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Chemistry of the Guttiferae of Ceylon*

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Abstract : The structure elucidation of the compounds isolated from the bark and timber extractives of different species of the family Guttiferae belonging to the genera, *Calophyllum* L., *Kayea* Wall, *Mesua* L., and *Garcinia* L., is discussed. The account includes the chemistry of xanthones, coumarins, bark acids, biflavanoids and triterpenes.

In Ceylon there are over 3300 flowering plant species belonging to 1294 genera and 192 families. Of these about 830 plant species belonging to 342 genera and 94 families are endemic to Ceylon. Our programme on the chemical investigation of the endemic plants envisages the study of the extractives obtained from the bark, timber and flowers or fruits with different solvents and isolation of pure compounds and chemically identifying them. Besides it is planned to carry out pharmacological studies on the total extracts and pure constituents.

Figure 1 shows the distribution of the endemic species in Ceylon.

The present review will be confined to the results we have obtained on the species of the family Guttiferae found in Ceylon. The family Guttiferae and its tribes[24] are given in *Chart A*.

There is difference of opinion about the inclusion of the genus *Hypericum* L. in the family Guttiferae (Clausiaceae). The family derives its name from *gutta* or gamboge, a yellow resinous juice that can be tapped from *Garcinia hanburyi* (Cambodia, Siam). Gamboge first reached Europe at the beginning of the 17th century. It is used medicinally as a laxative and provides a bright yellow pigment. *Garcinia mangostana* L., the mangostin tree, provides one of the most highly prized edible fruits of the tropics. Another tropical delicacy, the mamey

*Based on the *Kandiah Memorial Lecture*, of the Institute of Chemistry, Ceylon, delivered on July 14, 1972.

Figure 1. Distribution of the endemic species in Ceylon

Chart A Guttiferae

Subfamily	*Tribe*	*Genera*
1. Kielmeyeroideae	Kielmeyereae Caraipeae	*Kielmeyera* Mart., *Marila* Sw., *Caraipa*
2. Calophylloideae	Calophylleae Endodesmieae	*Mesua* L., *Mammea* L., *Calophyllum* L., *Kayea* Wall, *Endodesmia* Benth.
3. Clusioideae	Clusieae Garcinieae	*Clusia* L., *Chrysoclamys* Poepp. *Tovimita* Auble *Garcinia* L., *Pentaphalangium* Warb., *Allanblackia* Oliv., *Rheedia* L.,
4. Moronobeoideae		*Pentadesma* Sabine, *Moronobea* Auble, *Montrouziera* Planch, *Platonia* Mart., *Symphonia* L.
5. Lorostermonoideae		*Lorostemon* Duck
6. Hypericoideae	Vismieae Cratoxyleae Hypericeae	*Vismia* Vand, *Psorospermum* Spach, *Haronga* Thou. *Cratoxylon* Blume *Ascyrum* Vand, *Hypericum* L.

or St. Domingo apricot, is the fruit of *Mammea americana*, a large tree of the West Indies and now widely cultivated in many tropical countries. The seeds of the butter tree or tallow tree of tropical Africa, *Pentadesma butyracea* Sabine are the source of an edible fat used for cooking, called Lamy or Kanga or Sierra Leone Butter and used for soap, margarine and candles. In Ceylon all parts of the tree and seeds of *C.inophyllum* L. (*S* — domba)[4] have medicinal uses and medicinal uses have also been reported from *C.apetalum* Willd (whole plant), *C.tomentosum* Wight (seeds), *Garcinia indica* Chois (bark oil, fruits), *Garcinia mangostana* L. (pericarp), *Garcinia echinocarpa* Thw. (seed oil) etc. and several other *Calophyllum* L. species (known generally in *Sinhala* as " Kina ") are used for timber purposes.

Most plants belonging to this family are trees and are generally confined to the warm humid tropics. Some of the genera and species of this family have been found to be endemic to certain land masses e.g. *Kielmeyera* is confined to Brazil and other South American countries and *Symphonia* is confined to Africa and to the Malagasy Republic.[16] It is possible that all these plants originated from a common stock that existed before the continental drift and after separation of the continents those plants became confined to different land masses where they underwent different generic changes to give rise to different species. The spread of these species was checked by various barriers such as

oceans, large mountains and large patches of dryland and hence these new species became confined to land masses only. In Ceylon there are about 22 plants belonging to the subfamilies Calophylloideae and Clusioideae (*Chart B*).

Chart B GUTTIFERAE SPECIES IN CEYLON

HYPERICUM L.	*H. mysurense* Wight & Arn	
	H. japonicum Thunb	
	H. humifusum L.	
MESUA L.	*M. ferrea* L.	
	M. ferrea L. (form *M.salicina* Pl. & Tr.)	Ia
	* *M. thwaitesii* Planch & Triana	I
CALOPHYLLUM L.	*C. soulattri* Burm.f.	
	* *C. calaba* L.	II
	C. pulcherrimum Wall ex Choisy	III
	C. inophyllum L.	IV
	C. tomentosum Wight	
	* *C. bracteatum* Thw.	V
	* *C. thwaitesii* Planch & Triana	VI
	C. trapezifolium Thw.	VII
	* *C. cuneifolium* Thw.	VIII
	* *C. cordato-oblongum* Thw.	IX
	* *C. walkeri* Wight	X
KAYEA Wall	* *K. stylosa* Thw.	XI
CLUSIA L.	*C. rosea* Jacq	XII
GARCINIA L.	*G. cambogia* (Gaertn) Desr.	
	G. morella Desr.	
	G. echinocarpa Thw.	XIII
	* *G. terpnophylla* Thw.	XIV
	G. spicata (Wight & Arn) Hook.f.	
	G. tinctoria (DC) W. F. Wight	
	G. mangostana L.	

* Endemic species

We have investigated all but one of the endemic species which we have not been able to locate yet.†

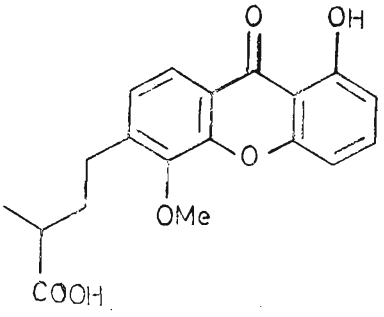
Over 57 Guttiferae species[7] have been investigated in the literature, without the *Hypericum* species, *Kielmeyera* (9 species) *Calophyllum* (20 species), and *Garcinia* (16 species) being the major ones. We have investigated 9 *Calophyllum*, 3 *Garcinia*, 2 *Mesua*, 1 *Kayea* and 1 *Clusia* species.

Xanthones

Over 80 different xanthones[7] have been isolated from the different species. Xanthones with isopentenyl and geranyl substituents are common in the family

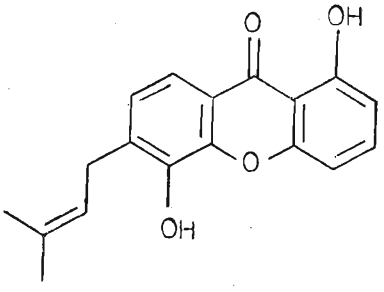
† Professor A. Kosterman has provided this species recently and it is being investigated.

Guttiferae. Scheinmann[7] and others have suggested that substituents other than oxygen may have chemotaxonomic significance. One or two isopentenyl side chains may be present eg. Scriblitifolic acid (1), guanandin (2) and mangostin (3)



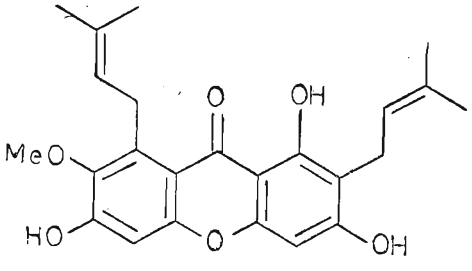
SCRIBLITIFOLIC ACID

1



GUANANDIN

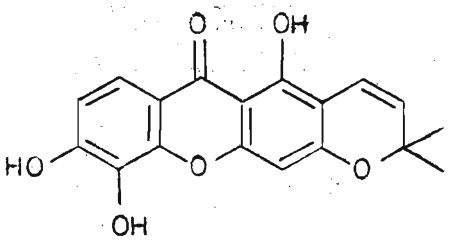
2



MANGOSTIN

3

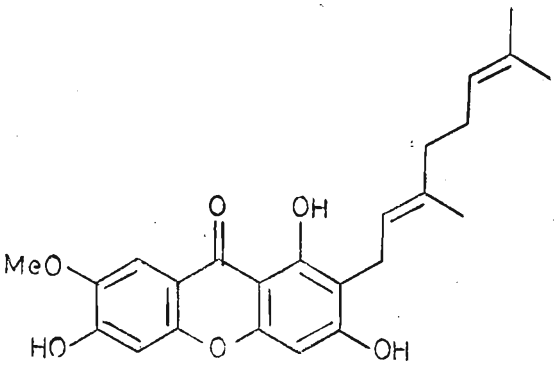
In some cases one or two of these isopentenyl substituents are modified by terminal cyclisation on to the *o*-hydroxy group to give a chromene system as in the case of jacareubin (4)



JACAREUBIN

4

The geranyl substituents are found mostly in the genus *Garcinia* L. In some cases these substituents remain unmodified as for example cowaxanthone (5) and cowanin (6) but in others the substituents undergo cyclisation as in the case of gambogic acid (7a) and morellin (7b).

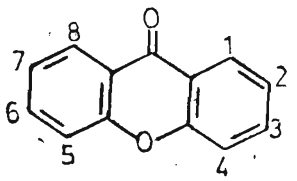


COWAXANTHONE

5

The different xanthones isolated from the various species can be classified according to their oxygenation pattern. Of these we have isolated 22 different xanthones of which seven are new xanthones and some are being reported for the first time in certain genera. The structures of the known xanthones and the frequency of their isolation are listed in *Table* 1.

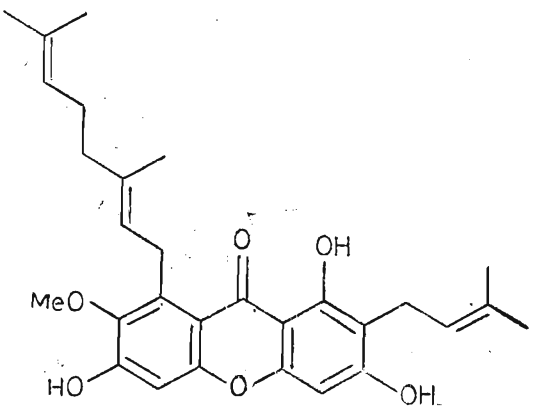
Table 1



Xanthone	Plant isolated from in Ceylon*
Mono oxygenated	
2-hydroxyxanthone 4-hydroxyxanthone 2-methoxyxanthone	I(a)
Di-oxygenated	
1, 5-dihydroxyxanthone	I, Ia, V, VI, VII, X, XIII, XIV (*from* 6 *other species by others*)
1, 5-dihydroxy-6 (3′-methylbut-2-enyl) xanthone (guanandin)	II, VIII (*from* 3 *species by others*)
1-hydroxy-5-methoxy-6 (3′-carboxylbutyl) xanthone (scriblitifolic acid 1)	II, VIII (*from* 1 *species by others*)
1, 7-dihydroxyxanthone	I, Ia, II, V, VI, VII, VIII, X, XIV (*from* 12 *species by others*)
Tri-oxygenated	
1, 3-dimethoxy-5-hydroxyxanthone	I (*from* 1 *species by others*)
1,3,5-trihydroxy-2-(3-methyl-but-2-enyl) xanthone	VIII (*from* 1 *species by others*)
6-deoxyjacareubin	II, V, VIII (*from* 7 *species by others*)
1,6-dihydroxy-5-methoxyxanthone (buchanoxanthone)	II, VIII (*from* 4 *species by others*)
1, 5, 6-trihydroxyxanthone	I, I(a), II (*from* 8 *species by others*)
2, 8-dihydroxy-1-methoxyxanthone	II (*from* 1 *species by others*)
Tetraoxygenated	
Jacareubin (4)	II, V, VI, VII, VIII, X (*from* 8 *species by others*)
Mangostin (3)	XIV (*from* 1 *species by others*)

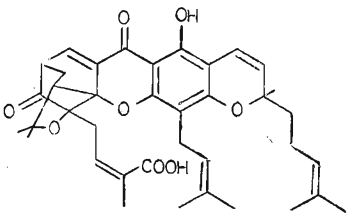
* Roman numerals refer to names of Guttiferae species given in *Chart B*

A brief outline of the methods used in fixing the structures of the new xanthones follows. From the barks of the *Calophyllum* species we have isolated for the first time three new di-isoprenylated xanthones, calabaxanthone, thwaitesixanthone and trapezifolixanthone. Of these calabaxanthone,[31] (9) isolated from *C. calaba L.*, showed in its uv, a resemblance to osajaxanthone(8).



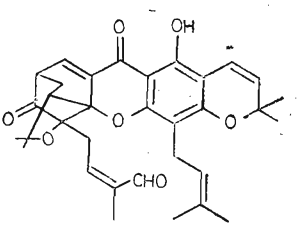
COWANIN

6



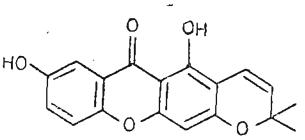
GAMBOGIC ACID

7a



MORELLIN

7b



OSAJAXANTHONE

8

This was further confirmed by comparison of the uv spectrum of tetrahydro-calabaxanthone with dihydro-osajaxanthone (*Table* 2).

