Chemical Investigation of Ceylonese Plants. Part I. Extractives of *Calophyllum calaba* L. and *Calophyllum bracteatum* Thw. (Guttiferae)

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The bark and timber extracts of *C. calaba* L. and *C. bracteatum* Thw. have been studied. A new di-isoprenylated xanthone, named calabaxanthone, isolated from the barks of both species has been shown to be 5-hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (II). The bark extracts of both species have yielded taraxerol (Ia) and taraxerone (Ib). The bark extracts of *C. bracteatum* Thw. have also yielded calophyllolide (XI). The timber extracts of *C. calaba* L. have given a hitherto unknown xanthone 1,8-dimethoxy-2-hydroxyxanthone (VII), eight other known xanthones, β-sitosterol, and stigmasterol. The timber extracts of *C. bracteatum* Thw. have given two new xanthones 1,3,5-trihydroxy-2-methoxyxanthone (XII) and 1,8-dihydroxy-3,5,7-trimethoxyxanthone (XVI), four other known xanthones, betulinic acid, β-sitosterol, and stigmasterol. Structures of the new xanthones have been elucidated.

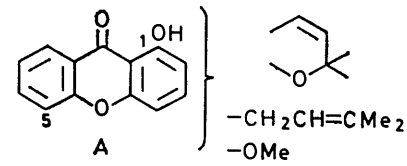
THE flora of Ceylon consists of over 3300 plant species of which about 830 are endemic.[1] Studies have been initiated mainly on the endemic plants and in this series the main chemical results will be presented. This paper reports results of the studies on *Calophyllum calaba* L.[2] and *Calophyllum bracteatum* Thw.[3]

Bark Extractives of C. calaba *L.*—The cold light petroleum extract of the powdered bark of *C. calaba* L. was concentrated and filtered. The mixture of solids was then separated on an alumina column, and three triterpenoid compounds were isolated. One was shown to be taraxerol (Ia)[4,5] (m.p., acetate, mixed m.p., i.r., and rotation) and another to be taraxerone (Ib)[5] (m.p., mixed m.p., i.r., and rotation). Taraxerol was oxidised with chromic acid to taraxerone and was shown to be identical with the isolated product. The third compound with a molecular formula $C_{30}H_{50}O$, m.p. 195°, has been recently characterised as β-simiarenol.†

The oil obtained from the filtrate when eluted with benzene on a silica gel column gave a yellow compound. It was insoluble in sodium hydroxide and failed to give a colouration with ferric chloride. The u.v. data and i.r. absorption at 1645 cm^{-1} indicated that it was a xanthone; it was therefore named calabaxanthone (II). The u.v. spectrum showed, besides the usual bands associated with the xanthone group, an intense absorption at 287 (ε 77,750) nm. A similar spectrum has been recorded for osajaxanthone (Vc).[6]

Hydrogenation of calabaxanthone yielded a tetrahydro-derivative whose u.v. spectrum showed a hypsochromic shift, indicating that one of the olefinic double bonds of calabaxanthone was conjugated with the aromatic nucleus.[7] The u.v. spectrum of tetrahydrocalabaxanthone showed a close resemblance to the u.v. spectrum of dihydro-osajaxanthone and derivatives of 1,3,7-trihydroxyxanthone, thus pointing to a similar oxygenation pattern for tetrahydrocalabaxanthone (Table 1).

A sharp low-field signal at τ −3·70 in the n.m.r. spectrum indicated a hydrogen-bonded phenolic proton. This was further supported by the conditions for methylating and acetylating[6] calabaxanthone. Therefore a hydroxy-group should be present at C-1 which is *peri* to the carbonyl group. The sharp singlet at τ 8·56(6H) and two doublets at τ 3·22(1H) and 4·58(1H, J_{AB} 9·6 Hz) revealed the presence of a dimethylchromen ring.[8,9] Two sharp singlets at τ 8·18(3H) and 8·34(3H) due to the olefinic methyl groups; a triplet at τ 4·76(1H) due to an olefinic proton α to a methylene group and a doublet at τ 5·87(2H) due to a methylene group indicated the presence of a 3,3-dimethylallyl side chain in the compound. The methoxy-group appeared as a singlet at τ 6·16(3H). The above data can be summarised by the partial structure A.



A

The three aromatic protons appeared as two singlets at τ 2·73(2H) and 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,[10] there is a proton *para* to C-1 of xanthone. Since there is only a single proton in the

† S. Selliah and M. U. S. Sultanbawa, unpublished results.

[1] (*a*) H. Trimen, 'Hand Book of the Flora of Ceylon,' Dulau, London, 1893—1900, vols. I—V; (*b*) A. H. G. Alston, 'Supplement to Trimen's Flora,' Dulau, London, 1931; (*c*) M. U. S. Sultanbawa and G. P. Wannigama, *Proc. Ceylon Assoc. Adv. Sci.*, 1969, **25**, 89.

[2] R. Somanathan and M. U. S. Sultanbawa, *Proc. Ceylon Assoc. Adv. Sci.* 1969, **25**, 91.

[3] R. Somanathan and M. U. S. Sultanbawa, *Proc. Ceylon Assoc. Adv. Sci.*, 1970, **26**, 75.

[4] S. Burrows and J. C. E. Simpson, *J. Chem. Soc.*, 1938, 2042.

[5] K. Takeda, *J. Pharm. Soc. Japan*, 1941, **61**, 63.

[6] M. L. Wolform, F. Komitsky, jun., and J. N. Looker, *J. Org. Chem.*, 1965, **30**, 144,

[7] M. L. Wolform, W. D. Harris, G. F. Johnson, J. E. M. Mahan, S. M. Moffatt, and B. S. Wildi, *J. Amer. Chem. Soc.*, 1946, **68**, 406.

[8] W. D. Ollis and I. O. Sutherland, 'Recent Developments in the Chemistry of Natural Phenolic Compounds,' eds. W. D. Ollis, Pergamon, London, 1961, p. 74.

[9] M. L. Wolform and F. Komitsky, jun., 'Recent Progress in the Chemistry of Natural and Synthetic Colouring Matters and Related Fields,' eds. T. S. Gore, B. S. Joshi, S. V. Sunthanker, and B. D. Tilak, Academic Press, New York, 1962, p. 287.

[10] F. E. King, J. T. King, and L. C. Manning, *J. Chem. Soc.*, 1957, 563.

phloroglucinol nucleus which is assigned to C-4 of xanthone, the 2,2-dimethyl-2*H*-pyrano-ring must be attached to the 2,3-positions of the xanthone ring giving a linear tetracyclic compound.

