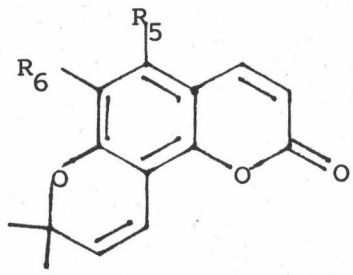
angular furanocoumarins

Furanocoumarins are easily recognized by their coupled furan proton doublets ($J \sim 2.5$ Hz), H-a at δ 6.7-7.2 and H-b at 7.5 -7.7. H-b is nearly invariant within this narrow range for all furanocoumarins and so is not useful in further characterization; however H-a provides information on the position of benzene substituents through long-range coupling effects[35].

By far the most useful such coupling is that between H-a and H-8 ($J \sim 1$ Hz) in linear furanocoumarins, or H-a and H-6 ($J \sim$ 1 Hz) in angular derivatives. In practice this causes the H-a signal to appear as a close-packed double-doublet, and indicates the presence of hydrogen at C-8 (or C-6, as the case may be); substituents at C-5 do not interfere. The chemical shift of H-a relative to that of H-3 can sometimes be used to indicate the presence of an isobergaptol derivative, since only in this case does the H-6 proton (if present) appear between the two signals. The postion of oxygen substituents in furanocoumarins can sometimes be confirmed by UV spectrometry[32].



linear pyranocoumarins



angular pyranocoumarins

Pyranocoumarins exhibit pyran doublets (J = 10 Hz) at 6.3-6.9 (H-a) and δ 5.3-5.8 (H-b). These compounds also show H-a long-range coupling with H-8/H-6 but the coupling constant is sometimes so small that the effect is of doublful diagnostic value. The gem dimethyl groups resonate together, very close to δ 1.46, but this along is not sufficient indication of a pyran ring[4].

At this point a short digression about long-range coupling[36,37] in coumarin spectra is in order. As mentioned above, coupling between H-a and H-8 of linear furanocoumarins (or H-a and H-6 of angular compounds) can be used to determine substitution patterns, and an analogous coupling occurs in pyranocoumarins. Besides these, long-range couplings (J = 0.6-1.0 Hz) between H-4 and H-8 are clearly visible in the spectra of linear furanocoumarins. Since angular derivatives do not show this coupling, these can sometimes be recognized in this way, especially if H-a/H-6 coupling is clearly present.

Table 6 is summarized over one hundred coumarins and their derivatives' characteristics to form a systematic examination of NMR spectra of natural coumarins

Table 6 Systematic examination of NMR spectra of natural coumarins[2]

1. Region δ6.0–8.5[a]				
1-a	Two doublets	J≈9.5 Hz	δ6.1–6.4, 7.5–7.9	Coumarin nucleus without oxygen at C-5	
1-b	Two doublets	J≈9.5 Hz	δ6.1–6.4, 7.9–8.2	Coumarin nuclues with oxygen at C-5	
1-c	Two doublets	8.5 Hz	δ6.6–6.9, 7.1–7.5	8-Substituted coumarin	
1-d	Two doublets	2.5 Hz	Both δ<7.0	5-7-substituted coumarin	check 1-b
1-e	Two doublets	2.5 Hz	δ6.7–7.2, 7.5–7.7	Furanocoumarin with benzene ring substituted ortho to furan oxygen	
1-f	Doublet and double doublet	2.5 Hz 2.5,1 Hz	δ7.5–7.7 δ6.7–7.2	Furanocoumarin unsubstituted ortho to furan oxygen	
1-g	Doublet	10.0 Hz	δ6.3–6.9	Pyranocoumarin	check 2-a
1-h	Doublet	5.0 Hz	δ6.5–7.1	Khellactone ester	check 2-b
1-i	Doublet	6.5 Hz	δ6.5–7.1	Hydroxycolumbianetin ester	check 2-b
1-j	Singlet	-	δ7.4–7.6	Possible 3-alkylcoumarin	
1-k	Quartet of Quartets	7.1 Hz	δ5.9–6.2	Angelate ester	check 3-g
1-l	Multiplet	-	δ6.1–6.3	Possible 1,1-dimethylallyl group	
2. Region δ 2.5–6.0[b]				
2-a	Doublet	J=10.0 Hz	δ5.3–5.8	Pyranocoumarin	check 1-g
2-b	Doublet	5.0 Hz or 6.5 Hz	δ5.1–5.5	Khellactone ester Hydroxycolumbianetin ester	check 1-h check 1-i
2-c	Quartet of Quartets	7.1 Hz	δ5.9–6.2	Angelate ester	check 3-g
2-d	Quartet	1.0 Hz	δ5.4–5.8	Senecioate ester	check 3-f
2-e	Quarter of Triplets	1.7 Hz	δ5.1–5.8	Prenyl group	check 3-b
2-f	2H Doublet	7 Hz	δ3.3–3.7 or 4.5–5.0	C-prenyl group O-prenyl	check 3-b
2-g	2H Multiplet	-	δ4.7–5.3	Terminal CH_2	
2-h	2H Multiplet	-	δ2.8–4.7	Prenyl epoxide or diol; or dihydropyranocoumarin with oxygen at C-b	check 3-c
2-i	Multiplet	-	δ3.0–3.9 or 5.1–5.5	As for 2-h Downfield position only when ester group is present	check 3-c
2-j	2H Doublet	8 Hz	δ3.0–3.4	Dihydrofuranocoumarin	check 2-k
2-k	2H Triplet	8 Hz	δ4.6–5.0	Dihydrofuranocoumarin	check 2-j
2-l	Singlet	-	δ3.8–4.4	Aromatic methoxyl	
3. Region δ 0.5–2.5[c]				
3-a	Singlet	-	δ2.0–2.2	Acetate ester	
3-b	Two doublets	J≈1 Hz	δ1.6–1.9	Prenyl group. These doublets sometimes have the appearance of singlets	check 2-c, 2-f
3-c	Two singlets	-	δ1.2–1.9	Prenyl epoxide or diol; or dihydrofuran or dihydropyran rings	
3-d	3H signals	-	δ<1.2	Saturated hydrocabon methyls, as in dihydroangelate or isovalarate	
3-e	6H singlet	-	δ1.45–1.65	Possible pyranocoumarin or 1,1-dimethylallyl group	check 1-f, 1-j
3-f	Two doublets	J≈1 Hz	δ1.8–2.1	Senecioate ester	check 2-d
3-g	Multiplets	-	δ1.8–2.1	Angelate ester	cheak 2-c

a Phenolic hydroxyls may appear as singlets in this range, and if $CDCl_3$ is the sample solvent a chloroform singlet will be present near δ7.25

b If d_6-DMSO is the sample solvent a DMSO multiplet will be present near δ2.50 and a broad singlet due to solvent water may be visible at δ3–4.

c Alcoholic hydroxyls usually appear in this region.