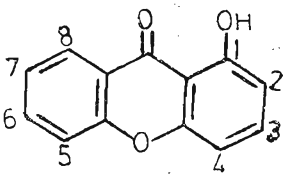
Table 2. Comparison of u.v. absorption maxima, nm (ε x 10⁻³)

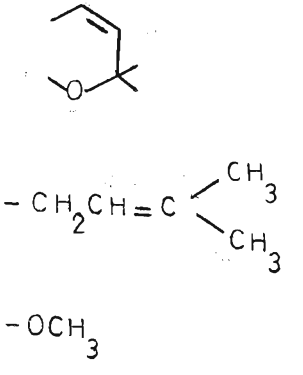
							Solvent
Osajaxanthone	238 (19.0)	249 (18.0)	286 (47.10)		339 (8.2)	382 (4.8)	*a*
Osajaxanthone mono methyl ether	240 (21.4)	248 (19.9)	286 (43.6)		339 (9.0)	375 (4.5)	*a*
Calabaxanthone	240 (34.01)		287 (77.25)	292sh (72.21)	314 (28.23)	384 (8.42)	*b*
Tetrahydrocalaba-xanthone	241 (63.36)	262 (64.84)			316 (36.43)	369 (9.11)	*b*
Dihydro-osajaxanthone	237 (30.9)	262 (33.48)			316 (16.22)	380 (6.25)	*a*
Dihydro-osajaxanthone monomethyl ether	233 (31.62)	259 (39.81)			316 (16.59)	372 (6.16)	*a*
1, 3-dihydroxy-7-methoxyxanthone	235 (28.28)	259 (32.36)			311 (13.8)	369 (6.31)	*a*
1-hydroxy-3, 7-dimeth oxyxanthone	231 (34.71)	259 (38.55)			292 (24.36)	369 (6.78)	*a*
1-hydroxy-3, 7-dimeth oxy-2-(1, 1-dimethyl allyl)-xanthone	233 (34.63)	264 (37.18)			311 (18.32)	372 (6.84)	*a*
2 -(3,3-dimethylallyl)-1,3,7-trihydroxy-xanthone	241 (33.87)	263 (32.89)			314 (17.14)	337 (6.50)	*a*

Solvent a- ethanol, *b*- methanol

The NMR spectrum gave information for the presence of

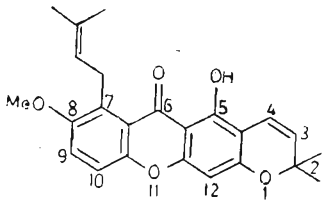
(*a*) dihydro pyran ring
(*b*) isopentenyl group
(*c*) OMe group





$-OCH_3$

The three aromatic protons appeared as two singlets at τ 2.73 (2H) and τ 3.77 (1H). The high chemical shift of the signal at τ 3.77 (1H) indicated that this proton should be located in the electron rich phloroglucinol ring of calabaxanthone. As the compound gave a positive Gibbs test, a proton para to xanthone C-1 position should be free. Since there is only a single proton in the phloroglucinol nucleus which is assigned to xanthone C-4 position the 2,2-dimethyl-2*H*-pyrano ring must be attached to the 2,3-position of the xanthone ring giving a linear arrangement of the 2*H*- pyrano ring with respect to the rest of the molecule



CALABAXANTHONE

9

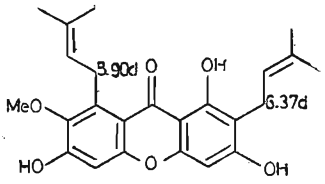
Further confirmation of this linear arrangement of the 2,2-dimethyl-2*H*-pyrano ring was obtained from the proton shifts observed on its acetate (*Table* 3)

Table 3. Chemical shift differences

	C_4 -H	C_3 -H	C_{12} -H
Calabaxanthone	3.25	4.43	3.77
Calabaxanthone acetate	3.46	4.24	3.31
Diamagnetic ($\Delta\tau$)	+ 0.21		
Paramagnetic ($\Delta\tau$)		—0.19	—0.44

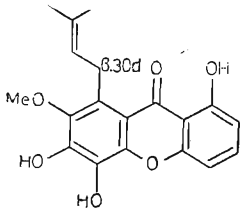
A positive diamagnetic shift and a negative paramagnetic shift for a linear 2*H* - pyrano ring proton and negative paramagnetic shift for the C_{12} aromatic proton has been recorded for similar systems[3].

The position of the isopentenyl group was fixed by comparison of the -CH_2- NMR signals of the isopentenyl group with the same group in mangostin (3) and celibixanthone (10).



MANGOSTIN

3

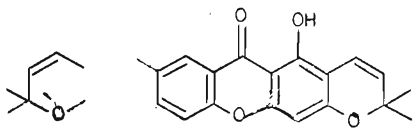


CELIBIXANTHONE

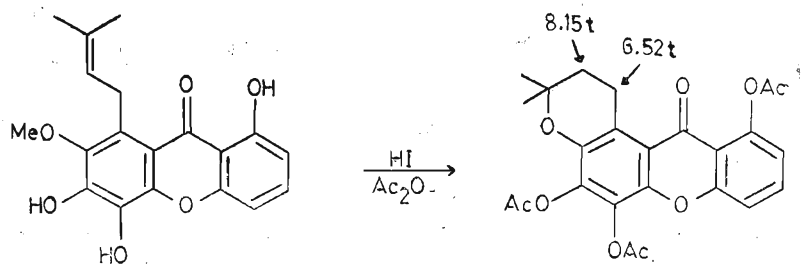
10

On the above basis the structure of calabaxanthone becomes 2,2-dimethyl-5-hydroxy-8-methoxy-7- (3-methylbut-2-enyl)-2*H*,6*H*-pyrano- (3,2-b) xanthen--6-one (9).

For the structure of thwaitesixanthone isolated from *C. thwaitesii* Planch & Triana we need only consider the establishment of the two dihydro pyran rings. Similar evidence as for calabaxanthone gives the partial structure :

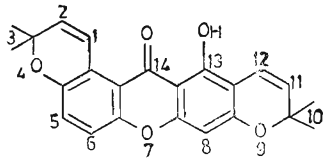


The other 2,2-dimethyl-2*H*-pyrano ring could be attached in two ways to give a linear or an angular molecule. Evidence which supported an angular fusion was (*a*) the low τ 1.98 (1H) for one of the doublets in thwaitesixanthone and its methyl ether (*b*) the NMR spectrum of tetrahydrothwaitesixanthone contained three sets of triplets at τ 6.50(2H), τ 7.28(2H) and τ 8.18(4H) Two of the three values are similar to those of tetrahydrocalabaxanthone τ 7.28(2H) and τ 8.18(2H). The other pair was similar to those found for ring closed celibixanthone (11) as shown below :



10 11

On this basis thwaitesixanthone was formulated as 13-hydroxy-3, 10-tetramethyl -3*H*, 10*H*, 14*H*-dipyrano [3,2,*a* :2′, 3′-*i*]-xanthen-14-one (12)



THWAITESIXANTHONE

12

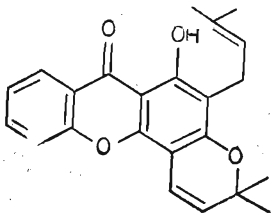
The above structure was confirmed by studying the proton shifts on the acetate, of thwaitesixanthone. (*Table* 4)

Table 4. Chemical shift differences

	C_{12} –H	C_{11} –H	C_8 –H	C_1 –H	C_2 –H
Thwaitesixanthone	3.26	4.41	3.72	1.98	4.19
Thwaitesixanthone acetate	3.50	4.28	3.33	2.04	4.23
Diamagnetic ($\Delta\tau$)	+0.24			+0.06	+0.05
Paramagnetic ($\Delta\tau$)		—0.13	—0.39		
	linear		*angular*		

A positive diamagnetic shift and a negative paramagnetic shift for a linear 2,2-dimethyl-2*H*-pyrano ring proton and a negative paramagnetic shift for C-8 aromatic proton is in agreement with the linear ring. The positive shift for the other ring protons may be indicative of the angular placement of the other pyrano ring.

Trapezifolixanthone (13) isolated from *C. trapezifolium* Thw. on similar arguments would be formulated in two ways by the 2,2-dimethyl-2*H*-pyran-ring being fused at C_2 or C_4. If it was fused at C_4 as in structure (14)



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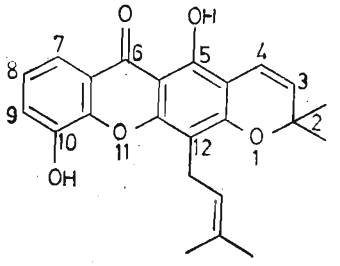
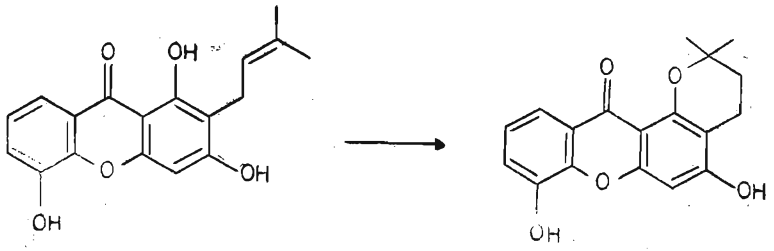


TRAPEZIFOLIXANTHONE

14

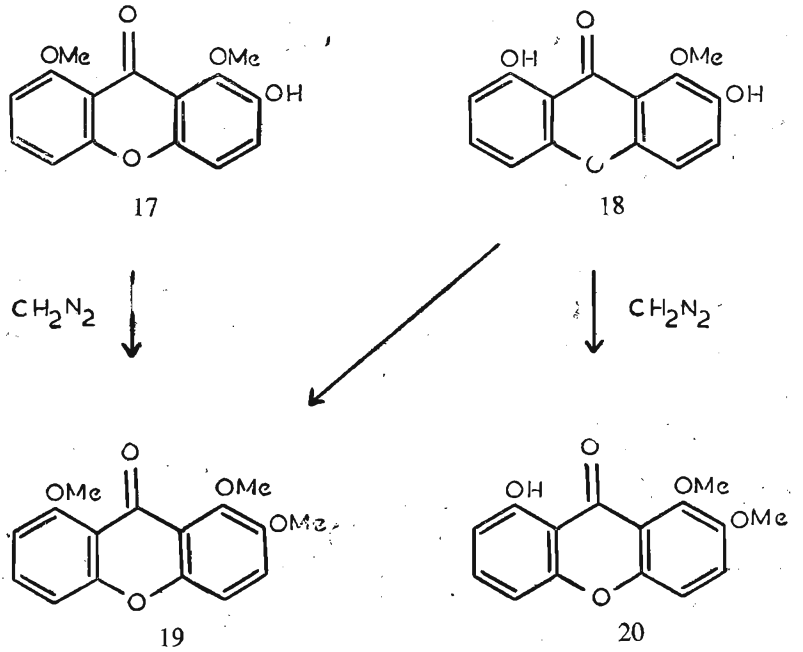
the methylbut-2-enyl side chain should be located at C_2 and the compound would ring close to form a dihydropyran type of compound on boiling with formic acid. However, trapezifolixanthone failed to cyclise under the conditions and hence it was concluded that it had 2,2-dimethyl-2*H*-pyrano ring fused to C_2 with 2-methylbut-2-enyl group in position C_4. Its structure therefore, becomes 5, 10-dihydroxy- 2,2-dimethyl-12- (3-methylbut-2-enyl)-2*H*, 6*H*-pyrano -[3,2-*b*] xanthene-6-one (13). This structure has been confirmed by synthesis by Professor Jain (*personal communication*) at the University of Jammu, India.

This method has been used by Selliah[29] to establish the structure of 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (15) by cyclising it to (16) with formic acid at room temperature.



15 16

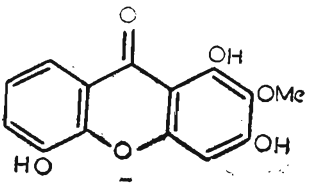
The chemical method used can be illustrated with 2-hydroxy- 1,8-dimethoxyxanthone[31] (17). Thus methylation of compound (17) with diazomethane and methylation of 2,8-dihydroxy-1-methoxyxanthone (18) with dimethyl sulphate converted both into 1,2,8-trimethoxyxanthone (19).



This suggested that compound (17) was a 1,2,8-trioxygenated xanthone. The NMR spectrum indicated the presence of two methoxy groups at τ 6.22 (3H) two *AB* doublets at τ 2.68 (1H) and τ 2.80(1H) (J_{AB} 8.4 Hz), two doublets at τ 3.08 (1H) and τ 2.98 (1H) (J_{AB} 8.4 Hz), and a triplet at τ 2.3 (1H J 9 Hz). This suggested that there were two ortho protons in one ring and three vicinal protons in the other. The fact that the compound was fully methylated with diazomethane suggested that the hydroxy group was free and not chelated to the carbonyl group. This observation was further confirmed by the u.v. spectrum which was unaltered by the addition of alcoholic aluminium chloride. 8-hydroxy-1, 2-methoxyxanthone (20) obtained from 2,8-dihydroxy-1-methoxy xanthone (18) by methylation of diazomethane was found to be different from compound (17) which must, therefore, be 2-hydroxy-1, 8-dimethoxyxanthone.