Further confirmation of this linear arrangement of the 2,2-dimethyl-2*H*-pyrano-ring was obtained from the proton shifts observed for its acetate. A positive diamagnetic shift and a negative paramagnetic shift for linear chromen protons and a negative paramagnetic shift for the C-12 aromatic proton has been recorded for similar systems.[11,12]

TABLE 1

Comparison of u.v. absorption maxima [$\lambda_{max.}$/nm ($10^{-3}\varepsilon$)]

								Solvent
Osajaxanthone (Vc)	238 (19·0)	249 (18·0)		286 (47·10)		339 (8·2)	382 (4·8)	*a*
Osajaxanthone monomethyl ether	240 (21·4)	248 (19·9)		286 (43·6)		339 (9·0)	375 (4·5)	*a*
Calabaxanthone (II)	240 (34·01)			287 (77·25)	292sh (72·21)	314 (28·83)	384 (8·42)	*a*
Tetrahydrocalabaxanthone	241 (63·36)		262 (64·84)		316 (36·43)		363 (9·11)	*b*
Dihydro-osajaxanthone	237 (30·9)		262 (33·48)		316 (16·22)		380 (6·25)	*b*
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-dimethoxyxanthone	231 (34·71)		259 (38·55)		292 (24·36)		369 (6·78)	*a*
2-(1,1-Dimethylallyl)-1-hydroxy-3,7-dimethoxyxanthone	233 (34·63)		264 (37·18)		311 (18·32)		372 (6·84)	*a*
2-(3,3-Dimethylallyl)-1,3,7-trihydroxyxanthone	241 (33·87)		263 (32·89)		314 (17·14)		337 (6·50)	*a*

[a] Ethanol. [b] Methanol.

TABLE 2

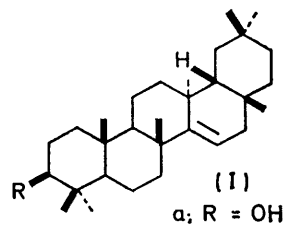
Chemical shift differences

	4-H	3-H	12-H
Calabaxanthone (II)	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

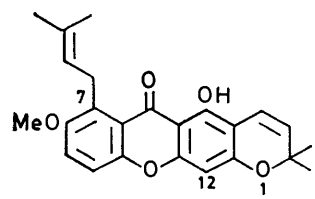
The position of the 3-methylbut-2-enyl side chain was assigned on the following basis. In the n.m.r. spectrum of calabaxanthone the methylene protons of the 3-methylbut-2-enyl side chain attached to the aromatic ring appeared at τ 5·87 similar to the position of the signals in related compounds like mangostin [13] (τ 5·90) and celibixanthone [14] (τ 6·03) which have the 3-methylbut-2-enyl side chain at C-8 (xanthen numbering) and much lower than the position of signals for methylene groups at other positions.[15] In addition, if C-8 was unsubstituted, there should have been an aromatic proton with a low-field signal [15] which is absent in calabaxanthone. On the above basis the structure of calabaxanthone becomes 5-hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)-2*H*,6*H*-pyrano[3,2-*b*]-xanthen-6-one (II).

This structural pattern would also explain the insolubility of the compound in sodium hydroxide, the failure to give a positive ferric chloride test and absence of any effect in the presence of aluminium chloride on the u.v. spectrum, and its high R_F value in benzene.

The n.m.r. spectrum of the methyl ether of calabaxanthone was very similar to that of calabaxanthone with 5-OMe group appearing at τ 6·18 and, as expected, the signal for 4-H appeared at a lower value, τ 3·49. The



(I)
a; R = OH
b; R = =O



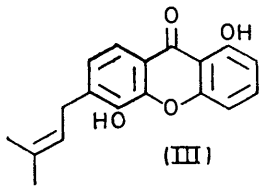
(II)

n.m.r. data of tetrahydrocalabaxanthone is also in agreement with this structure.

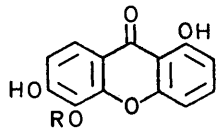
Timber Extractives of C. calaba *L.*—The finely powdered timber was extracted with cold light petroleum (b.p. 40—60°), and filtered. The filtrate contained mainly β-sitosterol and waxy material and this was not further investigated. The yellowish residue was taken up in ether and separated into sodium hydroxide soluble and insoluble fractions. From the alkali soluble fraction a bright yellow solid was isolated and characterised as guanandin (III) [16] which was confirmed by mixed m.p. and i.r. comparison with an authentic sample. The

[11] A. Arnone, G. Cardillo, L. Merlini, and R. Mondelli, *Tetrahedron Letters*, 1967, 4201.
[12] W. M. Bandaranaike, L. Crombie, and D. A. Whiting, *J. Chem. Soc.* (*C*), 1971, 804.
[13] P. Yates and G. H. Stout, *J. Amer. Chem. Soc.*, 1958, **80**, 1691.
[14] G. H. Stout, V. F. Stout, and M. J. Welsh, *Tetrahedron*, 1965, **19**, 667.
[15] B. Jackson, H. D. Locksley, and F. Scheinmann, *J. Chem. Soc.* (*C*), 1967, 2500.
[16] D. De Barros Correa, O. R. Gottlieb, and M. Taveira Magalhaes, *Anais Acad. brasil. Cienc.*, 1966, **38**, 296.

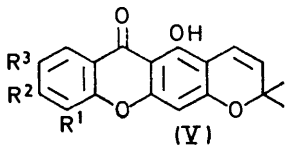
sodium hydroxide-insoluble fraction yielded a white crystalline solid which on g.l.c. examination was found to be mixture of β-sitosterol and stigmasterol with the former being the major component.



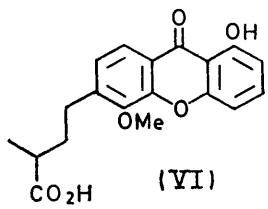
(III)



(IV)
a; R = Me
b; R = H



(V)
a; $R^1 = OH, R^2 = R^3 = H$
b; $R^1 = R^2 = OH, R^3 = H$
c; $R^1 = R^2 = H, R^3 = OH$



(VI)

The hot benzene extract of the timber gave on concentration a bright yellow solid which on elution with benzene on a silica gel column gave a yellow pigment which was characterised as 1,6-dihydroxy-5-methoxyxanthone (IVa) from the u.v., i.r., and n.m.r. data and confirmed by mixed m.p. comparison with an authentic sample. The filtrate of the benzene extract was washed with 10% sodium carbonate solution followed by 10% sodium hydroxide solution. The sodium hydroxide-soluble portion on acidification yielded five yellow pigments which were separated by silica gel chromatography. Four of them have been characterised as 1,7-dihydroxyxanthone (euxanthone),[17] guanandin (III), 6-deoxyjacareubin (Va) [16] and 2,8-dihydroxy-1-methoxyxanthone (VIII) [18] by spectral data which were confirmed by mixed m.p. comparison with authentic samples. The structure of the fifth pigment is under investigation.

The sodium carbonate solution on acidification yielded three yellow pigments which were separated by silica gel chromatography and characterised by spectral data.