The progression in coumarin-structure determination in NMR has been achieved using[4]:-

1. Solvent induced shifts.

2. Long-range coupling constants. - They could be of great diagnostic value in determining substitution patterns, however is not a very trustworthy tool, because, in general, couplings with very small values are likely to be missed in routine work (especially at low signal to noise ratio) due to which its practical value is rather limited.[2]

3. Nuclear Overhauser effect. - NOE has been used for the confirmation of the stereochemistry and structural information of coumarins.[4]

4. Lanthanide shift reagents. - It has been used to differentiate between the possible isomers of some coumarins.[4]

III. Biosynthesis of coumarins

The coumarins are typical metabolic products of higher plants; the simple ones are formed from the corresponding substituted *trans*-cinnamic acid derivatives. 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 coumarinic acid glycoside, which is structurally derived from *cis*-cinnamic acid. By enzymic elimination of glucose, free coumarinic acid is formed which cyclizes spontaneously to coumarin.[4]

Coumarins, to the biochemist, are lactones of phenylpropanoic acids, a class which derives from the shikimic acid pathway via the protoaromatic amino acids phenylalanine and, in a few plants, tyrosine.[38] *Trans*-cinnamic acid formed the enzyme mediated deamination of phenylalanine[39] so that shikimic acid, phenylalanine, and *trans*-cinnamic acid are common precursors of this coumarin nucleus (Fig. 1).[40-42] Cinnamic acid, however, represents a branch point in the elaboration of coumarin itself and probably other coumarins lacking 7-oxygenation, on the one hand, and the 7-hydroxycoumarins based on umbelliferone[43,44] on the other. The latter group constituting the vast majority of the class. *ortho*-Hydroxylation of *trans*-cinnamic acid leads to coumarin itself (Fig. 1), via a light-catalysed *trans-cis* isomerization, and lactone ring formation which can be formally represented as a dehydration but in novobiocin, lactone ring closure is not by dehydration

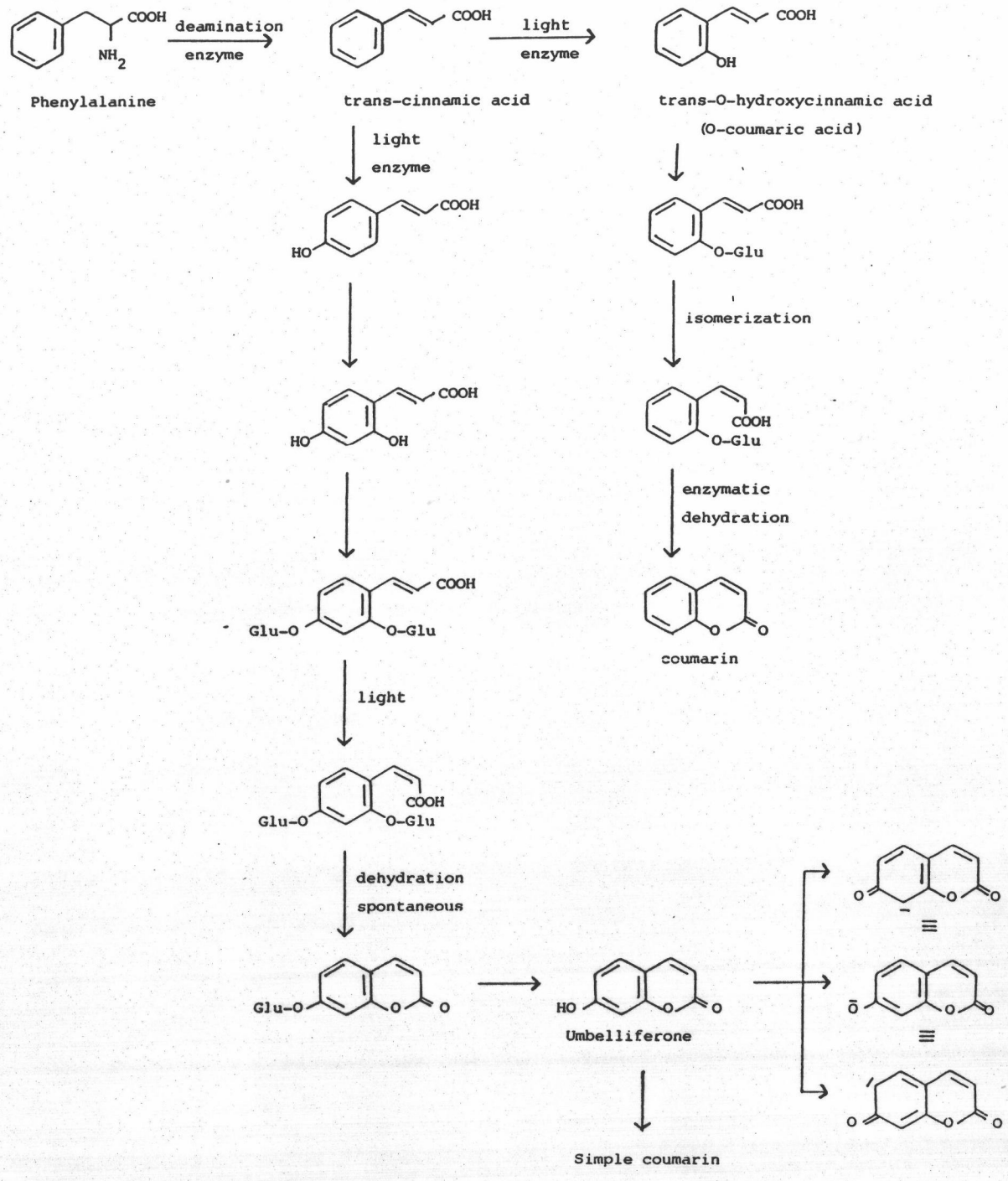


Fig. 1 Pathways from phenylalanine to coumarins[5,6,75]

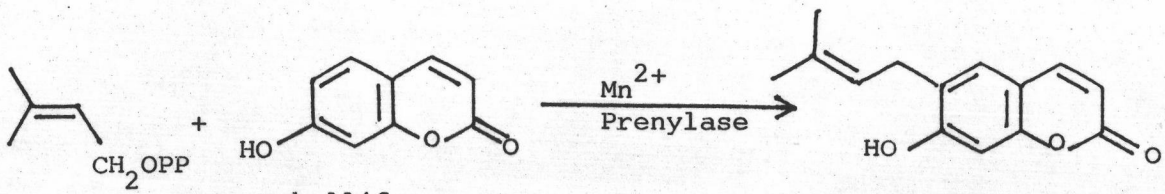
but by an oxidative mechanism.[47,48] *para*-Hydroxylation of *trans*-cinnamic acid is a necessary prerequisite for synthesis of the 7-hydroxycoumarins, via *o*-hydroxylation and lactonization as before (Fig. 1). The *ortho*-hydroxylations of cinnamic and *p*-hydroxycinnamic acids are mediated by different enzymes,[49,50] which are apparently seldom found together in the same species, so that coexistence of coumarin and the umbelliferones is an exceptional phenomenon.

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 coumarin[51], herniarin,[52] and umbelliferone[53] have all been shown to occur in the form of glycosides of the corresponding *cis*-*o*-hydroxycinnamic acids. This reaction is light-dependent and spontaneously.[4] However, glucosidases are also present which gain access to these substrates upon disruption of the cells, and the liberated aglycones by enzymatic elimination of glucose. Free coumarinic acid is formed which cyclizes spontaneously of the lactone ring to coumarins.[54] The pungent odour of coumarin associated with new-mown hay originates in this way.

Current evidence, admittedly incomplete, suggests that oxygenation of the benzene ring of simple plant coumarins tends to occur before the benzopyrone nucleus has been elaborated.[3]

The major feature in the diversification of simple coumarins in both Rutaceae and Umbelliferae is the widespread incorporation of prenyl units. Prenylation has been demonstrated to occur at the

umbelliferone stage.[55] In *Ruta graveolens* L. the additon of the dimethylallyl unit at C-6 appears to be specifically controlled by the enzyme dimethylallylphosphate (prenyl) transferase,[56,57] which has a requirement for Mn^{2+} (Fig. 2) to form demethylsuberosin. It does not, however, transfer a prenyl group from dimethylallyl pyrophosphate to C-8 of umbelliferone to form osthenol. It failed to used 7-methoxycoumarin (herniarin) as substrate. It is also inactive against pyrophosphate of O-prenylating and two or three isoprenoid units-geranyl and farnesyl.[56] This transferase is chloroplast enzyme,[57] a point of interest in the light of earlier demonstration that hydroxylase mediating the committed step in the elaboration of coumarin and the 7-oxygenated coumarins are also chloroplast enzyme.[49,50]



umbelliferone demethylsuberosin

Fig. 2 Formation of demethylsuberosin by a prenylase from *Ruta graveolens* L.[6].