The structure of the tetraoxygenated compound from *C. bracteatum* Thw. was assigned as the u.v. spectrum showed characteristic xanthone peaks and i.r. spectrum had strong bands at 3275 cm^{-1} (free OH) and 1652 cm^{-1} (chelated C = O). The NMR spectrum indicated the presence of one methoxy group and four nuclear protons. On methylation with diazomethane a trimethoxy compound and with dimethyl sulphate a tetramethoxy compound were formed indicating the presence of three free hydroxy groups and one of which is chelated, and therefore at C-1. This was further supported by the bathochromic

shift of maxima in the uv spectrum on addition of aluminium chloride. Since the compound was stable to alkali, C-2 and C-4 are not substituted. Furthermore, the original u.v. spectrum was unaltered by the addition of NaOAc-H_3BO_3 which ruled out the possibility of ortho-hydroxy groups. The presence of hydroxy group at C-3 *or* C-6 allows a xanthone to be ionized by sodium acetate, and the original uv spectrum of the compound (21) was altered in the longer wavelength region on the addition of sodium acetate which indicated the presence of a hydroxy group at C-3, -5 or -6.



21

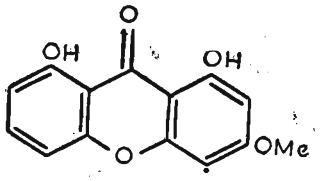
The NMR spectrum of 1-hydroxy-2, 3, 5-trimethoxy xanthone, obtained by the methylation of compound (21) with diazomethane showed resonances at τ 3.49 (4H,*s*), τ 2.78 (6- and 7-H, *m*) and τ 2.29 (8-H, *q*) and these values are very similar to that of the signals of the compound (21). The tetramethyl ether of (21) was shown to be identical with 1, 2, 3, 5-tetramethoxyxanthone by comparison with an authentic sample (mixed mp and NMR). This established the oxygenation pattern of the compound as that of a 1, 2, 3, 5-tetraoxygenated system.

One hydroxy group has already been assigned to C-1 and since there was no shift of the uv absorption with NaOAc—H_3BO_3 the methoxy group was assigned to C-2. On biosynthetic evidence, a phloroglucinol oxygenation pattern is required for the xanthone nucleus and hence the second hydroxy group is likely to be at C-3. Based on the above observations the structure of the new xanthone was deduced as 1, 3, 5- trihydroxy-2-methoxyxanthone[31] (21).

The structure of the pentaoxygenated compound from *C. bracteatum* Thw. was assigned as follows:

The uv and ir spectra indicated that it was a xanthone. The NMR spectrum showed signals at τ -3.2(1H) and τ -0.3(1H) for two hydroxy-groups and at τ 6.07 (3H,*s*), τ 6.15 (3H, *s*) and τ 6.25(3H,*s*) for three methoxy groups. The aromatic protons appeared as two doublets at τ 2.55 (1H) and τ 2.85 (1H) (J 9 Hz) and a sharp singlet at τ 3.37(1H). The di-acetate had a similar NMR spectrum for the aromatic protons and the three methoxy groups and the acetyl signals were observed at τ 7.49 (3H) and τ 7.66 (3H). The coupling pattern shown in the NMR spectra of the parent compound and its di-acetate suggested the presence of two aromatic protons in one ring and a single proton in the other. The high values of the aromatic proton signals in the NMR indicated that C-1 and C-8 of the xanthone nucleus were substituted.

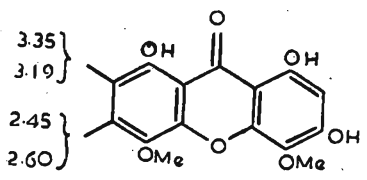
The addition of aluminium chloride produced a bathochromic shift of the maxima in the uv spectrum, thereby locating one of the hydroxy groups at C—1. The addition of NaOAc and NaOAc—H_3 BO_3 did not effect the uv spectrum. The compound was also stable to alkali. Therefore the second hydroxy group could not be at C-2, -4 or -5. The negative values observed for the two hydroxy groups in the NMR spectrum suggested that they are probably located at C-1 and C-8, as has been reported by Markham[26] for 1,8-dihydroxy-xanthones. This is confirmed by the absence of signals in the NMR chaiacteristic of nuclear protons at C-1, or C-8. On biosynthetic evidence, one aromatic ring in the xanthone nucleus should have a phloroglucinol oxygenation pattern. Therefore one oxygen function a methoxy group, is likely to be located at C-3. Hence the partial structure for the compound could be formulated as B:



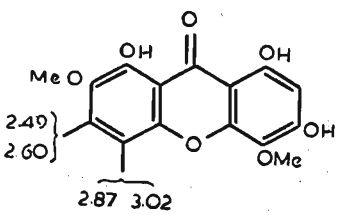
B

OMe at C-2 or -4
OMe at C-5 or -7

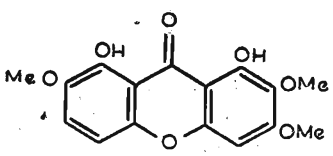
The other two methoxy groups have to be assigned to C-7 or -5 and C-2 or -4. The NMR signal of H-2 always appears at somewhat higher fields than that of H-4, for a given set of hydroxy and methoxy substituents. The exact values depend on the type of substituent in each ring but in general H-2 appears at τ 3.5 - 3.7 and H-4 at τ 3.2 - 3.5. On this basis the singlet at τ 3.37 was assigned to H-4 and the methoxy group at C-2. By comparison of the τ values observed for H-5 and -6 and H-6 and -7 in compounds (22a) and (22b), the methoxy group was assigned to C-7. Based on the above arguments, the structure is 1,8-dihydroxy-2,3,7-trimethoxyxanthone (23)



22a



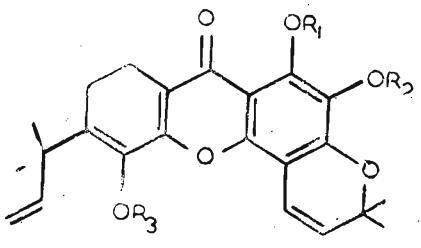
22b



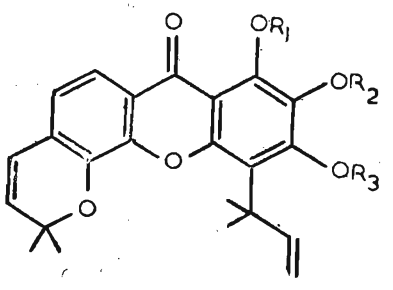
23

Kayeaxanthone[29] obtained from the bark of *Kayea stylosa* Thw. showed the u.v. maxima at 260^{sh} (log ε 4.29), 269 (4.31), 292 (4.63) and 368 (3.42) nm and strong ir absorption at 1644 (chelated carbonyl) and 3250 cm^{-1} in the IR These spectra suggested that the pigment was axanthone.

The NMR spectrum showed the presence of a 1,1-dimethyl-prop-2-enyl side chain, as in the case of symphoxanthone and globuxanthone, the olefinic protons appearing as an ABX system at τ 3.51, 5.01, and 5.15. The signals of the gem dimethyl appear at τ 8.25. The NMR spectrum also showed two doublets at τ 2.05 (J 9 Hz) and τ 2.90 (J 9 Hz) due to two aromatic protons. The chemical shift of the low field doublet showed that it could only arise from a proton next to a xanthone carbonyl.



24



25

a. $R_1 = H, R_2 = R_3 = Me$
b. $R_2 = H, R_1 = R_3 = Me$
c. $R_3 = H, R_1 = R_2 = Me$

This must be either C-1 or C-8 position of the xanthone nucleus. The adjacent position C-2 (or C-7) must bear the other aromatic proton to account for the magnitude of the coupling. Also visible in the NMR spectrum were doublets at τ 3.46 (J 10 Hz) and τ 4.32 (J 10 Hz) and a sharp singlet at τ 8.41 (6H). These signals indicated the presence of a 2,2-dimethyl chromene system. The fact that the chromene double bond was conjugated with xanthone system was shown by the change of uv pattern of this pigment on complete hydrogenation. The presence of 2,2-dimethyl chromene system was also shown by an intense M-15 peak at *m/e* 407 (base peak) in the mass spectrum of kayeaxanthone. The peak was most probably due to the stable benzopyrylium ion formed from the 2,2-dimethyl chromene system.

The NMR spectrum of the methyl ether of kayeaxanthone showed the presence of three methyl groups while that of kayeaxanthone showed two methyl groups. This indicated the presence of one hydroxy group and two methoxy groups in kayeaxanthone.

Having identified the various substituents it was now left to orientate them about the xanthone nucleus. The two aromatic protons could occupy as mentioned above either of the equivalent positions 8 and 7 or 1 and 2 of the xanthone nucleus.

2-Methoxy-1,3,5-trihydroxyxanthone (21) was the only xanthone isolated from the timber extract. Biosynthetic analogy with this xanthone suggested the same oxygenation pattern for the bark chromenoxanthone**. Further the u.v. pattern of the hydrogenated chromenoxanthone is very similar to that of some of the known 1, 2, 3, 5-tetraoxygenated xanthone systems. (*Table* 5).

Table 5. Comparison of u.v. absorption maxima λ_{max} (ethanol) (log ε)

Kayeaxanthone hydrogenated	217 (3.89)	244 (3.92)		262sh (3.82)	301 (3.83)	353 (3.55)
2-Methoxy-1, 3, 5-trihydroxyxanthone	222 (3.91)	244 (3.93)		262sh (3.87)	314 (3.57)	365 (3.56)
1-Hydroxy-2,3,5-trimethoxyxanthone	220	242	253	258sh	302	370

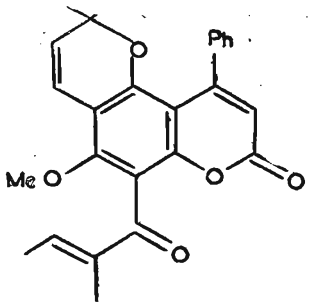
In keeping with the above arguments six possible structures (24a, b, c, and 25a, b, c) can be written for kayeaxanthone. The NMR spectrum of this xanthone did not show a low field signal of a chelated hydroxy group. Also the uv spectrum remained unchanged on addition of $AlCl_3$. Further methylation of the pigment with diazomethane as well as dimethyl sulphate gave the same methyl ether. All these observations indicated that position C-1 must bear a methoxy group rather than a hydroxy group. This rules out structures (24a) and (25a). The structure (25 c) is very unlikely as the uv spectrum of the original pigment remained unchanged on addition of sodium acetate. All attempts to cyclise the chromenoxanthone failed. This showed that 1,1-dimethyl-prop-2-enyl side chain cannot occupy a position ortho to a hydroxy group, since otherwise it would cyclise easily under mild acid conditions to give a dihydrofuran ring. This experiment rules out structures (24c) and (25c) leaving only (24b) and (25b) as the possible structures of this kayeaxanthone.

Further structural investigation of this xanthone is being delayed for want of plant material and due to the extreme difficulty in isolating the same. One method of distinguishing between the above two structures would be the use of nuclear overhauser effect technique.

** This argument may not be valid and is being further tested at the moment.

Coumarins

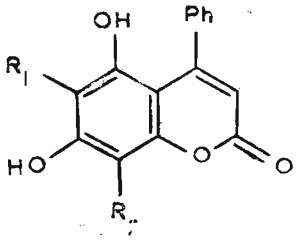
More than 50 coumarins have been isolated and characterised from various species of Guttiferae. The coumarins have been isolated mostly from the seeds and bark extracts. All the coumarins found in Guttiferae have a 5,7-oxygenated pattern and often have alkyl, acyl, hydroxyl group as substituents. There are also a few cases of coumarins with a methoxy group as a substituent, for example calophyllolide[28] (26)



26

Crombie[9] and co-workers have broadly classified these coumarins into a 6-acyl class and an 8-acyl class, largely based on the differences in base shifts in the ultra violet spectra of these compounds. The coumarins which belong to the 6-acyl class have an acyl substituent in position 6 as for example, mesuol (27e) and the coumarins which belong to the 8-acyl class have an acyl substituent in position 8 as for example mammea A/BB (27d).