TABLE 3

U.v. absorption maxima [$\lambda_{max.}$/nm (10^{-3} ε)] of of 1,2,8-trioxygenated xanthones in ethanol

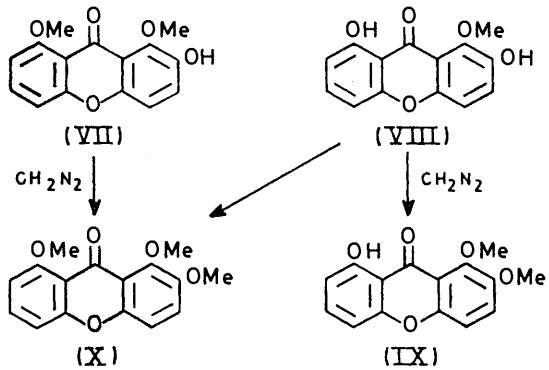
1,2,8-Trihydroxyxanthone	241 (26·0)	265 (36·4)	290 (7·8)	338 (8·8)
2,8-Dihydroxy-1-methoxyxanthone (VIII)	238 (26·8)	262 (32·8)	290 (5·2)	322 (4·4)
8-Hydroxy-1,2-dimethoxyxanthone (IX)	241 (49·5)		284 (8·1)	311 (9·5)
2-Hydroxy-1,8-dimethoxyxanthone (VII)	242 (30·3)	257 (31·2)	285 (5·7)	315 (5·3)

Two of them were identified by comparison with authentic samples as scriblitifolic acid (VI) [19] and 1,6-dihydroxy-5-methoxyxanthone (IVa).[15] The third compound was a new natural product (VII).

[17] D. B. Spoelstra and M. J. Van Royen, *Rec. Trav. chim.*, 1929, **48**, 370.
[18] O. R. Gottlieb, M. Taveira Magalhaes, and G. M. Stefani, *Tetrahedron*, 1966, **22**, 1785.

The n.m.r. and i.r. spectra of compound (VII) indicated that it was a xanthone and the u.v. spectrum resembled the related 1,2,8-trioxygenated pattern (Table 3).

Furthermore, methylation of compound (VII) with diazomethane and methylation of 2,8-dihydroxy-1-methoxyxanthone (VIII) with dimethyl sulphate converted both into 1,2,8-trimethoxyxanthone (X). This



(VII) (VIII) (X) (IX)

suggested that compound (VII) was a 1,2,8-trioxygenated xanthone. The n.m.r. spectrum indicated the presence of two methoxy-groups at τ 6·22(3H) and 6·15(3H), two AB doublets at τ 2·68(1H) and 2·80(1H, J_{AB} 8·4 Hz), two doublets at τ 3·08(1H) and 2·98(1H, J_{AB} 8·4 Hz), and a triplet at τ 2·31(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-dimethoxyxanthone (IX) obtained from 2,8-dihydroxy-1-methoxyxanthone (VIII) by methylation with diazomethane was found to be different from compound (VII) which must, therefore, be 2-hydroxy-1,8-dimethoxyxanthone.

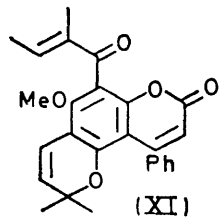
The sodium hydroxide-insoluble fraction of the benzene extract contained only β-sitosterol and waxes similar to that of the light petroleum extract.

The hot chloroform extract of the timber gave a brown solid which on silica gel chromatography gave two yellow pigments characterised by the spectral data as 1,5,6-trihydroxyxanthone (IVb) and jacareubin (Vb) which were confirmed by mixed m.p. comparison with authentic samples.

Bark Extractives of C. bracteatum *Thw.*—From the cold petroleum extract of the bark colourless flakes of calophyllolide (XI) [20,21] crystallised out. It was characterised by spectral data which were confirmed by mixed m.p. with an authentic sample. Calophyllolide has been isolated from the seed kernel of *C. inophyllum* L. by

[19] B. Jackson, H. D. Locksley, and F. Scheinmann, *J. Chem. Soc.* (*C*), 1967, 785.
[20] J. Polonsky, *Bull. Soc. chim. France*, 1957, 1079; 1958, 929.
[21] S. K. Nigam, C. R. Mitra, G. Kunesch, B. C. Das, and J. Polonsky, *Tetrahedron Letters*, 1967, 2633.

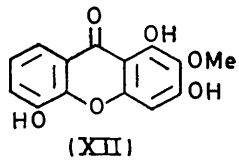
Polonsky,[20] but had not been reported from the bark. The light petroleum extracts were washed with sodium carbonate and sodium hydroxide solutions. The sodium hydroxide-insoluble fraction from light petroleum gave



(XI)

on silica gel chromatography calabaxanthone (II), taraxerol (Ia), taraxerone (Ib), β-sitosterol, and a triterpene, m.p. 195°. The last compound was shown to be identical with the β-simiarenol isolated from *C. calaba* L.

Timber Extractives of C. bacteatum *Thw.*—The chloroform extract of the timber gave a hot benzene-soluble fraction from which a yellow, solid mixture separated out. This was separated on a silica gel column to give a bright yellow solid, m.p. 252°. The u.v. spectrum showed characteristic xanthone peaks and the i.r. spectrum had strong bands at 3275 (free OH) and 1652 cm^{-1} (chelated C=O). The n.m.r. 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 was formed indicating the presence of three free hydroxy-groups and one which is chelated, and therefore at C-1. This was further supported by the bathochromic shift of maxima in the u.v. spectrum on addition of aluminium chloride. Since the compound was stable to alkali,[22] 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 a hydroxy-group at C-3—C-6 allows a xanthone to be ionised by sodium acetate [22] and the original u.v. spectrum of the compound (XII) was altered in the longer



(XII)

wavelength region on the addition of sodium acetate which indicated the presence of a hydroxy-group at C-3, -5, or -6.

The n.m.r. spectrum of the 1-hydroxy-2,3,5-trimethoxyxanthone,[23] obtained by the methylation of compound (XII) with diazomethane showed resonances at τ 3·49(4-H, s), 2·78(6- and 7-H, m), and 2·29(8-H, q) and these values are very similar to that of the signals of the compound (XII). The tetramethyl ether of (XII) was shown to be identical with 1,2,3,5-tetramethoxyxanthone by comparison with an authentic sample (mixed m.p. and n.m.r.). 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 u.v. 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 (XII).

The filtrate of the benzene extract was washed with 10% sodium carbonate and then with 10% sodium hydroxide. The sodium carbonate-soluble fraction was separated on silica gel to give three bright yellow pigments and two colourless solids. Two of the yellow pigments were shown to be 1,3,5-trihydroxy-2-methoxyxanthone (XII) and 1,5-dihydroxyxanthone (mixed m.p. and i.r.).