The mechanism of prenylating of umbelliferone is visualized as involving the formation of the stable anion which will permit the eletrophilic attack of prenyl-carbonium ion at either C-6 or C-8 to yield C-prenyl coumarins or on the phenoxide to give O-prenyl compounds (Fig. 1). Perhaps the role of the prenylating enzyme(s) is to localize the charge on the anion and thereby direct the attack of the prenyl unit. From tracer experiment have demonstrated that demethylsuberosin is a precursor of the linear

furanocoumarins and that osthenol is a precursor of the angular furanocoumarins.[58,59,60]

The nature of the pathways between demethylsuberosin and marmesin, and between osthenol and columbianetin, is still not definitely established. It was suggested quite early in the biosynthetic studies that the intermediates in question are coumarins in which the double bonds of the prenyl side chains have been epoxidized to yield structure A and B.[55] The cyclized reaction was shown in Fig. 3, involving an attack of the phenol group on the epoxide ring, with a cyclization, and the formation of a substituted dihydrofuranocoumarin. There is no experimental evidence pertaining to this step, and it is still possible that diols such as C, formed by hydrolysis of the analogous epoxides, participate.

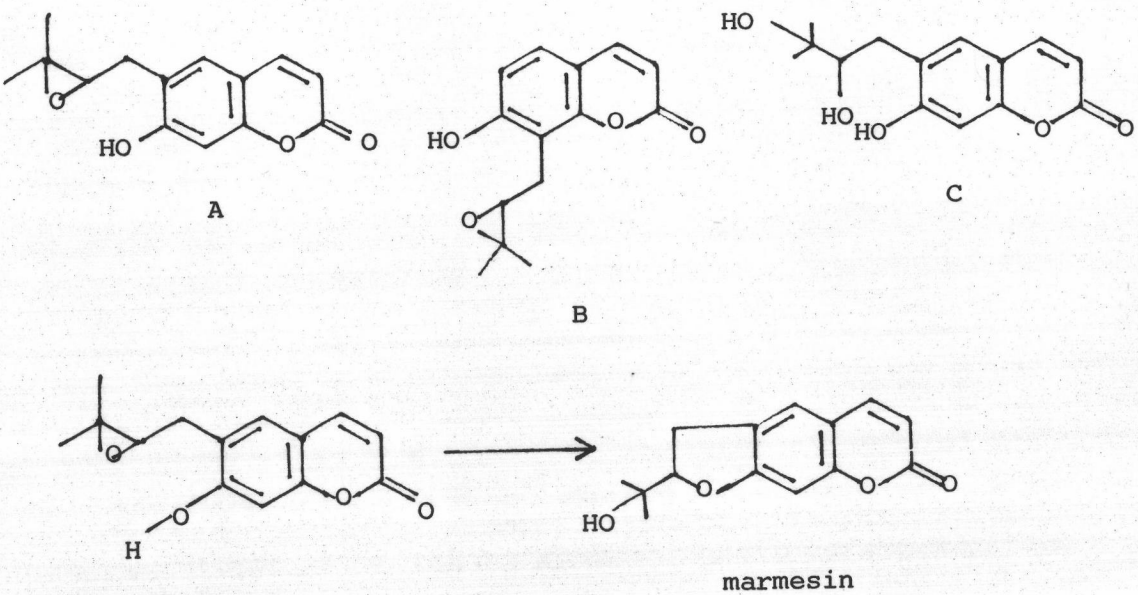
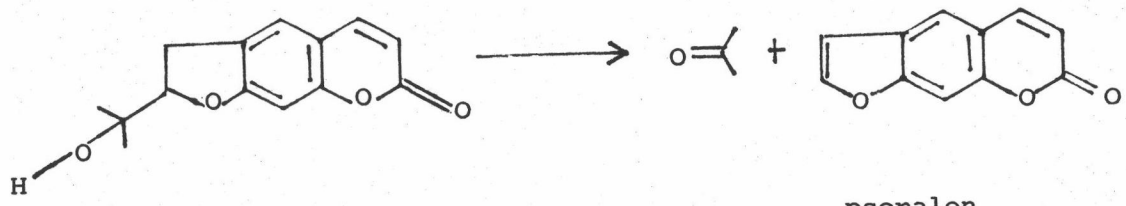


Fig. 3 Hypothetical cyclization reaction leading to marmesin formation.[6]



marmesin psoralen

Fig. 4 Mechanism proposed by Birch et al.[61] for the conversion of marmesin to psoralen[6]

The nature of this mechanism, in which a carbonium ion is generated at C-4' followed by a 1,3-elimination (isopropyl side chain) form marmesin and columbianetin as shown in Fig. 4,[61] the 3-carbon side chain being converted to acetone in the process and the double bond introduced to form the furan ring. To date there is no in vivo experimental support for this hypothesis.

Although no detailed investigation into the formation of pyranocoumarins have yet been reported the observation[59] that demethylsuberosin is heavily incorporated into 3',4'-dihydroxanthyletin suggests a pathway analogous to that for furanocoumarins (Fig. 5,6) is in operation. It has been noted that, as anticipated, the configuration of the C-prenyl epoxide intermediate is retained during the formation of pyranocoumarins (Fig. 7). The xanthyletin angular pyranocoumarins are probably the product of cyclization of a C-6 prenyl unit and a free C-5 hydroxy substituent.

Whilst the general mechanism of the cyclization of furan and pyran rings is now understood much of the detail remains unresolved. One of the foremost questions to be the likely intermediate, the epoxide or the diol (Fig. 7). In vitro experiments[62]