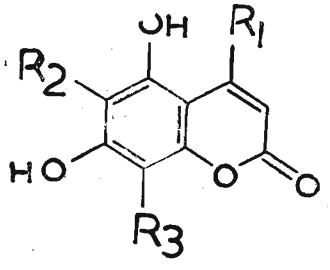
Members of these two classes also differ in their colour reaction with neutral ferric chloride. These coumarins may also be divided into another two groups namely a 4-phenyl group : (27)



27

	R_1	R_2
(*a*) Mammea A/AA (mammeisin)	$CO.CH_2.CH.(CH_3)_2$	$CH_2.CH:C(CH_3)_2$
(*b*) Mammea A/AB	$CO.CH(CH_3).CH_2.CH_3$	$CH_2.CH:C(CH_3)_2$
(*c*) Mammea A/BA	$CH_2.CH:C(CH_3)_2$	$CO.CH_2.CH(CH_3)_2$
(*d*) Mammea A/BB	$CH_2.CH:C(CH_3)_2$	$CO.CH.(CH_3)CH_2.CH_3$
(*e*) Mesuol	$CO.CH.(CH_3)_2$	$CH_2.CH:C(CH_3)_2$

and a 4-alkyl group: (28)



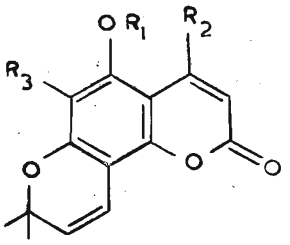
28

	$R_1 = CH_2CH_2CH_3$	R_2	R_3
(*a*)	Mammea B/BC	$CH_2.CH:C(CH_3)_2$	$CO.CH_2.CH_2.CH_3$
(*b*)	Mammein B/BA	$CH_2CH:C(CH_3)_2$	$CO:CH_2.CH(CH_3)_2$
(*c*)	Mammea B/BB	$CH_2CH:C(CH_3)_2$	$CO.CH(CH_3)CH_2CH_3$
(*d*)	Mammea B/AB	$CO.CH(CH_3)CH_2CH_3$	$CH_2CH:C(CH_3)_2$
(*e*)	Mammea B/AA	$CO.CH_2CH(CH_3)_2$	$CH_2.CH:C(CH_3)_2$
(*f*)	Surangin A	$CH_2.CH:C(CH_3)CH_2.CH_2.CH:C(CH_3)_2$	$CO.CH(CH_3)CH_2CH_3$
	$R_1 = CH(CH_3).CH_2.CH_3$		
(*g*)	Ferruol A	$CH_2CH:C(CH_3)_2$	$CO.CH(CH_3)CH_2.CH_3$
	$R_1 = CH_2CH_2CH_2CH_2CH_3$		
(*h*)	Mammea C/BB	$CH_2CH:C(CH_3)_2$	$CO.CH(CH_3)CH_2.CH_3$
	$R_1 = CH(O.CO.CH_3)CH_2CH_3$		
(*i*)	Surangin B	$CH_2.CH:C(CH_3)CH_2.CH_2CH:C(CH_3)$	$CO.CH(CH_3)CH_2CH_3$
(*j*)		$CH_2.CH:C(CH_3)_2$	$CO.CH_2.CH(CH_3)_2$
(*k*)		$CH_2.CH:C(CH_3)_2$	$CO.CH(CH_3)CH_2CH_3$

In fewer cases like surangin B(28i) acylated substituents are present in position 4 of the coumarin nucleus.

The isopentenyl substituents which are very common in the Guttiferae coumarins may occupy position 6 as in mammeisin (27a) or position 8 as in mammea B/BA (28b) depending on the position of the acyl group. Often

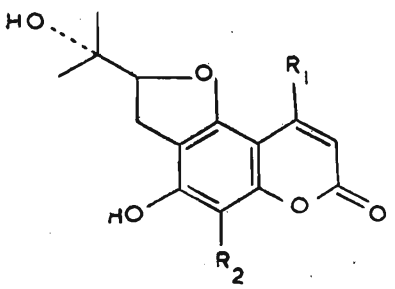
these isopentenyl side chains undergo cyclisation with the orthohydroxy group to give a chromene ring as in the case of mesuagin (29e)



29

	R_1	R_2	R_3
(*a*) * Mab 5	H	Ph	$CO.CH(CH_3)CH_2.CH_3$
(*b*) Mab 6	H	$CH_2CH_2CH_3$	$CO.CH(CH_3)CH_2.CH_3$
(*c*) Apetalolide	CH_3	Ph	$CO.C(CH_3):CH.CH_3$
(*d*) * Mammeigin	H	Ph	$CO.CH_2.CH(CH_3)_2$
(*e*) * Mesuagin	H	Ph	$CO.CH(CH_3)_2$

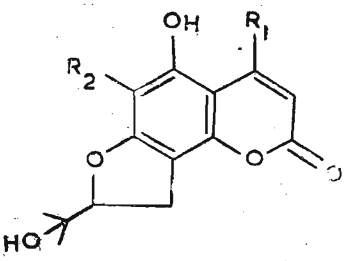
Such cyclisation can also lead to a hydroxyisopropyldihydrofuran system as in the case of coumarin (30)



30

R_1	R_2
$CH_2.CH_2.CH_3$	$CO.CH_2.CH(CH_3)_2$
$CH_2.CH_2.CH_3$	$CO.CH(CH_3).CH_2.CH_3$
$CH_2.CH_2.CH_3$	$CO.CH_2.CH_2.CH_3$

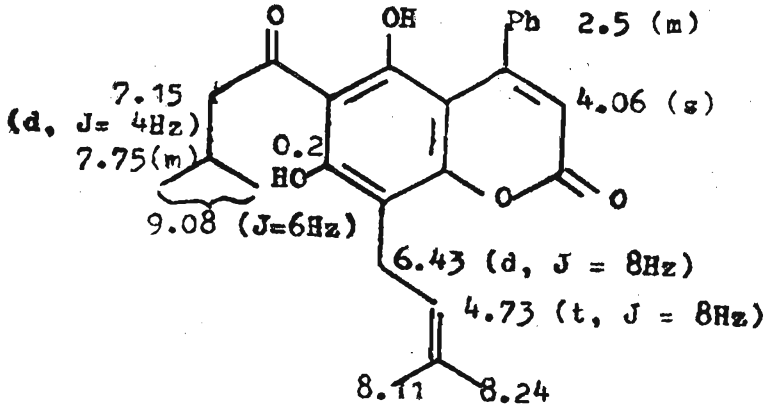
or to a 2,2-dimethyl-3-hydroxydihydropyran system as in the case of coumarin (31)



31

	R_1	R_2
(*a*) *	Ph	$CO.CH_2.CH(CH_3)_2$
(*b*) * Mab 3	Ph	$CO.CH(CH_3).CH_2.CH_3$
(*c*)	$CH_2.CH_2.CH_3$	$CO.CH_2.CH(CH_3)_2$
(*d*) Mab 4	$CH_2.CH_2.CH_3$	$CO.CH(CH_3).CH_2.CH_3$
(*e*) *	Ph	$CO.CH(CH_3)_2$

From the bark extracts of *Mesua thwaitesii* Planch & Triana a coumarin was isolated. Its uv spectrum showed a characteristic base shift for a 6 acyl coumarin and the NMR data fixed the structure as 4′, 5′-dihydro-5-hydroxy-5′-(1-hydroxy-1-methylethyl)-6-(3-methylbutyryl)-4-phenylfuro-[2′, 3′: 7,8] coumarin (31a)



4-phenyl-5,7-dihydroxy-6-isovaleryl-8-isopentenyl coumarin

32

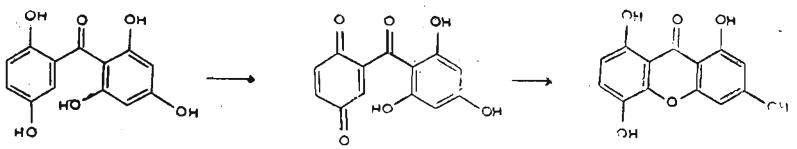
From the light petroleum extract of the seeds of *Mesua thwaitesii* Planch & Triana mammea A/AA (mammeisin) 4-phenyl-5, 7-dihydroxy-6-isovaleryl-8-isopentenyl coumarin (27a) and the coumarin isolated from the bark (31a)

were obtained in a pure state. The NMR data for mammeisin is given in (32). By a G.C. - mass spectrometric examination by Dr. D. E. Games[9] at the University of Wales, Cardiff, the presence of five other coumarins 29a, 29d, 29e, 31b and 31e were established. Coumarin (31e) is being reported for the first time.

Xanthone formation mechanisms

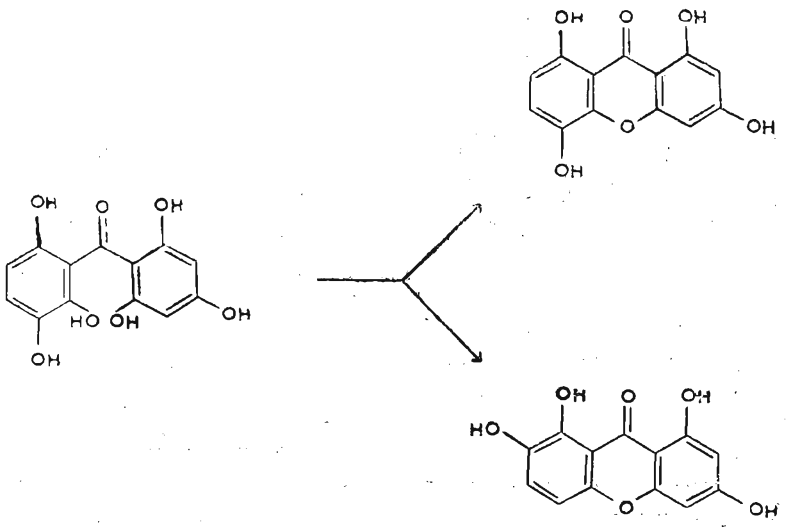
It has been established that xanthones in higher plants arise by condensation of acetate and shikimate derived moieties. It is believed that this condensation results in benzophenones which undergo intramolecular reaction to give the xanthones. Benzophenones are in fact known to co-occur with xanthones in some plants.

A number of mechanisms have been proposed for the intramolecular reaction of benzophenone leading to xanthone. One such mechanism involves a quinone intermediate[11] (*Scheme* 1)



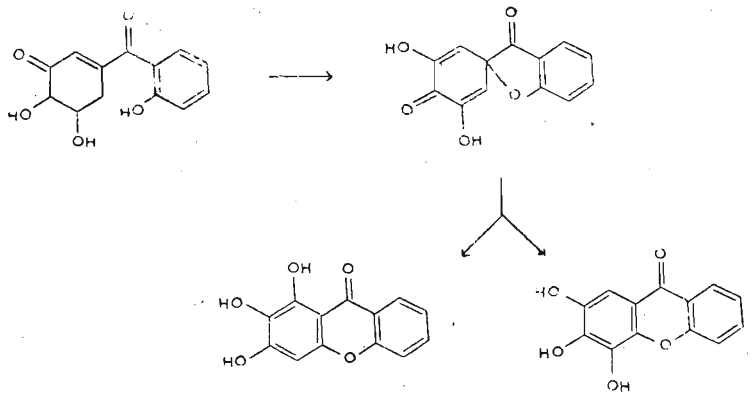
Scheme 1

Seshadri[26] and others postulated that xanthones could arise by dehydration between hydroxy groups of the acetate and shikimate derived moieties (*Scheme* 2).



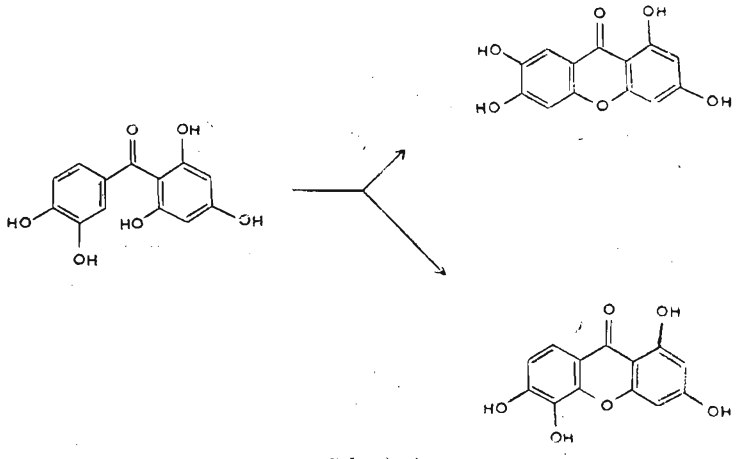
Scheme 2

A biogenetic pathway for xanthone formation from benzophenone involving a spirodienone intermediate (*Scheme* 3) has been suggested by Gottlieb[12]



Scheme 3

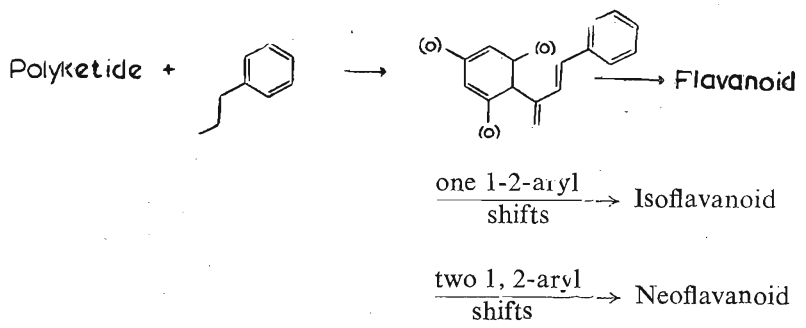
but Scheinmann[7] and co-workers favour a direct phenol oxidative coupling mechanism for the formation of xanthones from benzophenone (*Scheme* 4)



Scheme 4

Neoflavanoid formation mechanisms

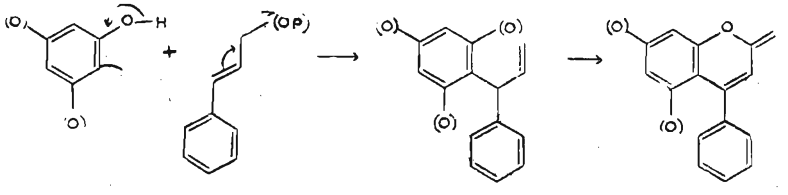
Various biogenetic theories have been proposed regarding the formation of neoflavanoids in plants. One such theory[36] suggests that these neoflavanoids arise by a biogenetic pathway where 1,2-aryl shifts from a chalcone precursor takes place (*Scheme* 5)



Scheme 5

This biogenetic pathway is similar to the one established for flavanoids and isoflavanoids. Seshadri[30] suggested that neoflavanoids could arise by another mode of attachment of the C_9 unit with the phenolic unit.