The u.v. and i.r. spectra of the third pigment indicated that it was a xanthone. The n.m.r. spectrum showed signals at τ −3·2(1H) and −0·3(1H) for two hydroxy-groups and at τ 6·07(3H, s), 6·15(3H, s), and 6·25(3H, s) for three methoxy-groups. The aromatic protons appeared as two doublets at τ 2·55(1H) and 2·85(1H) (J 9 Hz) and a sharp singlet at τ 3·37(1H). The diacetate had a similar n.m.r. pattern for the aromatic protons and the three methoxy-groups and the acetyl signals were observed at τ 7·49(3H) and 7·66(3H). The coupling pattern shown in the n.m.r. spectra of the parent compound and its diacetate suggested the presence of two aromatic protons in one ring and a single proton in the other. The high τ values of the aromatic proton signals in the n.m.r. indicated that C-1 and -8 of the xanthone nucleus were substituted.

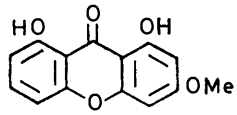
The addition of aluminium chloride produced a bathochromic shift of the maxima in the u.v. spectrum, thereby locating one of the hydroxy-groups at C-1. The addition of NaOAc and NaOAc–H_3BO_3 did not effect the u.v. spectrum. The compound was also stable to alkali. Therefore the second hydroxy-group could not be at C-2, -4, or -5. The negative values observed for the two hydroxy-groups in the n.m.r. spectrum suggested that they are probably located at C-1 and -8, as has been reported by Markham [24] for 1,8-dihydroxyxanthone. This is confirmed by the absence of signals in the n.m.r. characteristic of nuclear protons at C-1 or -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.

[22] O. R. Gottlieb, M. Taveira Magalhaes, M. Ottoni de Silva Pereira, A. Lins Mesquita, D. De Barros Correa, and G. G. de Oliveira, *Tetrahedron*, 1968, **24**, 1601.

[23] G. H. Stout, E. N. Christensen, W. J. Balkenhol, and K. L. Stevens, *Tetrahedron*, 1969, **25**, 1947.

[24] K. R. Markham, *Tetrahedron*, 1965, **21**, 3687.

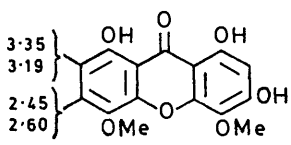
The other two methoxy-groups have to be assigned to C-7 or -5 and C-2 or -4. The n.m.r. signal of H-2 always appears at somewhat higher fields than that of H-4 [23,25] for a given set of hydroxy- and methoxy-substituents.



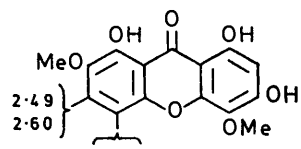
B

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

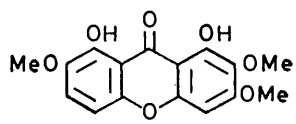
The exact values depend on the type of substituent in each ring but in general H-2 appears at τ 3·5—3·7 and H-4 at 3·2—3·5.[23] On this basis the singlet at τ 3·37 was assigned to H-4 and the methoxy-group to C-2. By comparison of the τ values observed for H-5 and -6 and H-6 and -7 in compounds (XIII) and (XIV), the methoxy-group was assigned C-7. Based on the above arguments, the structure is 1,8-dihydroxy-2,3,7-trimethoxyxanthone (XV).



(XIII)

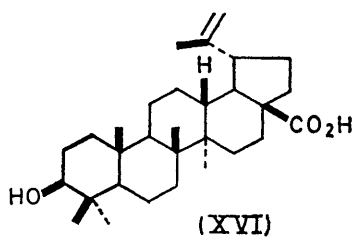


(XIV)



(XV)

One of the colourless solids was characterised as betulinic acid (XVI) [25] (mixed m.p., i.r., and n.m.r., and mass spectrum and mixed m.p. of methyl ester-acetate).



(XVI)

The sodium hydroxide-soluble fraction was separated on silica gel and gave 6-deoxyjacareubin (Va) and 1,7-dihydroxyxanthone (euxanthone) which were identified by mixed m.p. and i.r. comparison with authentic samples.

From the chloroform-soluble fraction, jacareubin (Vb) was characterised (mixed m.p., i.r., and n.m.r.) and further amounts of 1,3,5-trihydroxy-2-methoxyxanthone (XII) were isolated. This fraction gave in addition another pigment and a steroid which have not yet been characterised.

Jacareubin, which has been suggested by Scheinmann and his co-workers [26] to be characteristic of the genus *Calophyllum*, has been isolated in these two species as well, although its suggested precursor could not be detected after careful t.l.c. comparison of the extracts.

The isolation of 2,8-dihydroxy-1-methoxyxanthone (VIII) and 2-hydroxy-1,8-dimethoxyxanthone (VII) from the timber of *C. calaba* L. provides examples of the direct isolation of 1,2,8-trioxygenated compounds from the *Calophyllum* species, although such an oxygenation pattern has been recorded in the two compounds isolated from the *Kielmeyera* species, another genus of the Guttiferae. In *Calophyllum fragrans* Ridley [27] extracts the presence of 2,8-dihydroxy-1-methoxyxanthone has been shown by the isolation of 8-hydroxy-1,2-dimethoxyxanthone by use of diazomethane for the methylation of the crude extracts.

Likewise 1,3,5-trihydroxy-2-methoxyxanthone is the only tetraoxygenated compound with an oxygen function at C-2 from among *Calophyllum* species. The presence of 1,8-dihydroxy-2,3,7-trimethoxyxanthone is unique amongst the products obtained from the Guttiferae.

The isolation of calabaxanthone from the bark of the two species records for the first time a di-isoprenylated xanthone from the *Calophyllum* species. Polyisoprenylated xanthonoids like gambogic acid,[28] morellin,[29] deoxymorellin,[30] *etc.*, have been characterised from the barks of several *Garcinia* species of the Guttiferae. The sodium carbonate-soluble portion of the bark extracts contains compounds similar to blancoic acid,[31] papuanic acid,[32] *etc.*, from the t.l.c. pattern.

Nineteen xanthones have so far been reported from the *Calophyllum* species and comparison of the oxygenation pattern shows that only one 4-oxygenated compound, 4-hydroxyxanthone from *C. brasiliensis*, has so far been isolated. Ten of the reported compounds and four new xanthones have been isolated in the present investigation.

EXPERIMENTAL

U.v. spectra were recorded with a Unicam 800B spectrophotometer. The i.r., n.m.r., and mass spectral data were obtained from the Universities of Osaka, Sheffield, and Strathclyde. Rotations were determined with a Perkin-Elmer 141 polarimeter. Analytical and preparative t.l.c. were carried out with silica gel G (Merck). Separation by column chromatography was carried out by use of silica gel

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(Koch–Light) and neutral alumina (Merck). Acid-washed silica gel used for column chromatography was prepared by percolating the adsorbent in 2% acetic acid for 24 h, filtering, washing with water repeatedly, and drying at 110°. M.p.s were determined on a Kofler hot stage and are uncorrected. All R_F values are recorded on t.l.c. (thickness 0·25 mm). Elemental analyses were carried out at the CSIRO, Microanalytical Service, Melbourne, and at the University of Osaka.