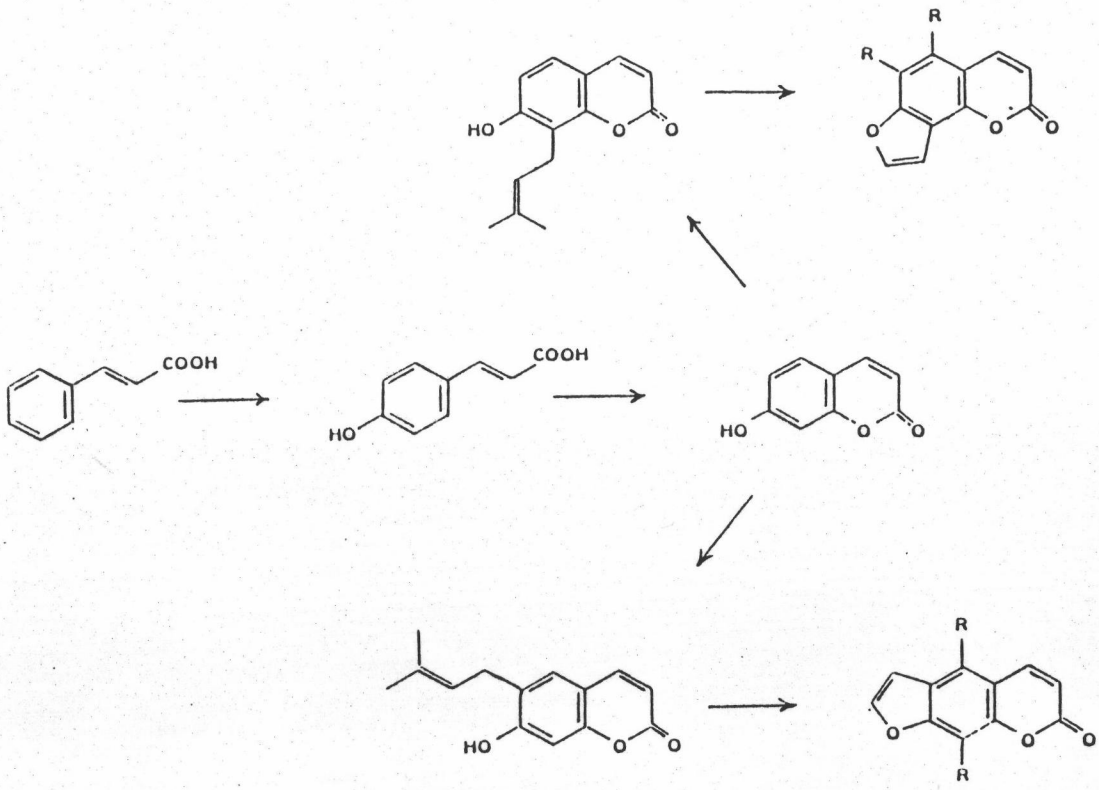


Fig. 5 Hypothesis of furanocoumarin biosynthesis in *Pimpinella magna*.[75]

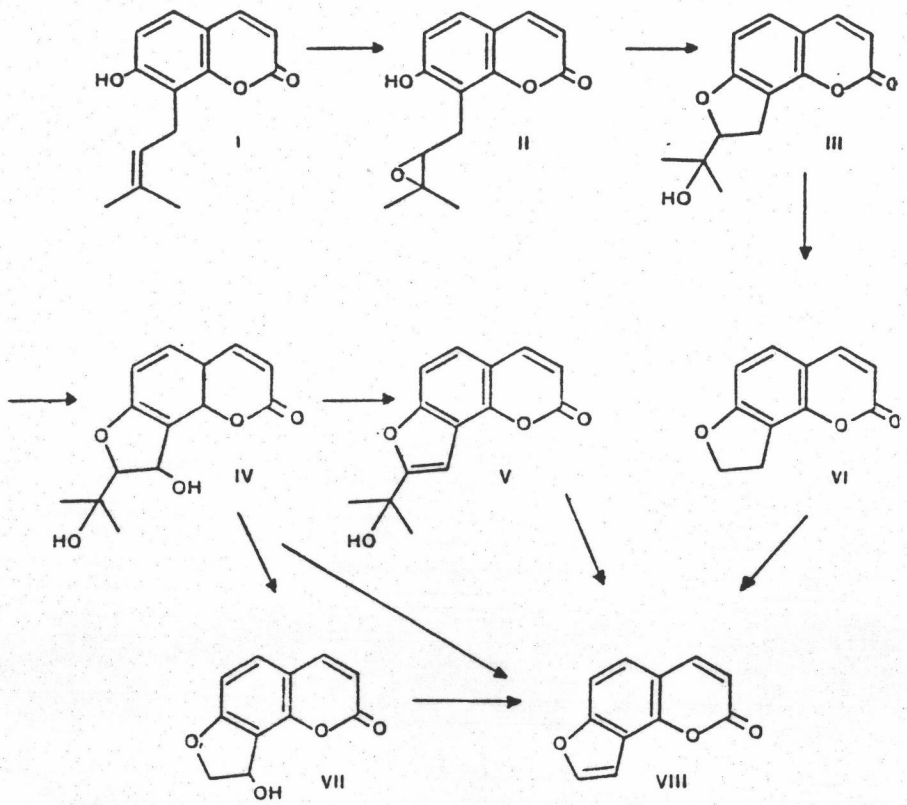


Fig. 6 Possible ways of formation of the furan ring in furanocoumarins.(75)

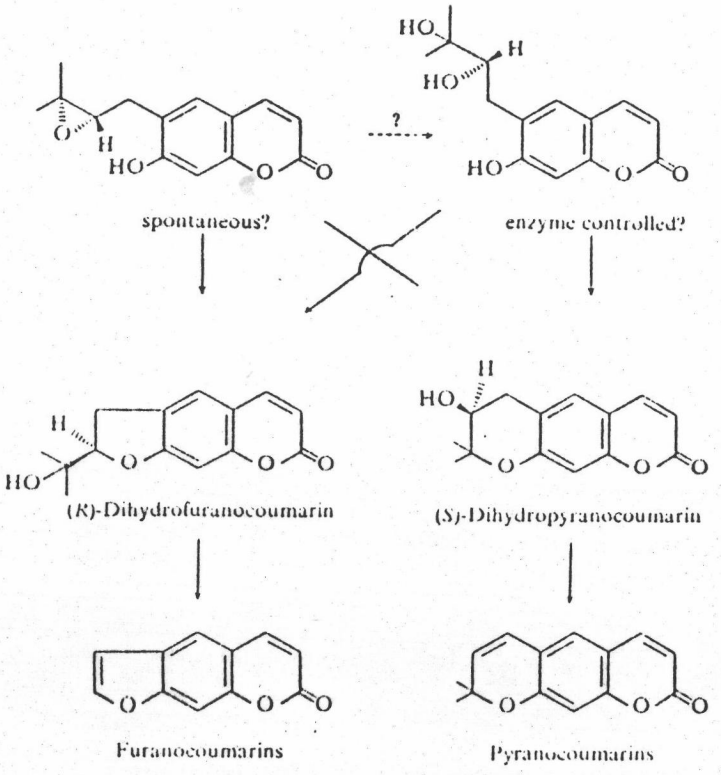


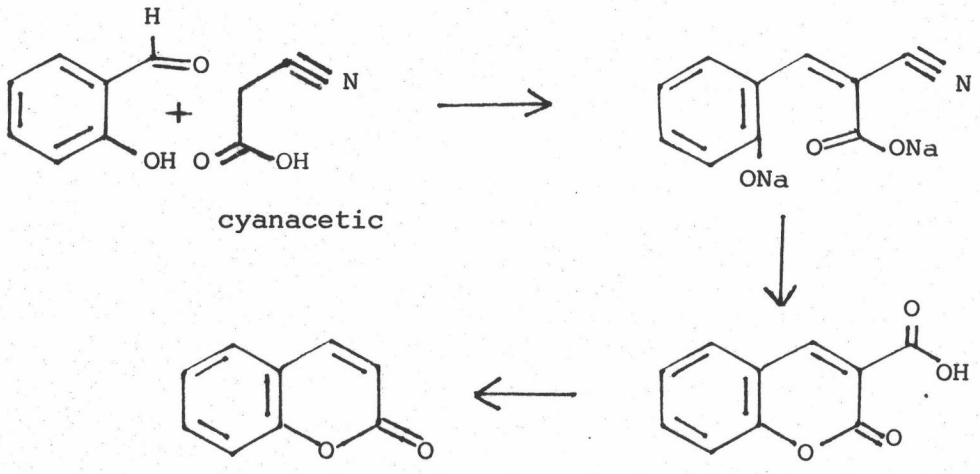
Fig. 7 Formation of linear furanocoumarins and pyranocoumarins form 6-C-isoprenyl coumarin precursor (after Grundon and McColl.)[76] An analogous pathway involving 8-C-isoprenyl coumarin precursors must also exist.[5]

have shown cyclization to occur spontaneously upon epoxidation of a prenyl group to which there is a free ortho hydroxy substituent and suggest the epoxide to be the more likely intermediate. The relative paucity of prenyl coumarins isolated with free *ortho*-hydroxy functions from the Rutaceae would seem to agree with this contention. A second unanswered question concerns the mechanism governing the choice of formation of either furano or pyrano coumarin rings. No enzyme system has yet been found to govern either and, in the light of the acknowledged spontaneity of cyclization, it seems feasible that none need exist. In this context the observation[62] that, in vitro, the furan ring was formed under neutral or basic conditions and the pyran ring under acidic conditions may will be significant. If this situation is paralleled in vivo then external factors aflecting the pH at the site of synthesis will obviously play a primary role in deciding the structures of the coumarins produced.