Ollis[27] and co-workers suggested that cinnamyl pyrophosphate could be the C_9 compound and this unit could alkylate a phenolic unit or its polyketide equivalent to form dalbergiones which in turn oxidise to give 4-phenyl coumarins (*Scheme* 6)

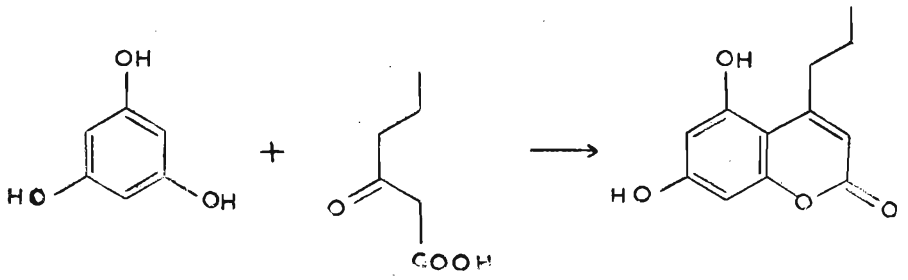


Scheme 6

Ollis, Gottlieb[13] and co-workers later suggested that isomerisation to 1-phenylallylpyrophosphate takes place before neoflavanoid formation.

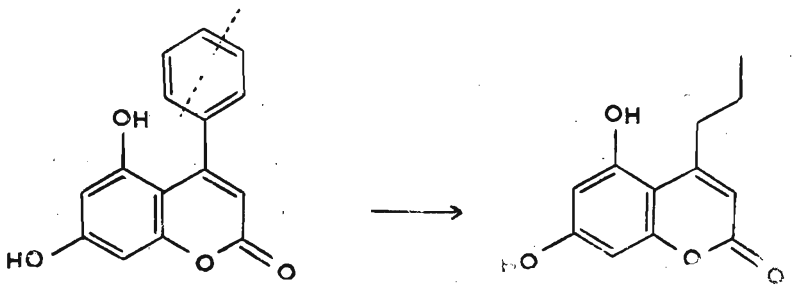
Kunesh and Polonsky[22] carried out feeding experiments on *Calophyllum inophyllum* L. seeds with specifically labelled phenyl alanine. They isolated calophyllolide (26) and ruled out the first theory where the biogenetic pathway is similar to that established for flavanoids and isoflavanoids. They also established that the C_9 unit in the formation of neoflavanoids is formed by shikimate-prephenic pathway. Later the same group performed some feeding experiments[23] using L-U- ^{14}C isoleucine, L-U- ^{14}C leucine and 1-^{14}C acetate and showed L - U - ^{14}C isoleucine is specifically incorporated into the tigloyl side chain of calophyllolide (26). They also noted that 1- ^{14}C acetate, apart from being incorporated into the phloroglucinol ring, was also incorporated into carbon atom C-3 and C-4 of the tigloyl grouping.

Apart from 4 phenyl coumarins, alkyl coumarins have been isolated from the Guttiferae species. Scheinmann[8] and co-workers have suggested two possible biogenetic pathways for their formation. One pathway involves an addition of a phenolic C_6 unit or its equivalent to a keto acid or its polyketide equivalent (*Scheme* 7)



Scheme 7

The second pathway is by biodegradation of the phenyl coumarin (*Scheme* 8) to give 4 alkyl coumarins.



Scheme 8

This pathway probably accounts for the fact that most alkyl substituents of the coumarins isolated from Guttiferae have not exceeded five carbon atoms and also these alkyl substituents may be branched as in ferruol A (28*g*). Feeding experiments using specifically labelled shikimate moieties may throw some light on the biogenesis of these four alkyl coumarins.

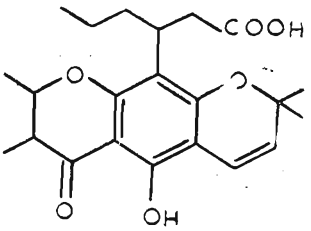
Cuneifolic acid

From the *Calophyllum cuneifolium* Thw. bark extracts, we have isolated a new acid which we have called cuneifolic acid. The uv spectrum resembles that of apetalic and blancoic acids (*Table* 6)

Table 6. Comparison of uv spectra λ_{max} (log ε) nm

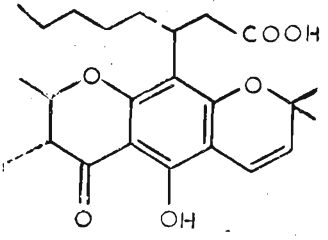
Cuneifolic acid	268 (4.49)	276 (4.53)	301 (4.01)	314 (4.03)	368 (3.77)
Apetalic acid[14](33)	268 (4.49)	276 (4.53)	301 (4.40)	315 (4.03)	368 (3.37)
Blancoic acid[35](34)	267 (4.60)	275 (4.62)	300 (4.05)	312 (4.07)	365 (3.33)

The NMR spectrum of cuneifolic acid gave valuable information on its structure especially when compared to those of apetalic (33), blancoic (34) and other related acids from species of *Calophyllum* L.



Apetalic acid
(2,3 methyl *cis*)

33



Blancoic acid
(2,3 methyl *trans*)

34

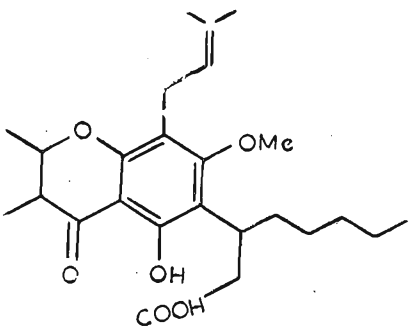
The doublets at τ 3.43 (J 10 Hz) and τ 4.43 (J 10 Hz) and the singlet at τ 8.58 are characteristic of a 2,2-dimethyl-chromene system. The presence of this system is also shown by an intense peak at *m/e* 373 (M-15), most probably due to M-15 pyrylium ion formed from 2,2-dimethylchromene. The presence of a propylpropionic acid side chain in this acid as in the case of apetalic acid (33) is shown by signals at τ 6.28 (1H,*m*), τ 7.25 (2H,*m*), τ 8.64 (4H broad) and τ 9.13(3H,*t*) in the NMR spectrum.

The presence of a 2,3-dimethylchromanone system is shown by the signals at τ 5.80 (1H,*m*), τ 7.32 (3H,*d*) and τ 8.82(3H, *d*). The NMR spectrum of this acid is somewhat similar to that of apetalic acid (33) suggesting close structural similarities between these two acids. But a significant difference is seen in the signals of the 2,3-dimethyl-chromanone system. In apetalic acid where the two methyl groups of the chromanone system have cis-relationship to each other, the multiplet of the C-2 proton of the chromanone system appears at τ 5.5 whereas in cuneifolic acid this multiplet appears at τ 5.80. Comparison of the chemical shifts of C-2 proton multiplet and C-2 and C-3 methyl signals

of the chromanone system of this acid with the chemical shifts of *cis*- and *trans*- 2,3-dimethylchromanone of known compounds (*Table* 7) suggested that the two methyl groups in cuneifolic acid have a trans-relationship to each other.

Table 7. Chemical shifts as τ values

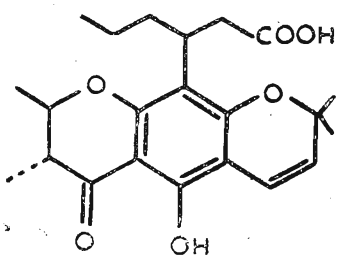
	C-2 proton	C-2 *and* C-3 methyls	
1. Papuanic acid [33](*trans*-) (35)	5.88	8.51	8.84
2. Isopapuanic acid[33] (*cis*-) (36)	5.44	8.63	8.82
3. Brasiliensic acid [34](*trans*-)	5.85	8.48	8.79
4. Isobrasiliensic acid [34](*cis*-)	5.50		
5. Apetalic acid (*cis*-) (33)	5.50	8.69	8.91
6. Blancoic acid (*trans*-) (34)	5.96	8.52	8.82
7. Cuneifolic acid (*trans*-)	5.80	8.50	8.82



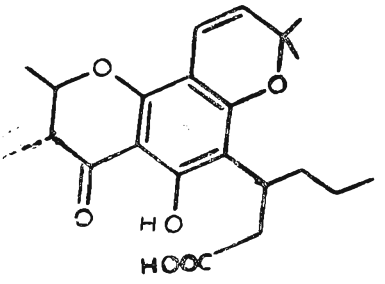
(35) Papuanic Acid (2, 3 methyl *trans*)

(36) Isopapuanic Acid (2, 3 methyl *cis*)

Having identified the various substituents of the *cis*-2,3-dimethylchromanone system it is now left to orientate them about the chromanone ring. Biosynthetic analogy with coumarins suggested a phloroglucinol oxygenation pattern for this acid. Further evidence of this oxygenation pattern comes from close similarity of the uv pattern of this acid to those of blancoic acid and apetalic acid. Placing the oxygen atoms in keeping with the above arguments two structures (37) and (38) are possible for cuneifolic acid.



37



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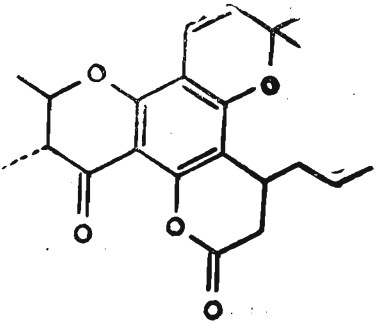


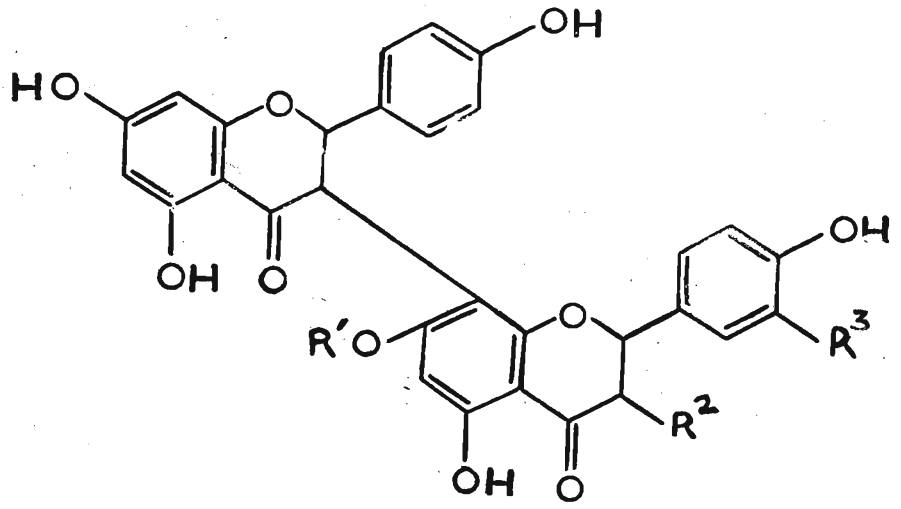
39

The two structures could be easily distinguished. Cuneifolic acid would readily lactonise to give the product (39) if it had the structure (38). NMR spectrum of the acetate of cuneifolic acid would also give some information on the orientation of the substituent about the chromane nucleus. Treatment of the acid with acetic anhydride and pyridine gave a gum. The gum which was homogeneous on TLC gave a strong peak at 1785 cm^{-1} in its ir spectrum. This absorption is characteristic of the phenyl esters. The shift of ir absorption from 1700 cm^{-1} in the acid to 1785 cm^{-1} in the gum strongly suggested that lactonization has taken place as in the case of papuanic acid (35). Since lactonization had probably taken place, cuneifolic acid must probably have the structure (38). Further confirmation of the structure of cuneifolic acid must await the NMR spectra of the gum.

Biflavanoids

Three types of biflavanoids have been described from the bark and timber of Guttiferae species.