C. calaba L. and *C. bracteatum* Thw. were obtained from the Dediyagala forest reserves, Ceylon. The timber and bark of each species were separated, chipped, and powdered in a mill and extractives were obtained successively with light petroleum, benzene, chloroform, and methanol. The solvents were removed under reduced pressure.

Extractives from Bark of C. calaba *L.—Isolation of taraxerol and taraxerone.* Cold extraction of the bark (1·9 kg) with light petroleum (b.p. 40—60°) (10 l) for 7 days deposited calabaxanthone (69 mg) which was filtered and on concentration of the filtrate a white solid (1·3 g) crystallised out. It was filtered and evaporation of the mother liquor afforded an oil (15 g). The solid (1 g) was chromatographed on neutral alumina (50 g). Elution with benzene gave taraxerone (40 mg), needles, m.p. 239—240° (from benzene), $[\alpha]_D^{20}$ +12° (*c* 1·00, $CHCl_3$) {lit.,[5] 241—243°, $[\alpha]_D^{23}$ +12·2° ($CHCl_3$)}, R_F 0·4 (benzene), and then taraxerol (420 mg), hexagonal crystals, m.p. 279—280° (from chloroform–acetone), $[\alpha]_D^{20}$ +5° (*c* 1·00, $CHCl_3$) {lit.,[5] 279—281°, $[\alpha]_D^{19}$ +3·1° ($CHCl_3$), R_F 0·58 ($CHCl_3$)}. Both were identified by comparison with authentic samples (mixed m.p. and i.r. spectra). Taraxerol gave an acetate with acetic anhydride–pyridine, m.p. 297—298°, $[\alpha]_D^{20}$ +13° (*c* 1·02, $CHCl_3$) {lit.,[5] 298—299°, $[\alpha]_D^{23}$ +13·8 ($CHCl_3$)}. Taraxerol (10 mg) was oxidised with chromium trioxide (10 mg) in pyridine (2 ml) at room temperature for 24 h. The mixture was diluted with water and extracted with ether. The extract was washed well with water, dried, and ether was removed. The product was crystallised from benzene and gave an undepressed mixed m.p. with the isolated taraxerone.

Isolation of calabaxanthone. The oil (15 g) was chromatographed on silica gel (600 g). Elution with benzene gave *calabaxanthone* (*ca.* 200 mg), yellow needles, m.p. 172° (from EtOH), R_F 0·8(benzene) [Found: C, 73·4; H, 6·2%; *M*(mass spectrometry), 392·16244. $C_{24}H_{24}O_5$ requires C, 73·5; H, 6·3%; *M*, 392·16236]. $\lambda_{max.}$ (ethanol) 240 (ε 34,100), 287(77,250), 292sh(72,210), 314(28,830), and 384(8420) nm, $\nu_{max.}$ (Nujol) 2930, 1648, 1604, 1582, 1313, 1288, 1254, 1230, 1168, 1140, 1113, 1082, 1018, 955, 890, 838, 820, 773, and 717 cm^{-1}, τ ($CDCl_3$, 100 MHz) −3·70(1H, s, 5-OH), 2·75(2H, s, 9- and 10-H), 3·25(1H, d, *J* 9·6 Hz, 4-H), 4·34(1H, d, *J* 9·6 Hz, 3-H), 3·75(1H, s, 12-H), 4·76(1H, t, =CH), 5·85(2H, d, CH_2), 6·12(3H, s, 8-OMe), 8·15 and 8·34(6H, s, $=CMe_2$), and 8·54(6H, s, $>CMe_2$). The u.v. absorption was unaltered in the presence of $AlCl_3$, NaOAc, or NaOH. The material was insoluble in sodium hydroxide. It gave a negative $FeCl_3$ test and positive Gibbs test.

Calabaxanthone monomethyl ether. Calabaxanthone (100 mg) was refluxed with dimethyl sulphate (2 ml), calcinated potassium carbonate (2 g), and dry acetone (100 ml) for 16 h and usual work-up gave *calabaxanthone monomethyl ether* which crystallised from acetone as needles, m.p. 116—119°, R_F 0·32(benzene) [Found: C, 73·9; H, 6·8%; *M*(mass spectrometry), 406. $C_{25}H_{26}O_5$ requires C, 73·9; H, 6·4%; *M*, 406], $\nu_{max.}$ (Nujol) 2942, 1647, and 1603 cm^{-1}, τ ($CDCl_3$, 60 MHz) 2·81(2H, s, 9- and 10-H), 3·26(1H, d, *J* 9·6 Hz, 4-H), 3·49(1H, s, 12-H), 6·18 and 6·10(6H, s, 5- and 8-OMe), 4·42(1H, t, =CH), 4·33(1H, d, *J* 9·6 Hz, 3-H), 5·88(2H, d, CH_2), 8·11 and 8·35(6H, s, $=CMe_2$), and 8·56(6H, s, 2,2-CMe_2).

Calabaxanthone acetate. Calabaxanthone (50 mg) was refluxed with acetic anhydride (1 ml) and pyridine (1 ml) for 16 h and usual work-up gave *calabaxanthone acetate*, colourless needles m.p. 147—148° (from MeOH), R_F 0·37 (benzene), τ ($CDCl_3$, 100 MHz) 2·75(1H, s, 9- and 10-H), 3·31(1H, s, 12-H), 3·46(1H, d, *J* 11 Hz, 4-H), 4·24(1H, d, *J* 11 Hz, 3-H), 4·72(1H, t, =CH), 5·89(2H, d, CH_2), 6·18(3H, s, 8-OMe), 7·50(3H, s, 5-OAc), 8·15 and 8·32(6H, s, $=CMe_2$), 8·52(6H, s, $>CMe_2$) (Found: C, 71·6; H, 5·9. $C_{26}H_{26}O_6$ requires C, 71·9; H, 6·0%).

Tetrahydrocalabaxanthone. Calabaxanthone (100 mg) in absolute alcohol (100 ml) was hydrogenated using pre-reduced palladised charcoal as catalyst, at atmospheric pressure and room temperature. Filtration and removal of solvent gave *tetrahydrocalabaxanthone* as a yellow solid which crystallised from EtOH as yellow needles, m.p. 152°, R_F 0·8(benzene), $\lambda_{max.}$ (ethanol) 242(ε 63,460), 262(64,800), 316(36,400), and 363(9110) nm, $\nu_{max.}$ (KBr) 3377, 2902, 1642, 1603, and 1579 cm^{-1} τ ($CDCl_3$, 100 MHz) −4·15(1H, s, 5-OH), 2·82(2H, s, 9- and 10-H), 3·80(1H, s, 12-H), 6·16(3H, s, 8-OMe), 6·60(2H, t, $ArCH_2$), 7·29(2H, t, chroman CH_2), 8·18(2H, t, chroman CH_2), 8·30—8·60(3H, c, CH_2CHMe_2), 8·64(6H, s, $>CMe_2$), and 9·01(6H, d, $CHMe_2$) [Found: *M*(mass spectrometry), 396. $C_{24}H_{28}O_5$ requires *M*, 396].