IV. Synthesis of coumarins

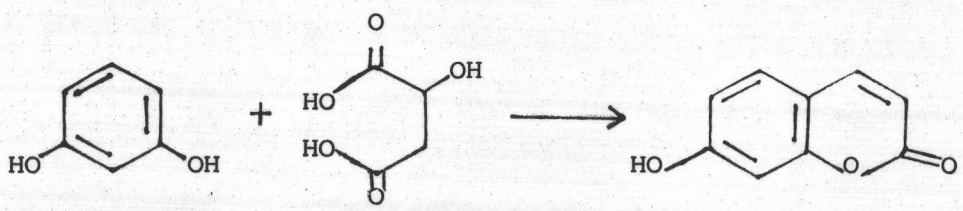
Perkin reaction The Perkin Reaction is the simplest method of preparing coumarins on a small scale. The investigation of Yanagisawa and Kondo[63] showed that by using this reaction, yield was only 27% when 90 g of salicylic aldehyde, 120 g of acetic anhydride and 150 g of anhydrous sodium acetate were heated for 2 hr. at 120℃ and then for 4 hr at 180-195℃ The use of a catalyst, the yield increased to 49% when 25 g of fused zinc chloride was added to the reacting substances, and to 70% when 2 g of iodine was added. The addition of oxides of Ni, Mn or Co has resulted in still better yield and fewer byproduct.

Several modifications have been effected in the Perkin coumarin synthesis. In the Knoevenagel modification, the acid component is malonic acid and the product, the corresponding coumarin-3-carboxylic acid, is in an almost quantitative yield. The reaction is done by heating the appropriate aldehyde, malonic acid and aniline hydrochloride on a steam bath for some hours. On decarboxylation of the acid, the coumarin is obtained. The difficulties of isolation due to excessive solubility, and easy decomposition and loss of malonic acid, have been serious hindrances to the adoption of this process on a technical scale. The difficulties have been overcome by substituting cyanacetic for malonic acid.[64]



coumarin coumarin-3-carboxylic acid

Pechmann reaction Another useful method for the preparation of coumarins is the Pechmann reaction, in which a phenolic compoind is condensed with a variety of compounds (usually β-ketonic esters) having a reactive methylene group. The condensing agents, such as sulfuric acid, hydrogen chloride, anhydrous aluminium chloride, phosphorous pentoxide, phosphoryl chloride, hydrogen fluoride, boron trifluoride etherate, polyphosphoric acid and cation exchange resins, (Duolite C-20, Amberilte IR-120 and Zeokarb 225) have been used by various workers.

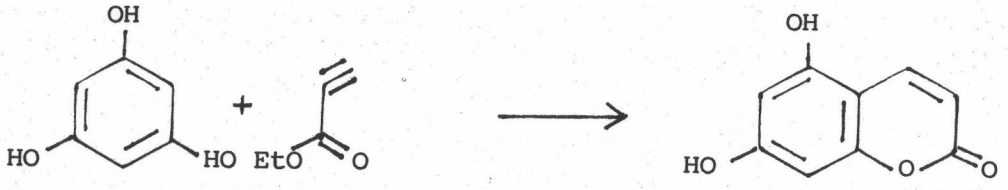


phenolic compound β-ketonic ester 7-hydroxycoumarin (umbelliferone)

Some of the more important syntheses of naturally occuring coumarins are given below.

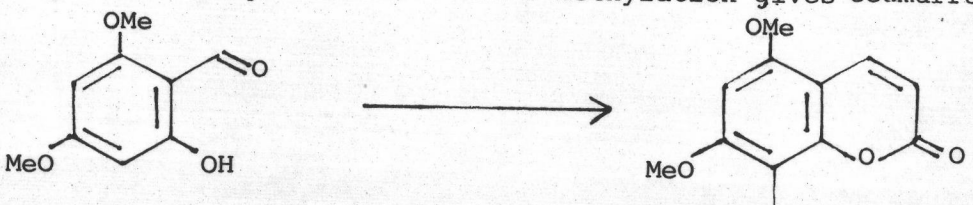
Simple coumarin derivatives

5,7-dihydroxycoumarin[65] 1,3,5-$C_6H_3(OH)_3.2H_2O$, $CH{:}CCO_2Et$ and $ZnCl_2$ are heated in a steam bath, cooled and treated with aq.HCl to yield 5,7-dihydroxycoumarin.

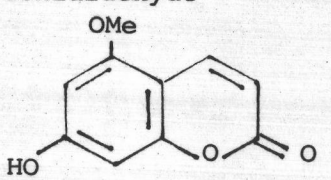


5,7-dihydroxycoumarin

Coumurrayin[66,67] 2-Hydroxy-4,6-dimethoxybenzaldehyde as its sodium salt is converted into 3-dimethylallyl derivative, which on Perkin condensation gives 5,7-dimethoxy-8-dimethylallyl coumarin. The sodium derivative of 7-hydroxy-5-methoxycoumarin is treated with 1-bromo-3-methyl-2-butene to give 7-hydroxy-8-(3-methyl-2-betenyl)-5-methoxycoumarin. This O-methylation gives coumurrayin