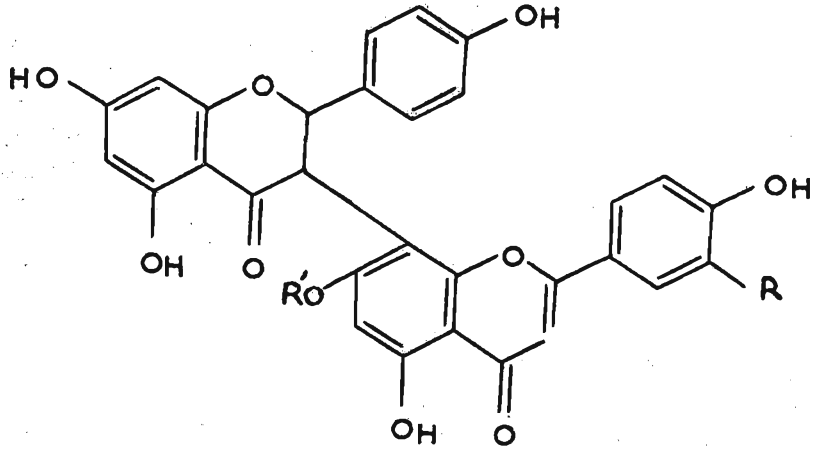
1. Biflavanoid type where both monomers are flavanones like naringenin, aromadendrin, taxifolin etc. *eg.* II-3, I-4′, II-4′ I-5, II-5, I-7, II-7 heptahydroxy-[I-3, II-8] biflavanone (40). These biflavanoids are referred to as GB biflavanoids[17].



40

(*a*) $R^1 = H$, $R^2 = OH$, $R^3 = OH$, GB-2
(*b*) $R^1 = H$, $R^2 = OH$, $R^3 = H$, GB-1 — II-3, I-4′, II-4′, I-5, II-5, I-7, II-7 heptahydroxy-[I-3, II-8] biflavanone
(*c*) $R^1 = R^2 = R^3 = H$, GB-1a
(*d*) $R^1 = R^2 = H$, $R^3 = OH$, GB-2a
(*e*) R^1 = glucose moiety $R^2 = H$, $R^3 = OH$

2. Flavanone : flavone type where one unit is naringenin (42) and the second unit is flavone, *e.g.* volkensiflavone[17] (41b).



41

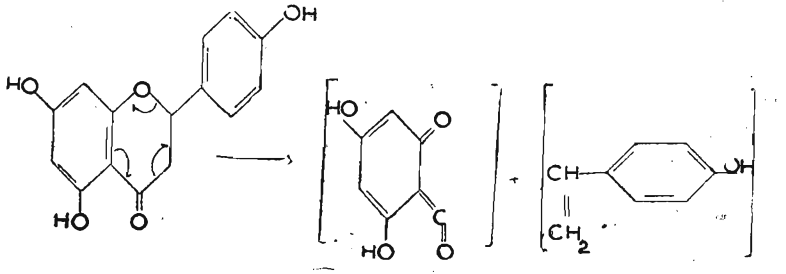
(*a*) R = OH, $R^1 = H$, morelloflavone
(*b*) $R = R^1 = H$ volkensiflavone: I-4′, II-4′, I-5, II-5, I-7, II-7 hexahydroxyflavanone [I-3, II-8] flavone
(*c*) R = H, R^1 = glucose moiety – spicataside
(*d*) R = OH, R^1 = glucose moiety – fugugiside

3. Biflavanoid glycosides eg. fugugiside[30] (41d)

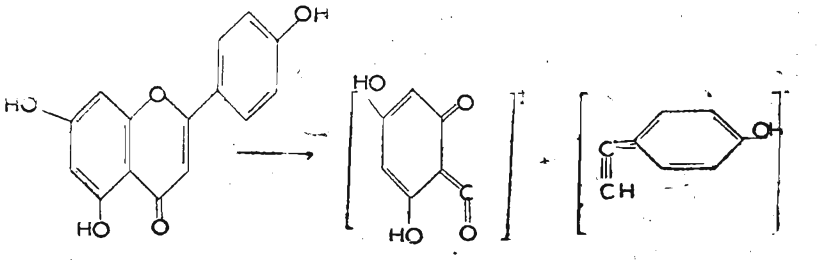
All the biflavanoids isolated from the timber, bark and root extracts of Guttiferae species have a [I-3, II-8] interflavanoid linkage.

The separation of the biflavanoids in the two species studied *Garcinia terpnophylla* Thw. & *Garcinia echinocarpa* Thw. presented considerable difficulties as no separation could be effected on silica gel or cellulose columns. Therefore partial separations were effected by cold aqueous sodium carbonate and borax extraction. The borax extracts were taken up in ethyl acetate and then partitioned in a counter current distribution unit. The fractions from these were then separated on a polyamide column to give each of the biflavanoids F_1 , F_2 and F_3 from *G. terpnophylla* Thw. in a pure state.

All three pigments from *G. terpnophylla* Thw. were pale yellow in colour and gave a deep red colouration with magnesium turnings and conc. hydrochloric acid. They all had similar uv patterns and their maxima showed, on addition of sodium acetate the bathochromic shifts characteristic of 5,7-dihydroxyflavanone system. Their molecular weights as determined by mass spectrometry indicated that they are flavanoid dimers. The study of the physical data particularly the mass spectra of pigments F_1 , F_2 and F_3 led to the identification of these flavanoid dimers. Simple flavones and flavanones are known to undergo a retro Diels-Alder fragmentation in their mass spectra as for example naringenin (42) and apigenin (43) (*Scheme* 9).



42



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Scheme 9

A similar process was observed in the case of volatile biflavanoids where two successive retro Diels-Alder fragmentation takes place *eg.* II-3, I-4′, II-4′, I-5, II-5, I-7, II-7 heptamethoxy-[I-3, II-8] biflavanone (*Chart* 1)

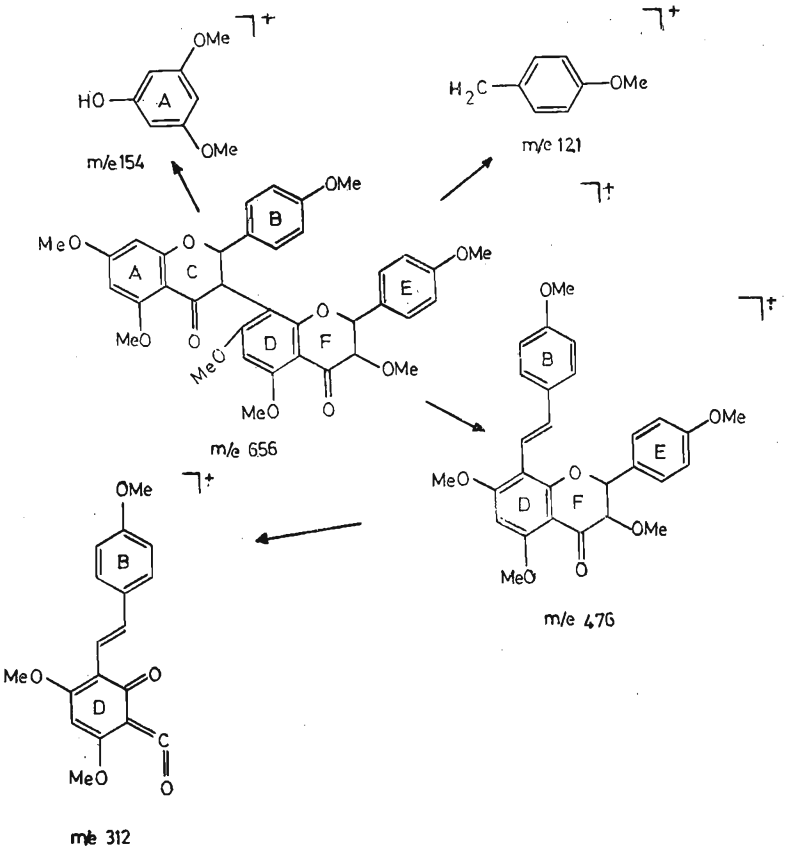


Chart 1

The base peak at *m/e* 312 is a direct result of the two successive retro Diels-Alder fragmentation of the molecular ion. The cleavage of ring C takes place first to give a fragment of *m/e* 476 (M-180) which then undergoes another retro Diels-Alder fragmentation to give the ion corresponding to *m/e* 312. Also observed in the spectra are p-methoxy benzyl ion of *m/e* 121 and phloroglucinol ion of *m/e* 154. The phloroglucinol fragment of *m/e* 154 is believed to be a result of thermal breakdown of the parent molecule.

The appearance of phloroglucinol fragment and p-methoxy benzyl fragment in the mass spectrum of the above heptamethyl ether confirms that

(*a*) at least one phloroglucinol ring is not included in the linkage of the two monomer units and that

(*b*) both heterocyclic rings have a methoxy phenyl substituent and these methoxy phenyl substituents are not involved in the linkage of the two flavanoid units. It has been observed that volatile biflavanoids like GB 1a (F_1) (*Chart* 2)

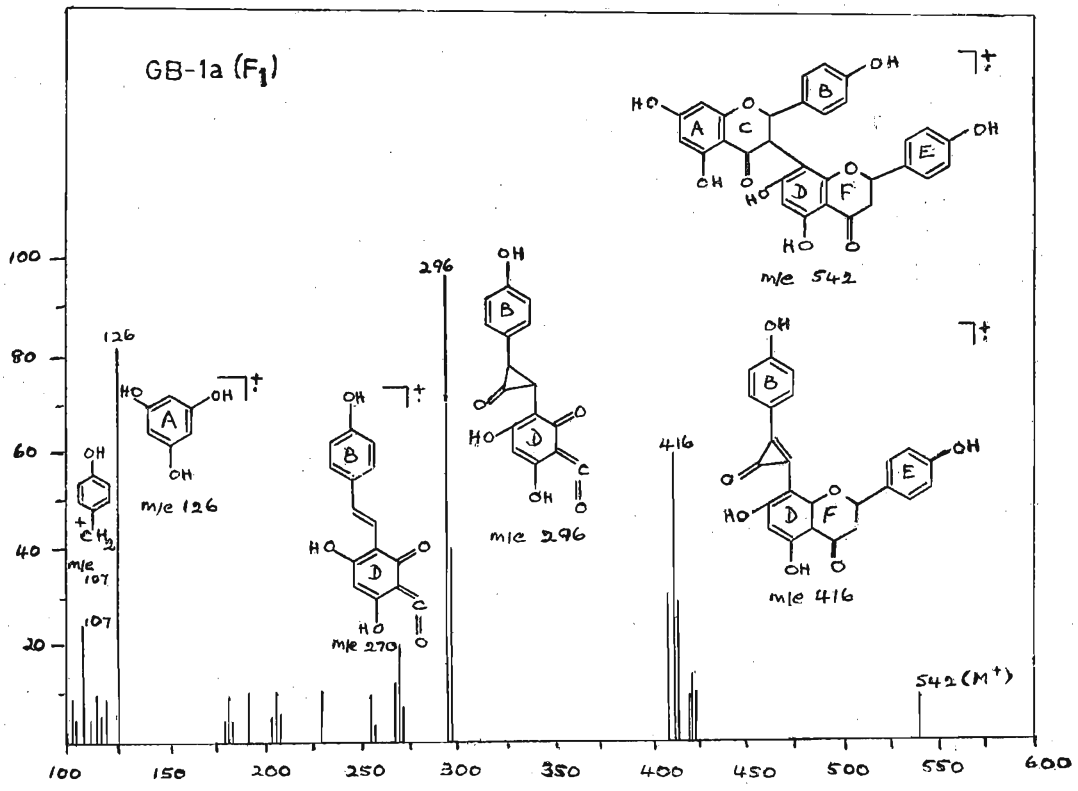


Chart 2

lose a molecule of phloroglucinol very readily from the parent ion and give rise to a base peak at *m/e* 126. The loss of phloroglucinol from the parent ion is often followed by a single retro Diels-Alder fragmentation.

The pigment F_1 showed the u.v. maxima at 228 (log ε 4.52), 290(4.49) and 335 (3.92) nm. In the mass spectrum the molecular ion appeared at *m/e* 542 (*Chart* 2). The peaks at *m/e* 126 and *m/e* 107 most probably due to phloroglucinol ion and p-hydroxybenzyl ions respectively, suggested that at least one phloroglucinol ring is not involved in the interflavanoid linkage and that

one of the hetrocyclic rings had a hydroxy phenyl substituent and this hydroxyphenyl substituent is not involved in the interflavanoid linkage. The u.v. pattern and the presence of these peaks in the mass spectrum indicated that F_1 was a GB flavanoid. The loss of elements of phloroglucinol from the parent ion gave an ion of *m/e* 416 which then underwent retro Diels-Alder fragmentation to give an ion of *m/e* 296. The absence of a peak due to a dihydroxybenzyl fragment at *m/e* 123 indicated that both heterocyclic rings probably had only hydroxyphenyl substituents. Also visible in the mass spectrum is a weak peak at *m/e* 270 which could have arisen from the molecular ion by two successive retro Diels-Alder fragmentations. Together with the other physical data, these data indicated that F_1 was I-4′, II-4′, I-5, II-5, I-7, II-7 hexahydroxy-[I-3, II-8] biflavanone (GB 1a)[26] (40c). This identification was confirmed by comparison with an authentic sample *i.r.* and TLC

From the spectroscopic and mass spectral data (*Chart* 3) F_2 was recognized as II-3, I-4′, II-4′ I-5, II-5, I-7, II-7 heptahydroxy-[I-3, II-8] biflavanone (GB 1)[17] (40 b).