Isolation of unidentified triterpene. Further elution with benzene gave more taraxerone (15 mg), taraxerol (100 mg) and another solid which gave crystals from chloroform–acetone, m.p. 195°, R_F 0·35(benzene), $[\alpha]_D^{20}$ +48·9° ($CHCl_3$) [Found: C, 84·3; H, 11·7%; *M* (mass spectrometry), 426. $C_{30}H_{50}O$ requires C, 84·6; H, 11·8%; *M*, 426], $\nu_{max.}$ (KBr) 3470, 2940, 1650, 1382, 1362, 1238, 1207, 1143, 1183, 1122, 1094, 1046, 974, and 803 cm^{-1}.

Extractives from Timber of C. Calaba *L.*—Cold extraction of the timber (4·1 kg) with light petroleum (b.p. 40—60°) (10 l) for 7 days gave on filtration crude material (254 mg) which was dissolved in ether and washed with sodium hydroxide to give crystals (100 mg), m.p. 136—137°. It was shown by g.l.c. to consist of β-sitosterol and stigmasterol, the former being the major constituent.

Isolation of guanandin. The sodium hydroxide washings were acidified (HCl), extracted with ether, the extracts were washed with water, dried and ether removed to give guanandin (30 mg) as pale yellow plates m.p. 205—208° (lit.,[16] 206—208°), R_F 0·83 [$CHCl_3$–MeOH (40 : 1)] identical with authentic sample (i.r., mixed m.p., and t.l.c.). The filtrate of the light petroleum extracts gave a waxy solid (3·0 g) which was not investigated further.

Hot extraction with benzene (10 l) for 7 days in a Soxhlet extractor gave a dark brown solid (17·5 g) from which waxes were removed with light petroleum. The solid was then separated into a cold benzene-soluble fraction and a residue. The cold benzene solution on concentration gave a yellow solid (1·5 g) which was filtered.

Isolation of 1,6-*dihydroxy*-5-*methoxyxanthone.* The solid was separated on silica gel (50 g) and on elution with benzene gave 1,6-dihydroxy-5-methoxyxanthone (420 mg), yellow needles, m.p. 242—245° (from acetone) (lit.,[15] 243—246°), R_F 0·35 [$CHCl_3$–MeOH(40 : 1)] (Found: C, 65·0; H, 3·7. Calc. for $C_{14}H_{10}O_5$: C, 65·1; H, 3·9%) [identical with an authentic sample (mixed m.p. and t.l.c.)]. Methylation with diazomethane gave 1-hydroxy-5,6-di-

methoxyxanthone, yellow needles, m.p. 180° (lit.,[33] 183—185°), R_F 0·88 [$CHCl_3$–MeOH(40 : 1)]. Methylation by refluxing with dimethyl sulphate and potassium carbonate in acetone gave 1,5,6-trimethoxyxanthone, m.p. 148—150° (lit.,[34] 150—151°), R_F 0·80 [$CHCl_3$–MeOH(40 : 1)] (Found: C, 67·3; H, 5·1. Calc. for $C_{16}H_{14}O_5$: C, 67·1; H, 4·9%).

Isolation of scriblitifolic acid. The cold benzene-soluble fraction (14 g) was dissolved in ether and washed with aqueous 10% sodium carbonate and 10% sodium hydroxide solutions. The ether layer was concentrated to give a tar (3 g) which was not further investigated. Acidification of the sodium carbonate solution with hydrochloric acid followed by the usual work-up gave material (5·2 g) which was chromatographed on silica gel (250 g). Elution with benzene gave more 1,6-dihydroxy-5-methoxyxanthone (230 mg). Elution with chloroform–benzene (1 : 1) gave scriblitifolic acid (500 mg), yellow needles; m.p. 164—167° (from benzene) (lit.,[20] 164—167°), R_F 0·25 [$CHCl_3$–MeOH(40 : 1)] identified by comparison with authentic sample (mixed m.p. and t.l.c.)].

Isolation of 2-hydroxy-1,8-dimethoxyxanthone. Elution with chloroform gave *2-hydroxy-1,8-dimethoxyxanthone* (210 mg), orange plates, m.p. 182—183° (from benzene), R_F 0·35 [$CHCl_3$–MeOH(40 : 1)] (Found: C, 66·2; H, 4·5. $C_{15}H_{12}O_5$ requires C, 66·2; H, 4·4%), $\lambda_{max.}$ 242(ε, 30,300), 257(31,200), 285(5700), and 315(5200) nm, $\nu_{max.}$ (Nujol) 3221, 1646, and 1603 cm^{-1}, τ [$(CD_3)_2SO$, 60 MHz] 1·58(1H, s, 2-OH), 2·31(1H, t, *J* 8·4 Hz, 6-H), 2·68(1H, d, *J* 8·4 Hz, 3-H), 2·80(1H, d, *J* 8·4 Hz, 4-H), 2·98(1H, d, *J* 8·4 Hz, 7-H), 3·08(1H, d, *J* 8·4 Hz, 5-H), and 6·15(6H, s, 1- and 8-OMe), *m/e* 272(M^+, 80%), 254(49), 229(53), 198(34), and 188(38).

2-Hydroxy-1,8-dimethoxyxanthone (100 mg) in methanol (20 ml) with ethereal diazomethane gave 1,2,8-trimethoxyxanthone (80 mg) which crystallised from cyclohexane–acetone as cubes, m.p. 150—152° (lit.,[18] 153—155°), R_F 0·73 (Found: C, 67·2; H, 5·0. Calc. for $C_{16}H_{14}O_5$: C, 67·1; H, 4·9%) [identical with 1,2,8-trimethoxyxanthone from 2,8-dihydroxy-1-methoxyxanthone (i.r. and mixed m.p.)].

Isolation of 6-deoxyjacareubin. Acidification of the sodium hydroxide washings with 10% hydrochloric acid followed by the usual work-up gave material (5·0 g) which was chromatographed on silica gel (250 g). Elution with benzene gave more guanandin (25 mg) and 6-deoxyjacareubin (35 mg). The latter was recrystallised from benzene and gave orange plates, m.p. 203—206° (lit.,[16] 211—213°), identified by comparison with an authentic sample (i.r. spectrum).

Isolation of 2,8-dihydroxy-1-methoxyxanthone. Elution with benzene–chloroform (9 : 1) gave 2,8-dihydroxy-1-methoxyxanthone (165 mg), yellow needles, m.p. 197—199° (from ethanol) (lit.,[18] 197—199°), R_F 0·73 [$CHCl_3$–MeOH(40 : 1)] (Found: C, 65·25; H, 3·8. Calc. for $C_{14}H_{10}O_5$: C, 65·1; H, 3·9%).