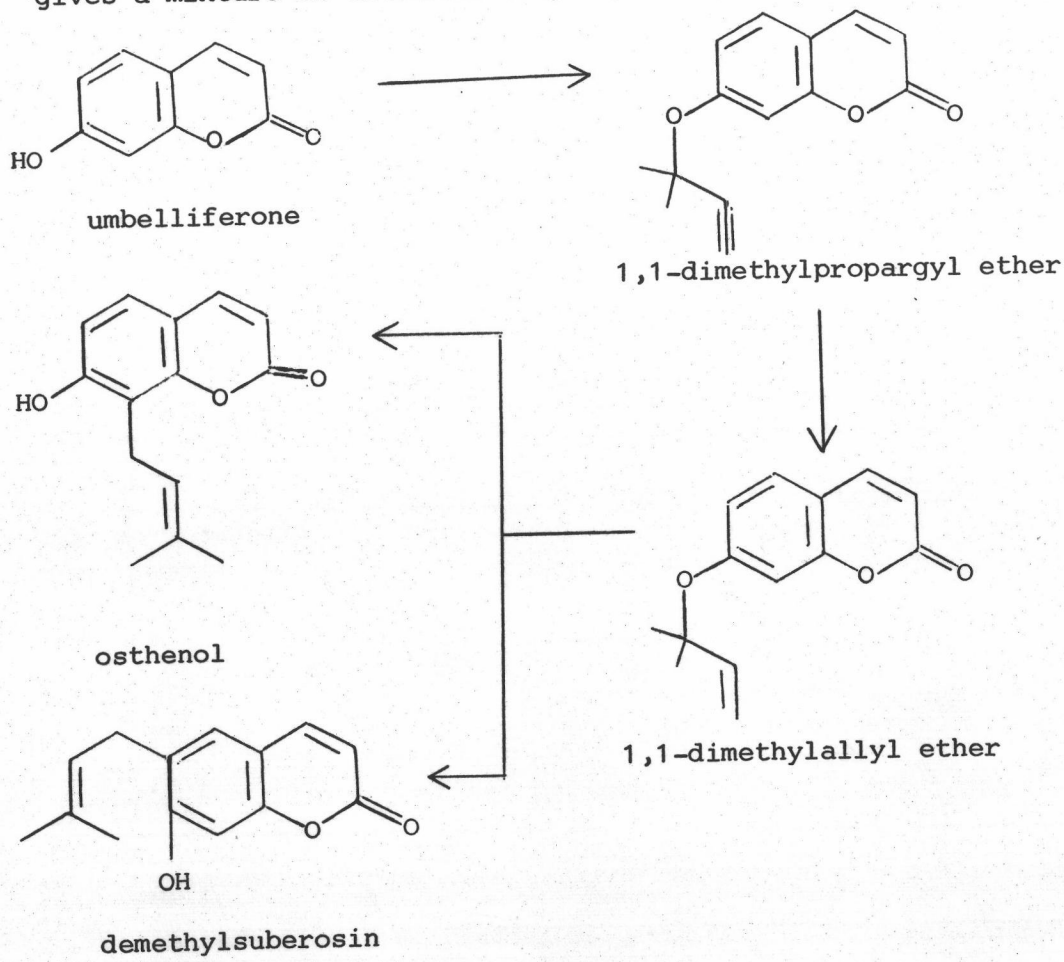
2-hydroxy-4,6-dimethoxy-benzaldehyde



7-hydroxy-5-methoxy-coumarin

coumurrayin

Osthenol and suberosin[68] Umbelliferone is treated with $ClCMe_2C{:}CH$ to give 1,1-dimethylpropargyl ether, which is subjected to partial hydrogenation to 1,1-dimethylallyl ether. This on pyrolysis gives a mixture of osthenol (major product) and demethylsuberosin.



umbelliferone

1,1-dimethylpropargyl ether

osthenol

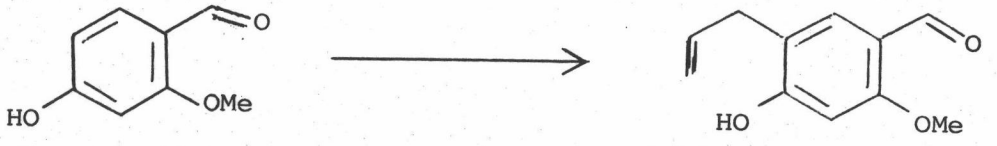
1,1-dimethylallyl ether

demethylsuberosin

Furanocoumarin derivatives

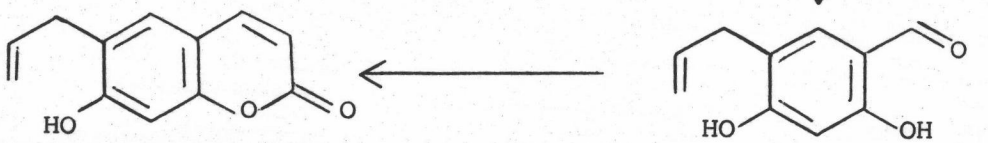
Psoralen[69] β-Resorcylaldehyde on allylation gives 4-O-allyl-β-resorcylaldehyde which on methylation yield the 2-O-methyl compound. This is under goes thermal rearrangement to give 2-O-methyl-5-allyl-β-resorcylaldehyde, which on demethylation affords 5-C-allyl-β-resorcylaldehyde. 6-C-allyl umbelliferone is obtained from it through Perkin reaction. The allyl group is

converted into the acetaldehyde by ozonolysis and cyclization to furan is achieved by the action of polyphosphoric acid.