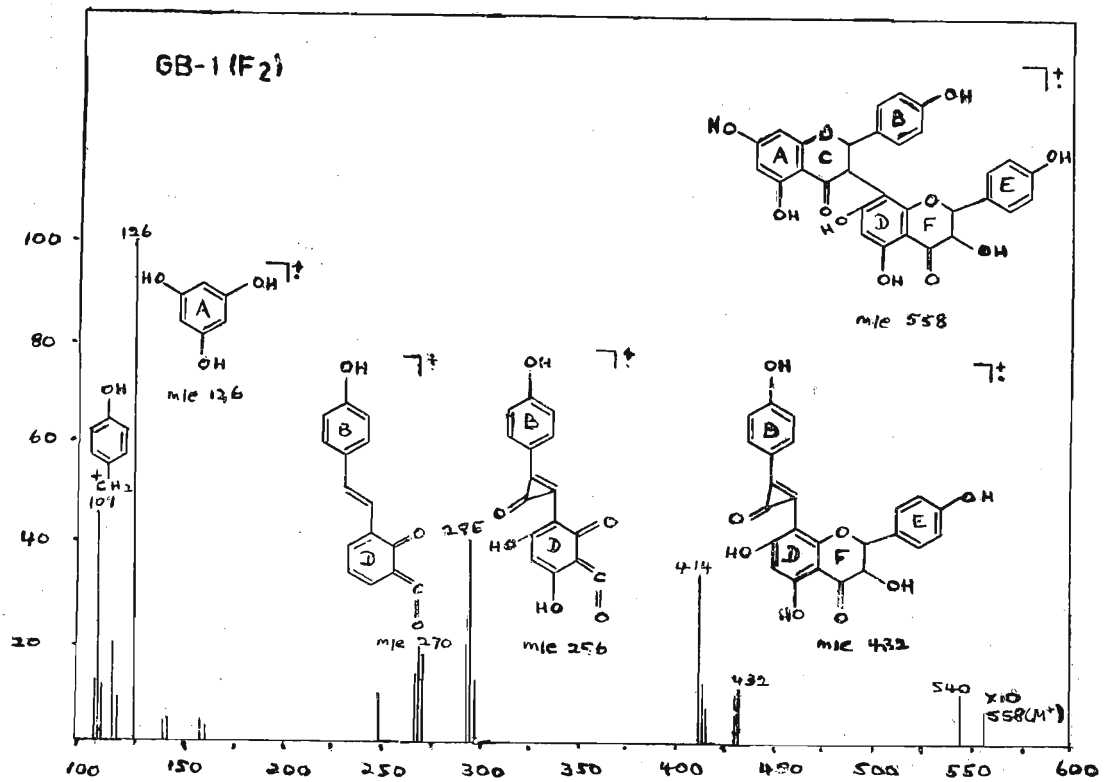


Chart 3

This identification was confirmed by comparison with an authentic sample *ir* and TLC)

The third biflavanoid F_3, in a similar manner (*Chart* 4) was identified as II-3, II-3′ I-4′ II-4′ I-5, II-5, I-7, II-7 octahydroxy [I-3, II-8] biflavanone (GB 2)[26] (40a) and confirmed by comparison with an authentic sample ir and TLC)

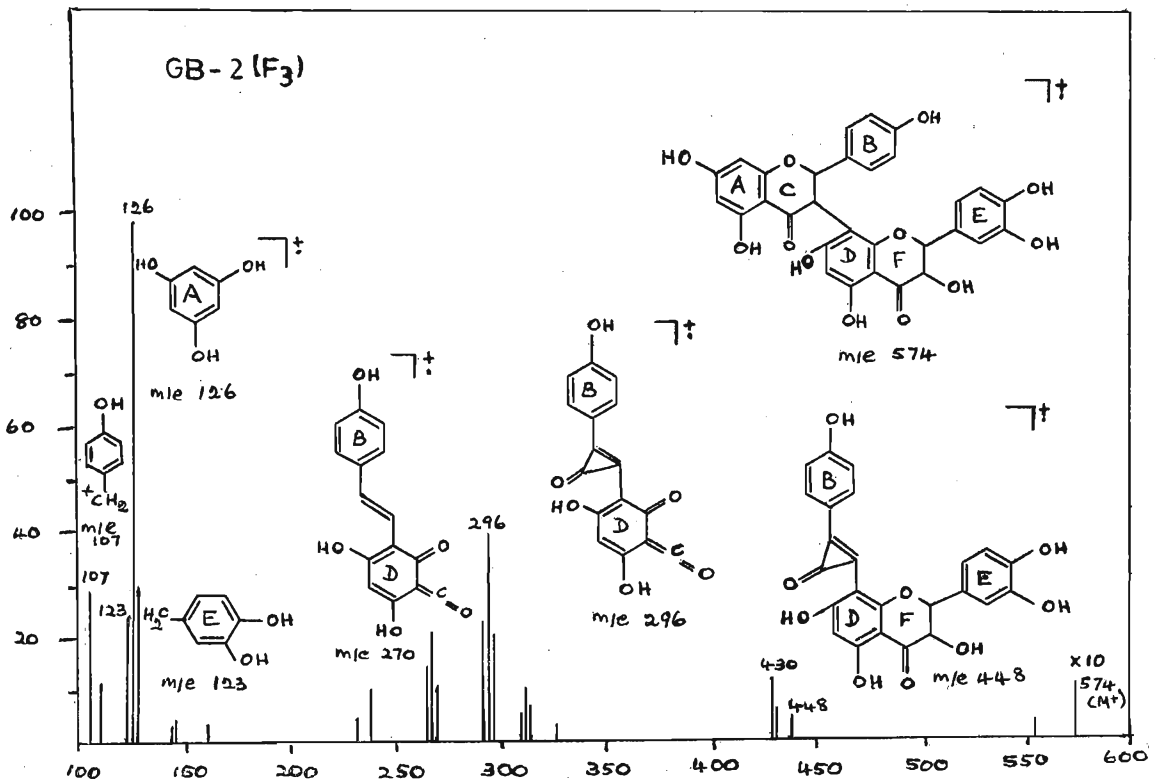


Chart 4

Both pigments F_4 and F_6 from *G. echinocarpa* Thw. gave green colouration with neutral ferric chloride solution and deep red colouration with magnesium turnings and conc. hydrochloric acid. Besides the pigments showed uv patterns similar to that expected for a combination of naringenin (42) and apigenin (43) (*Table* 8)

Table 8. uv absorption maxima λ_{max} ethanol

Flavanoids	uv maxima in ethanol λmax (log ε) nm			
Pigment F_4	225(4.60)	275(4.38)	289(4.44)	330(4.20)
Pigment F_6	225(4.57)	275(4.38)	288(4.33)	340(4.25)
Apigenin		268(4.23)		335(4.40)
Naringenin			288(4.25)	330(3.68)

The biflavanoid F_6, molecular weight 556, showed characteristic peaks at *m/e* 126, 107 and at *m/e* 121 in its mass spectrum (*Chart* 5)

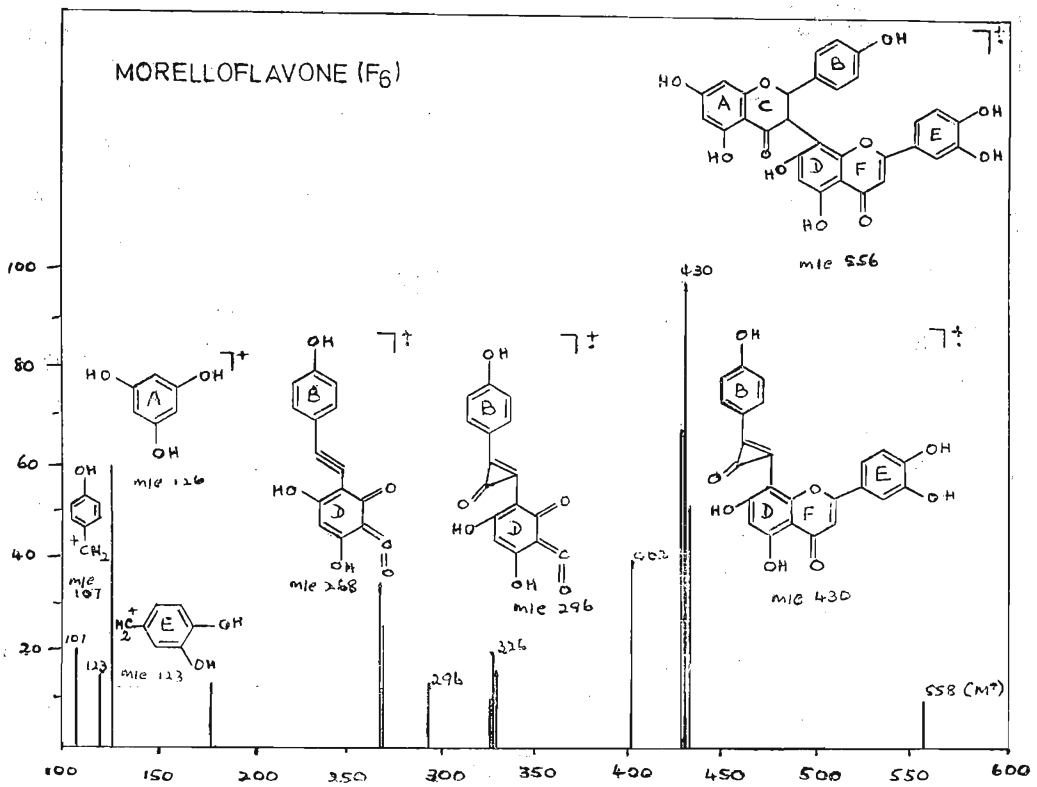


Chart 5

most probably due to phloroglucinol, parahydroxybenzyl and dihydroxybenzyl fragments respectively. These peaks and the uv data indicated that F_6 had the same carbon skeleton as the other Garcinia biflavancids. The uv pattern (*Table* 8) suggested that one of the monomers was flavanone. The peak at *m/e* 107 and *m/e* 121 indicated that while one of the heterocyclic rings had a *p*-hy-

droxybenzyl substituent, the other had a dihydroxyphenyl substituent. In the mass spectrum of this pigment the most intense peak was at *m/e* 430 which probably arises from the loss of elements of phloroglucinol from the parent ion. The fragment either loses elements of carbon monoxide to give a fragment at *m/e* 402 and then undergoes retro Diels-Alder reaction to give a fragment at *m/e* 265 or first undergoes a retro Diels-Alder fragmentation to give a fragment at *m/e* 296 which subsequently loses carbon monoxide. From these mass spectral fragmentation pattern and other physical data, the pigment was identified as II-3′, I-4′, II-4′, I-5, II-5, I-7, II-7-heptahydroxyflavanone [-I-3, II-8] flavone (morelloflavone)[19] (41a). This identification was confirmed by comparison with an authentic sample (mixed mp., ir and T.L.C.).

The biflavanoid F_4 from its mass spectral fragmentation (*Chart* 6) and other physical data was identified as I-4′, II-4′, I-5, II-5, I-7, II-7 hexahydroxyflavanone [I-3, II-8] flavone (volkensiflavone)[15] (41b) and confirmed by comparison with an authentic sample ir and TLC).

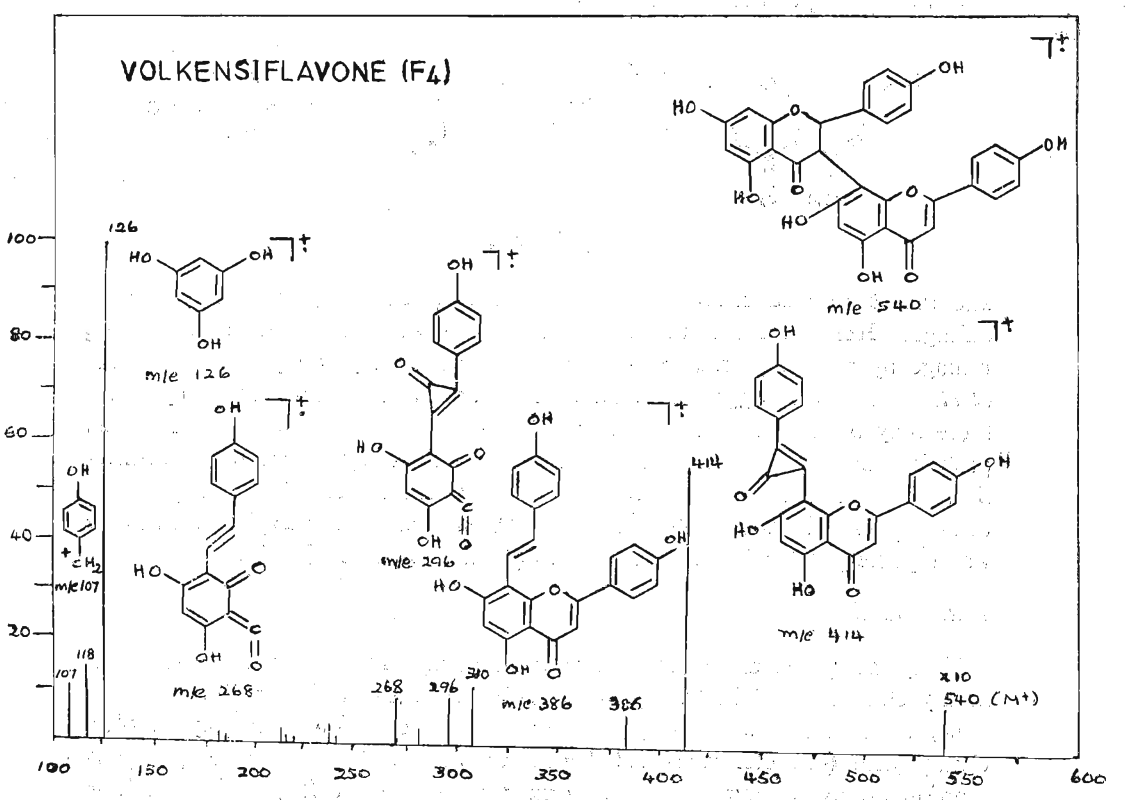
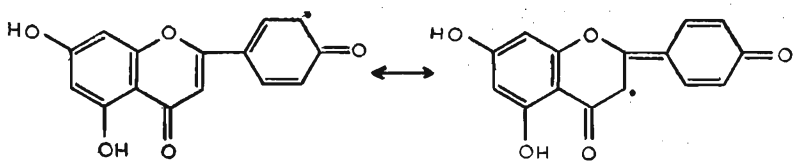


Chart 6

It has been suggested that biogenesis of these biflavanoids involves either a radical pairing or radical substitution of two complete embryonic flavanoid units[18]. The biogenetic pathway of flavanoids involves a combination of two fundamental pathways to aromatic compounds, namely shikimate and acetate pathways. The former pathway leads to the formation of ring B and C of the flavanoid. Studies using ^{14}C – labelled shikimic acid had shown that shikimic acid is incorporated in the ring B of quercetin. The ring A arises by the acetate pathway. The presence of hydroxy groups in positions 5 and 7 of the ring A in several flavanoids fits in with the phloroglucinol pattern. Also feeding experiments involving $C^{14} H_3 COOH$ and $CH_3 C^{14} OOH$ have shown that ring A of quercetin arises from acetic acid[5].