2,8-Dihydroxy-1-methoxyxanthone (100 mg) in methanol (20 ml) was treated with ethereal diazomethane and usual work-up gave yellow needles of 8-hydroxy-1,2-dimethoxyxanthone, m.p. 170—171° (lit.,[18] 173—174°) (Found: C, 66·30; H, 4·5. Calc. for $C_{15}H_{12}O_5$: C, 66·2; H, 4·45%).

2,8-Dihydroxy-1-methoxyxanthone (100 mg), potassium carbonate (2 g), and dimethyl sulphate (2 ml) in dry acetone (50 ml) were refluxed for 16 h and 1,2,8-trimethoxyxanthone (60 mg) was isolated and crystallised from cyclohexane–acetone as cubes, m.p. 150—152°.

Isolation of 1,7-dihydroxyxanthone (euxanthone). Elution with chloroform–benzene (9 : 1) gave euxanthone (131 mg) which was recrystallised from toluene to give yellow needles, m.p. 239—240° (lit.,[17] 239°), undepressed by admixture with an authentic sample.

Further elution with chloroform–benzene (9 : 1) gave an amorphous pigment (100 mg), m.p. 248—251°. It was insoluble in most organic solvents. An acetate was prepared with acetic anhydride–pyridine, m.p. 178—181°.

Isolation of jacareubin. Extraction of the timber with hot chloroform (10 l) for 7 days gave a brown solid (25 g) which was separated into a cold chloroform-soluble fraction and a residue. The residue (10·5 g) was separated on silica gel (200 g). Elution with chloroform–methanol (99 : 1) gave jacareubin, which was recrystallised from ethanol–water to give yellow needles, m.p. 254—256° (lit.,[35] 254—256°), identified by comparison with an authentic sample (mixed m.p. and i.r.).

Isolation of 1,5,6-trihydroxyxanthone. Elution with chloroform–methanol (98 : 2) gave 1,5,6-trihydroxyxanthone, which was recrystallised from ethyl acetate–chloroform as needles, m.p. 285—286° (lit.,[36] 287—288°), identified by comparison with an authentic sample (mixed m.p. and i.r.). It gave 1,5,6-trimethoxyxanthone with dimethyl sulphate and potassium carbonate in acetone, cream needles, m.p. 148—150° (lit.,[34] 150—151°).

Extractives from Bark of C. bracteatum *Thw.—Isolation of calophyllolide.* Cold extraction of the bark (2·7 kg) with light petroleum (b.p. 60—80°) (10 l) for 3 days gave a gum which was crystallised from methanol to give calophyllolide (0·617 g), m.p. 152—154° (lit.,[20] 152—154°) (Found: C, 75·1; H, 5·9. Calc. for $C_{26}H_{24}O_5$: C, 75·0; H, 5·8%). I.r. and n.m.r. spectra were identical with the recorded values and an authentic sample gave an undepressed mixed m.p.

Isolation of calabaxanthone, taraxerone, and taraxerol. Further extraction of the bark powder for 7 days with cold light petroleum (b.p. 60—80°) yielded a gum from which a yellowish solid (0·485 g) crystallised out. Evaporation of the mother liquor afforded an oil (16 g). The yellowish solid was chromatographed on silica gel (30 g). Elution with benzene gave calabaxanthone (47 mg), yellow needles, m.p. 172° (from ethanol); taraxerone, m.p. 239—240° (lit.,[5] 240—241°); and then taraxerol, m.p. 279—280° (lit.,[4] 279—282°). All three were identified by comparison with authentic samples (mixed m.p., t.l.c., and i.r. spectra).

Isolation of β-sitosterol. The oil (16 g) in ether was washed with 10% sodium carbonate and then with 10% sodium hydroxide and the neutral oil (12 g) was obtained on evaporation of the ether. The oil was separated on silica gel (300 g). Elution with benzene–light petroleum gave an oil (not investigated). Further elution with benzene gave a triterpene, m.p. 195°. It was shown to be identical with a triterpene from *C. calaba* L. (mixed m.p., t.l.c., and i.r. spectra). Elution with chloroform gave β-sitosterol, m.p. 136—137°.

Extractives from Timber of C. bracteatum *Thw.*—Cold extraction of timber (9·7 kg) with light petroleum (b.p. 60—80°) (10 l) for 7 days gave a crystalline solid (100 mg),

[33] B. Jackson, H. D. Locksley, and F. Scheinmann, *J. Chem. Soc.* (*C*), 1968, 2579.
[34] T. R. Govindachari, B. R. Pai, P. S. Subramaniam, U. R. Rao, and N. Muthukumaraswamy, *Tetrahedron*, 1967, **23**, 243.
[35] F. E. King, J. T. King, and L. C. Manning, *J. Chem. Soc.*, 1953, 3932.
[36] H. D. Locksley, I. Moore, and F. Scheinmann, *J. Chem. Soc.* (*C*), 1966, 430.

m.p. 136—137°, shown by g.l.c. to consist of β-sitosterol and stigmasterol, the former being the major constituent. The filtrate on evaporation gave waxy material (3 g) which was separated on silica gel (150 g). Elution with chloroform gave a waxy solid (31 mg), m.p. 78°, R_F 0·55 ($CHCl_3$), $\nu_{max.}$ (KBr) 2870, 1451, 1058, 753, and 731 cm^{-1}, *M*(mass spectrometry), 309.

The dried timber powder was extracted in a Soxhlet apparatus with hot chloroform for 7 days to give a dark gummy solid (40 g). On extraction of this with cold light petroleum (b.p. 60—80°) it gave a waxy material (10 g) which on t.l.c. showed the presence of β-sitosterol. The hot benzene-soluble fraction of the chloroform extract gave fraction (*a*), a yellow solid (0·850 g), on concentration, fraction (*b*), a gummy solid (23 g), on evaporation of the extract, and fraction (*c*), an insoluble residue (6 g).

Isolation of 1,3,5-*trihydroxy*-2-*methoxyxanthone*. Fraction (*a*) was separated on acid-washed silica gel by elution with chloroform–ethanol (24 : 1) to give 1,3,5-*trihydroxy*-2-*methoxyxanthone* (0·221 g), yellow needles, m.p. 252° (from ethanol–water), R_F 0·5 [$CHCl_3$–HOAc(92 : 8)] [Found: C, 61·2; H, 3·6%; *M*(mass spectrometry), 274. $C_{14}H_{10}O_6$ requires C, 61·3; H, 3·65%; *M*, 274], $\lambda_{max.}$ (ethanol) 223(ε 8270), 240(8560), 262sh(7580), 315(7580), and 365(3790) nm, $\lambda_{max.}$ (EtOH with $AlCl_3$) 243(ε 5280), 265(6000), 275sh(4450), and 334(4800) nm, $\lambda_{max.}$ (EtOH with NaOAc) 222(ε 7600), 240(3500), and 350(3600) nm, no alteration was observed in the presence of NaOAc–H_3BO_3, $\nu_{max.}$ (Nujol) 3275, 2930, 1657, and 1616 cm^{-1}, τ [$(CD_3)_2CO$, 100 MHz] −3·06(1H, s, 1-OH), 2·35(1H, q, 8-H), 2·74(2H, m, 6- and 7-H), 3·49(1H, s, 4-H), 6·12(3H, s, 2-OMe).