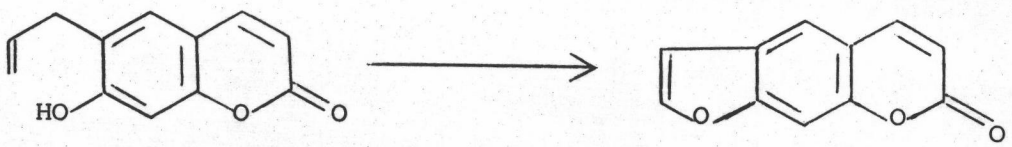
2-O-methyl compound

2-O-methyl-5-allyl-β-resorcylaldehyde



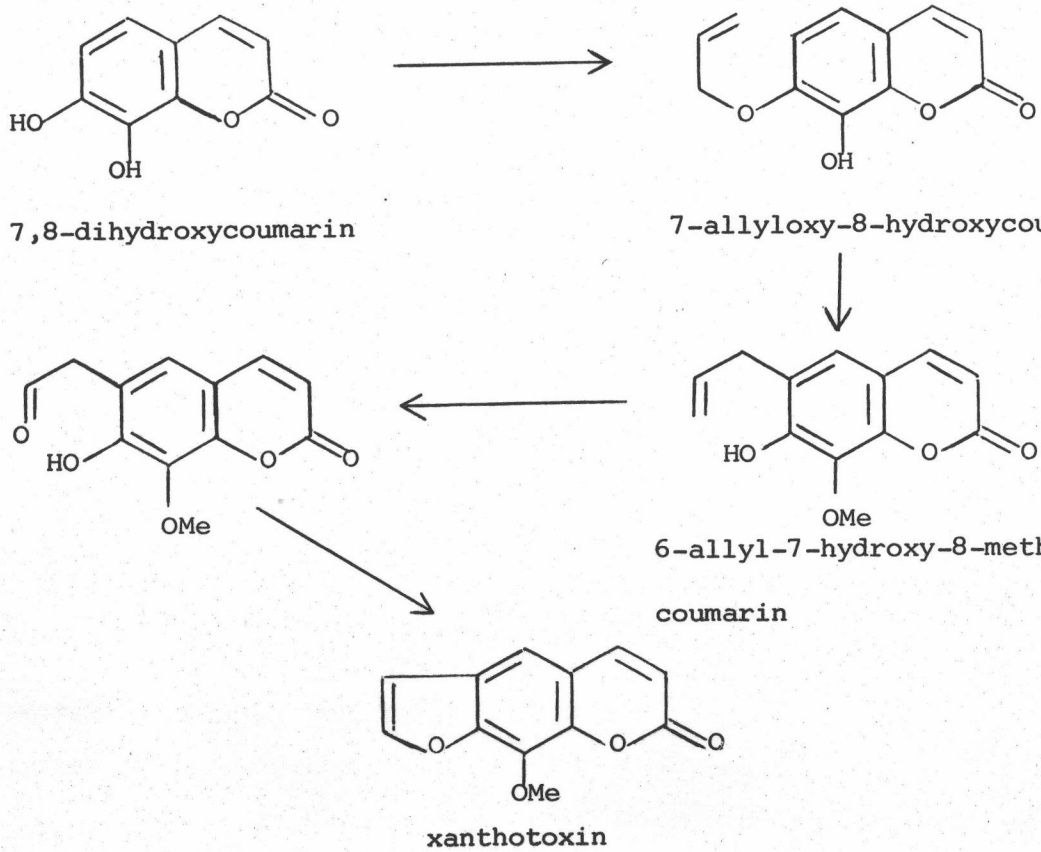
6-C-allyl umbelliferone

5-C-allyl-β-resorcylaldehyde

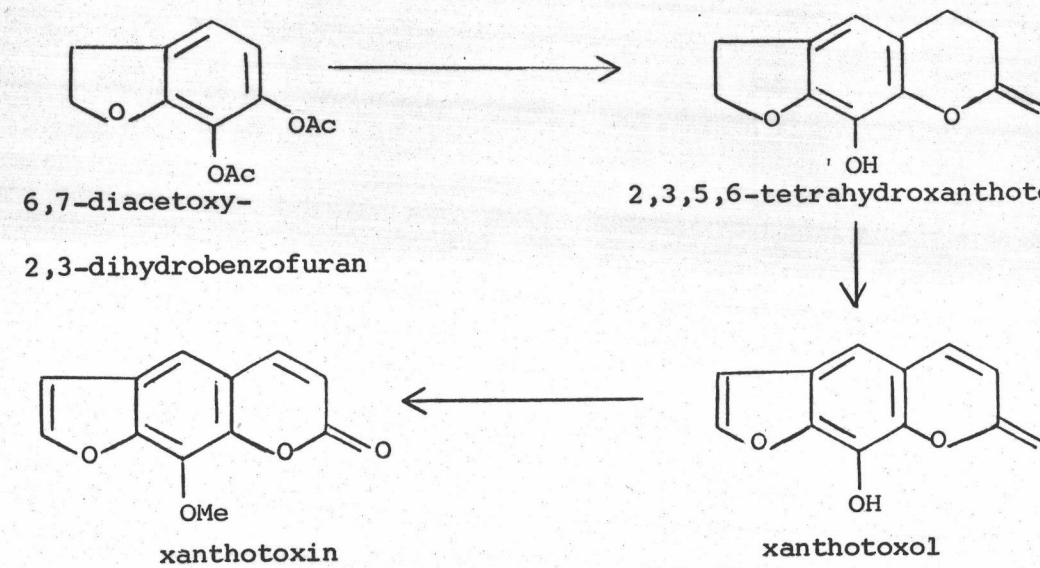


psoralen

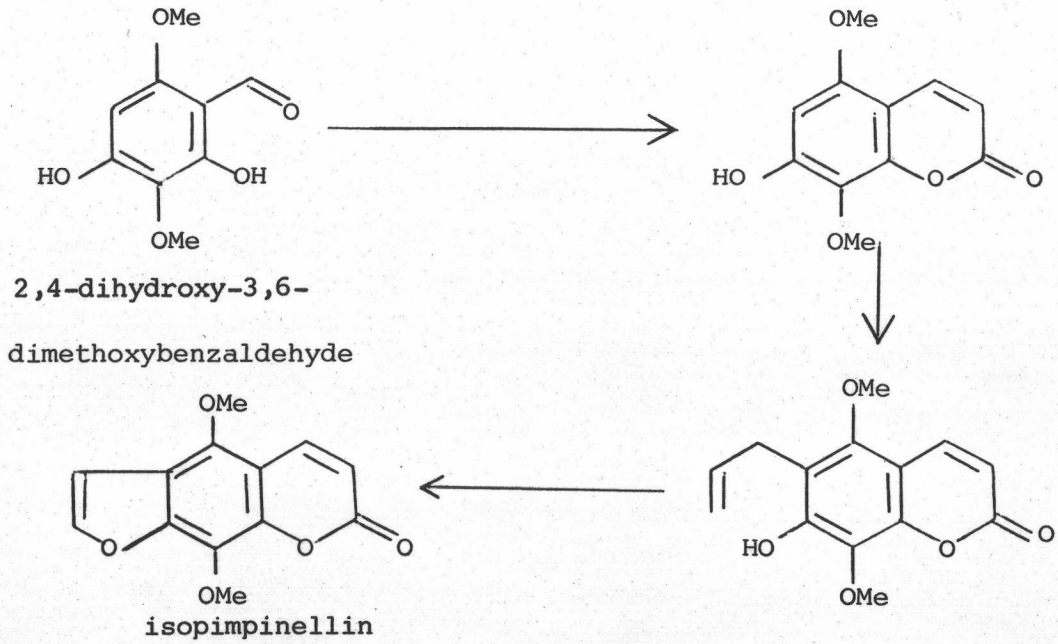
Xanthotoxin[69] 7,8-Dihydroxycoumarin on partial allylation affords 7-allyloxy-8-hydroxycoumarin. 7-allyloxy-8-hydroxycoumarin thus obtained is methylated and the methyl ether undergoes Claisen migration to give 6-allyl-7-hydroxy-8-methoxy-coumarin. Oxidation using ozone yield an intermediate acetaldehyde derivative, which is cyclized by means of polyphosphoric acid.



Xanthotoxol and xanthotoxin[70] 6,7-Diacetoxy-2,3-dihydrobenzofuran is treated with acrylonitrile in the presence of anhydrous zinc chloride and hydrochloric acid to give 66% of 2,3,5,6-tetrahydroxanthotoxol, which on dehydrogenation with Pd/C in boiling Ph_2O gives xanthotoxol. Final methylation with CH_2N_2 gives xanthotoxin.



Isopimpinellin[71] In the total synthesis of isopimpinellin from 2,4-dihydroxy-3,6-dimethoxybenzaldehyde, the steps involved are Perkin condensation, deacetylation, allylation and subsequent Claisen migration. The final furan ring formation involves oxidation with osmium tetroxide-periodate and cyclodehydration by means of polyphosphoric acid.

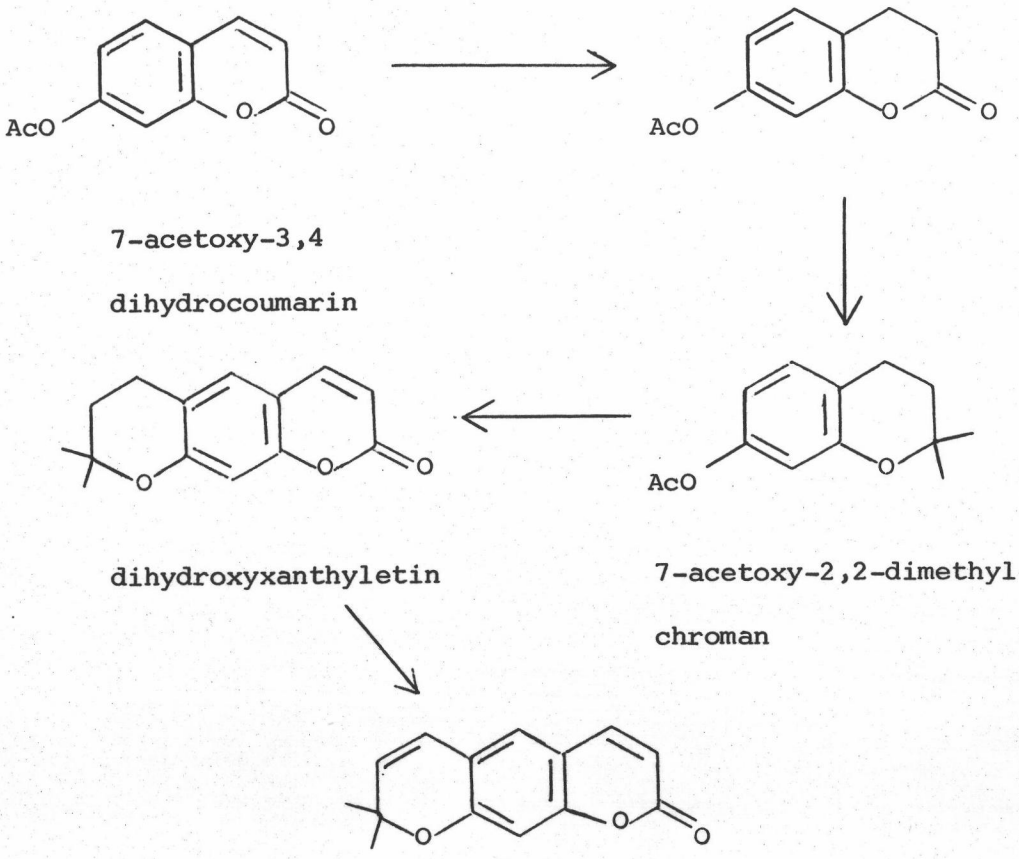


2,4-dihydroxy-3,6-dimethoxybenzaldehyde

isopimpinellin

Pyranocoumarin derivatives

Xanthyletin[72] 7-Hydroxy-2,2-dimethylchroman is obtained by the reaction of MeMgI with 7-acetoxy-3,4-dihydrocoumarin. Its condensation with methyl acrylate and anhydrous aluminium chloride and HCl afforded tetrahydroxanthyletin. Dehydrogenation over palladium-charcoal leads to the formation of dihydroxanthyletin. Bromination with N-bromosuccinimide followed by dehydrobromination with collidine yields xanthyletin