These biflavanoids are thought to arise when a quinone methide anion combined with an aryl system and the product enolises. Molyneux [25] *et. al.* have shown that in the case of apigenin the radical which is initially formed at C-4 oxygen atom on aerial alkaline oxidation is delocalised primarily to the C-3 and C-3′, positions (*Scheme* 10)

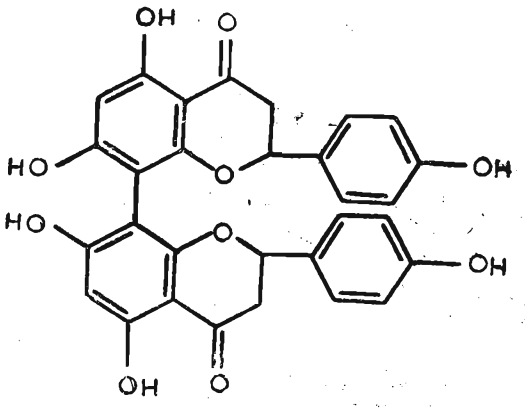


Scheme 10

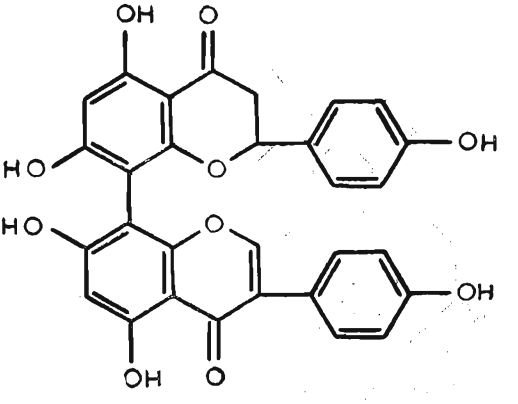
and that dimerisation leads to products with [3-3] and [3-3′] interflavanone linkage. Hence the formation of biflavanoids with [1-3, II-8] or [I-3, II-6] linkage by radical pairing in alkaline medium is very likely. Scheinmann *et al.*[18] however suggest that since Guttiferae timbers are invariably acidic there may be different modes of coupling under acidic conditions. Another probable mode of formation of these biflavanoids is by electrophilic substitution of a quinone methide radical on a phloroglucinol nucleus[5]. The presence of two other biflavanoids (44) and (45) in the stamens of *Mesua ferrea* L flowers was reported at the 8th IUPAC meeting[32] in New Delhi (1972).

Triterpenoids

Many triterpenes and steroids have been isolated from several species of the family Guttiferae. The triterpenes include members of the friedelane, oleanane, taraxerane, adianane groups etc. Friedelin (46), canophyllal (47), Canophyllol (48) etc. represent friedelane group. The oleanane group is represented by β-amyrin (49) and erythrodiol-3-acetate (50) while betulinic acid (51) and lupeol (52) represents the lupane group. Taraxerol (53) and tara-

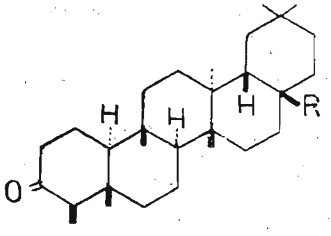


44 Mesua Ferrone A

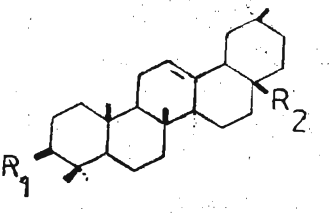


45 Mesua Ferrone B

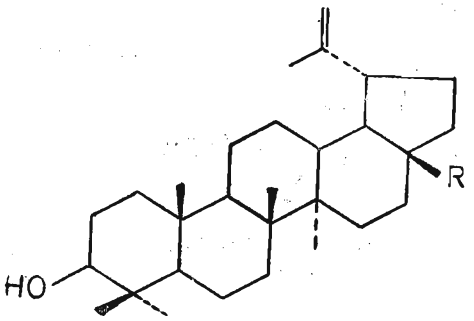
xerone (54) present in many *Calophyllum* L. species are members of the taraxerane group. An uncharacterised triterpene isolated from many *Calophyllum* L. species which is identified in the present work as simiaren-3 β-ol (55) belongs to the adianane group.



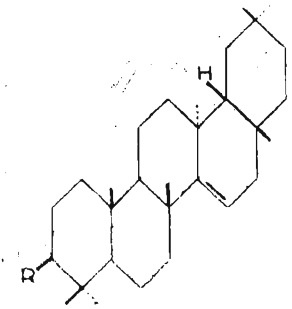
46 Friedelin R=CH_3
47 Canophyllal R=CHO
48 Canophyllol R=CH_2OH

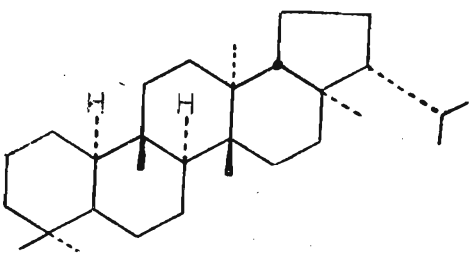


49 β Amyrin R_1=OH, R_2=CH_3
50 Erythrodiol-3-acetate
R_1=OAc, R_2=CH_2OH



51 Betulinic acid R=COOH
52 Lupeol R = CH_3





53 Taraxerol R = OH
54 Taraxerone R = O

55 β—simiarenol

In the plants investigated by us we have isolated friedelin (46), taraxerol (53), taraxerone (54) and β-simiarenol (55). The molecular formula of simiarenol was found to be C_{30} H_{50} O. The i.r. spectrum showed the presence of hydroxy group. This was also shown by its ready conversion into an acetate. The NMR spectrum of this triterpene showed the presence of eight methyl groups and a single olefinic proton (τ 4.40 complex multiplet) indicating the presence of a trisubstituted double bond. The signal at τ6.56 arises from a proton geminal to a hydroxy group. The small half width ($W_{\frac{1}{2}}$ 10 Hz) of this signal is suggestive for an equatorial proton.

The mass spectrum of this compound gave valuable information regarding its structure. Djerassi[6] and others have shown that the molecular ions of unsaturated triterpenes undergo a retro Diels-Alder fragmentation and furnish an intense peak. The base peak at *m/e* 274 of this triterpene could possibly arise from such a fragmentation. The mass spectral fragmentation of this

triterpene showed a close resemblance to that of glutinone[21] (57). This showed that the triterpene has some structural similarities to that of glutinone (57). The intense peak at *m/e* 274 suggested that the double bond is in position 5 and the peaks at m/e 152 and *m/e* 134 (*Chart* 7) are in accordance with the glutinone type of skeleton.

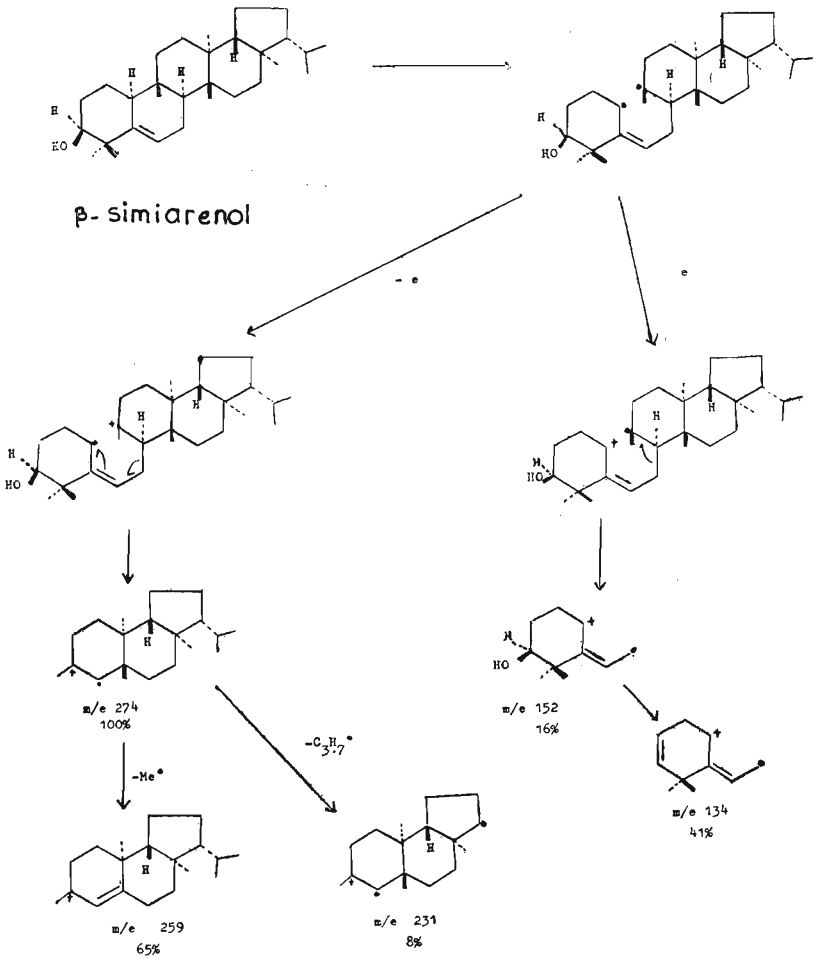
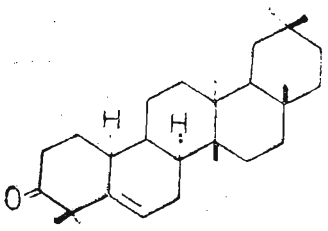


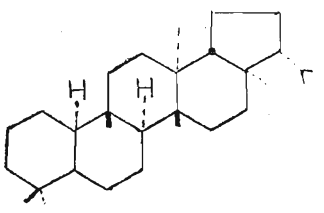
Chart 7

But a significant difference between the glutinone mass spectrum of this triterpene is the presence of M-43 fragment and a fragment at *m/e* 231 (*m/e* 274-43). These fragments suggested the presence of an isopropyl group. The presence of an isopropyl group in turn suggested that this triterpene belonged

to the adianane (58) series rather than the glutinone series[2]. From these data and from the m.p. and rotation, this triterpene was identified as simiaren-3β-ol[2] (55). This identification was confirmed by comparison with an authentic sample (mixed mp., ir and T.L.C.).



57 Glutinone



58 Adianane

The acetate of this triterpene was shown to be identical tc the acetate of simiaren-3β-ol by co mparison with an authentic sample (mixed mp., ir and T.L.C.). The triterpene was oxidised with chromium trioxide-pyridine and the oxidant was shown to be identical to simiarenone (56) by comparison with an authentic sample (mixed mp., ir and T.L.C.).

This is only a general survey of the solid constituents present in some members of the family Guttiferae. Several chemotaxonomic correlations have alsc come out, but this would take far too long a time for elaboration and I shall omit it for the present. These studies have thrown out fresh problems that should be undertaken here as it would be most appropriate. I may mention just a few of them as a stimulus for further research in this field. The first species studied *Calophyllum calaba* L. is found in the Southern Province, Batticaloa area and even in Passara at an elevation of 4000 ft. The form of these trees is different and chemical investigation should show up impcrtant differences. Another is *Garcinia echinocarpa* Thw. (madol) which also seems to be different according to the location. In the current literature too there is a controversy at the moment on the constituents in *C. inophyllum* L. We too have started a study on it but the results are incomplete.*

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* The presence of jacareubin has been established

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