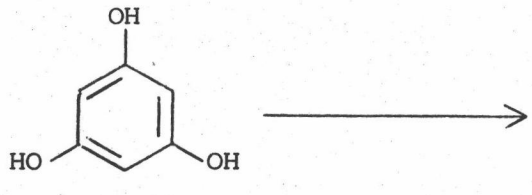
7-acetoxy-3,4 dihydrocoumarin

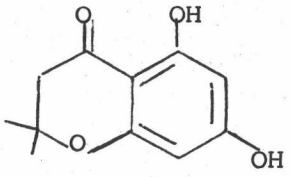
7-acetoxy-2,2-dimethyl-chroman

dihydroxyxanthyletin

xanthyletin

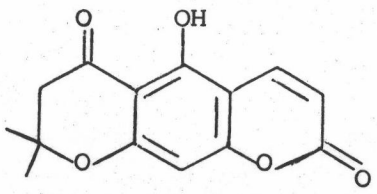
Clausenin and xanthoxyletin[22] 5,7-Dihydroxy-2,2-dimethyl-chroman-4-one, prepared from phloroglucinol and 1,1-dimethylacrylchloride is condensed with ethyl propiolate in the presence of zinc chloride. Of the two isomeric products A and B, A is identical with clausenin; on methylation with diazomethane and reduction of methyl ether with $NaBH_4$ it gives a mixture of two products. One of these on dehydration with $KHSO_4$ gives xanthoxyletin.





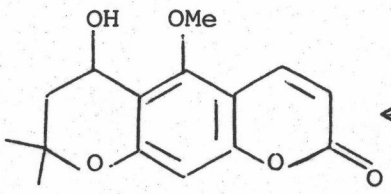
phloroglucinol

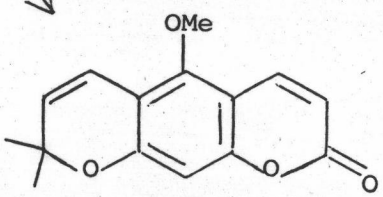
5,7-dihydroxy-2,2-dimethyl-chroman-4-one



A

clausenin



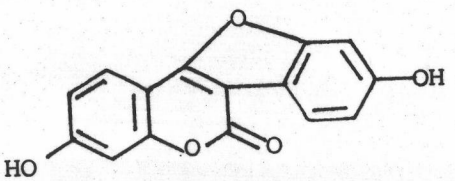


xanthoxyletin

V. Physiological properties of coumarins.

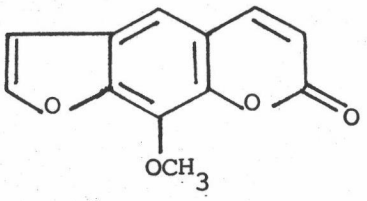
From the studied of structure-activity relationship, it was found that several physiological properties of coumarins were related with their structure. Coumarins exhibit many physiological properties as see below.

1. Oestrogenic activity - All the coumarins which have been shown to possess this property belong to the group of 3-phenyl coumarins. It is recognised that in these compounds the *trans*-stilbene character which makes a significant contribution to their spectral behaviour is also responsible for their oestrogenic activity.(1) Stilboestrol is the wellknown synthetic oestrogen. and coumestrol is one of the most potent which occurs in ladino clover and alfalfa.



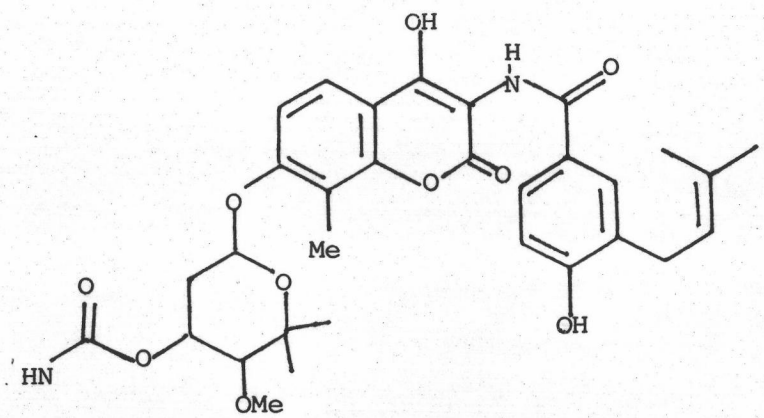
coumestrol

2. Phyto-sensitizing action - This property is due to the presence of simple linear furanocoumarins, angular furanocoumarins is much less active.(1) Phenolic furanocoumarins and more highly substituted compounds have found to be inactive.(5) Oral doses of xanthotoxin followed by controlled exposure to ultraviolet radiation have shown remarkable success in experimental treatment for the stubborn and disfiguring skin disease psoriasis.(6)



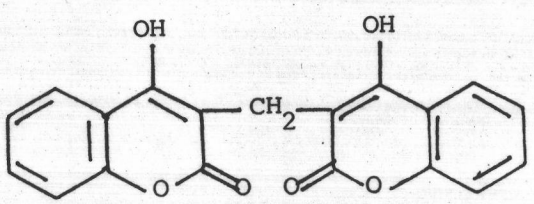
xanthotoxin

3. Antibiotic activity - The highly substituted coumarin, novobiocin, is commercial antibiotic and some related 3-amino-4-hydroxycoumarins, possess considerable antibacterial activity.(1)



novobiocin

4. Anticoagulant - Coumarins hydroxylated at C-4, such as dicoumarol, function as anticoagulants.(6)



dicoumarol

VI. Coumarins in Clausena species.

Table 7 is summarized of the compounds isolated from Clausena species.[7-29] It is found that most of coumarins in Clausena species are in the group of simple coumarin, furanocoumarin [psoralen type and dihydrofuranocoumarin (7,6)] and pyranocoumarin [xanthyletin type (linear and angular), dihydroxanthyletin type (linear)]

Table 7 Coumarins in *Clausena* species.

plant species	compound	reference
C.anisata Willd.	chalepin, osthol, imperatorin, xanthoxyletin, coumarrayin, 3-(1,1-dimethylallyl) xanthyletin	12,13
C.dentata (Will.) R.& S.	imperatorin, dentatin, nordentatin	20
C.excavata Burm.	clausarin, xanthoxyletin nordentatin, clausenidin	14
C.heptaphylla Wt.& Arn.	clausenin, clausenidin, dentatin	21-29
C.pentaphylla Oliv.	imperatorin, phellopterin, dihydrofuranocoumarin chalepin, chalepin, chalepensin, suberosin, indicolactonediol, byakangelicin, xanthotoxol, pubesinol, clausindine, 8-hydroxy-5-methoxypsoralen,	7-11
C.willdenvii (Roxb.)DC.	clausarin, dentatin, clausenidin, clausamarin A and B	17-19
C.willdenvii W.& A.	3-(1,1-dimethylallyl) xanthyletin	15,16