Methylation of 1,3,5-*trihydroxy*-2-*methoxyxanthone*. (a) (i) 1,3,5-Trihydroxy-2-methoxyxanthone (100 mg) in MeOH (50 ml) with diazomethane in ether gave 1-hydroxy-2,3,5-trimethoxyxanthone (90 mg) which crystallised from acetone as needles, m.p. 184° (lit.,[23] 189—190°). (ii) 1-Hydroxy-2,3,5-trimethoxyxanthone (90 mg) on methylation with dimethyl sulphate (2 ml) and calcined potassium carbonate (2 g) in acetone (50 ml) for 6 h and usual work-up gave 1,2,3,5-tetramethoxyxanthone, needles, m.p. 145—146° (from ethanol) (lit.,[23] 146—147°) [identical with authentic sample (i.r. and n.m.r. spectra)].

(b) 1,3,5-Trihydroxy-2-methoxyxanthone (100 mg) on methylation with dimethyl sulphate (2 ml) and calcined potassium carbonate (2 g) in acetone (50 ml) for 6 h and usual work-up gave 1,2,3,5-tetramethoxyxanthone, m.p. 145—146°.

The gummy solid (23 g) obtained in fraction (*b*) was dissolved in ether and washed with 10% sodium carbonate. During this process an insoluble sodium salt precipitated out and was filtered. The precipitate was acidified with dilute HCl and extracted with ether. The two extracts were combined, washed with water, dried ($MgSO_4$), and ether was removed to give a brown solid (12 g), fraction (*d*).

The ether solution of fraction (*b*) was then washed with 10% sodium hydroxide, the aqueous solution was acidified (4% HCl), extracted with ether, the extracts were washed with water, dried ($MgSO_4$), and ether was removed to give a residue (4 g), fraction (*e*).

Separation of Fraction (e).—*Isolation of* 6-*deoxyjacareubin*. The fraction (4 g) was separated on acid-washed silica gel (200 g). On elution with benzene, the product obtained was 6-deoxyjacareubin (50 mg), m.p. 206—208° (lit.,[16] 211—213°), R_F 0·45 [$CHCl_3$–MeOH(40 : 1)]. It was characterised by comparison with an authentic sample (mixed m.p., i.r., and n.m.r. spectra).

Isolation of 1,7-*dihydroxyxanthone*(*euxanthone*). Further elution with chloroform–benzene (1 : 1) gave euxanthone (10 mg), recrystallised from toluene to give yellow needles, m.p. 237—238° (lit.,[17] 239°). It was characterised by comparison with an authentic sample (mixed m.p. and i.r. spectrum). On further elution a brown oil was obtained.

Separation of Fraction (d).—The fraction (12 g) was separated on acid-washed silica gel (360 g) into four fractions. Fraction (i), obtained by elution with chloroform, gave a crystalline solid (35 mg) which was not further investigated. Fraction (ii) was obtained by further elution with chloroform.

Isolation of Betulinic Acid. Fraction (iii).—This was obtained by elution with chloroform–methanol (99 : 1) and gave betulinic acid (0·220 g), needles, m.p. 310—314° (from methanol), $[\alpha]_D^{28}$ +30° (*c* 1·5, $CHCl_3$) {lit.,[37] 316—318°, $[\alpha]_D^{22}$ +7·89(pyridine)} [Found: C, 78·8; H, 10·5%; *M*(mass spectrometry), 456. Calc. for $C_{30}H_{48}O_3$: C, 78·9; H, 10·5%; *M*, 456]. An authentic sample gave an undepressed mixed m.p. and had an identical i.r. spectrum. Betulinic acid (100 mg) gave, with acetic anhydride–pyridine (1 : 1) (2 ml) at room temperature, an acetate, m.p. 265—266°. The acetate (70 mg) in methanol (50 ml) was converted to the methyl ester with ethereal diazomethane. The methyl ester-acetate crystallised from methanol as needles, m.p. 199—200°, $[\alpha]_D^{28}$ +21° (*c* 1·7, $CHCl_3$) {lit.,[38] 201—202°, $[\alpha]_D^{22}$ +17° ($CHCl_3$)}.

Fraction (iv), obtained by further elution, gave 1,3,5-trihydroxy-2-methoxyxanthone (50 mg).

Isolation of 1,5-*dihydroxyxanthone*. Fraction (ii) was a solid (0·350 g) and it was separated on silica gel (25 g). On elution with benzene–chloroform (19 : 1) 1,5-dihydroxyxanthone (60 mg) was obtained, m.p. 262—265° (lit.,[33] 266—267°), R_F 0·75 [$CHCl_3$–MeOH (40 : 1)]. An authentic sample gave an undepressed m.p. and identical i.r. spectrum.

Isolation of 1,8-*dihydroxy*-2,3,7-*trimethoxyxanthone*. Further elution with benzene–chloroform (1 : 1) gave 1,8-*dihydroxy*-2,3,8-*trimethoxyxanthone* (56 mg), yellow needles, m.p. 195—198° (from ethanol), R_F 0·78 [$CHCl_3$–HOAc (92 : 8)] [Found: C, 60·1; H, 4·4%; *M*(mass spectrometry), 318. $C_{16}H_{14}O_7$ requires C, 60·3; H, 4·4%; *M*, 318], $\nu_{max.}$ (Nujol) 3362, 2918, 1656, 1580, 1374, 1306, 1203, 1142, 1101, 1058, 1004, 964, 814, 790, 757, and 722 cm^{-1}, $\lambda_{max.}$ (EtOH) 242(ε 12,390), 264(12,290), 307(9830), and 380(3720) nm, $\lambda_{max.}$ (EtOH with $AlCl_3$) 238(ε 11,470), 270(9670), 277(10,030), and 324(7170) nm, no alteration was observed on addition of NaOAc or H_3BO_3–NaOAc, $\lambda_{max.}$ (EtOH with NaOH) 248(ε 10,930), 282(13,260), 310sh(3580), and 420(2500) nm, τ [$(CD_3)_2SO$, 60 MHz] −3·2(1H, s, 1-OH), −0·3br(1H, s, 8-OH), 2·55(1H, d, *J* 9 Hz, 6-H), 2·85(1H, d, *J* 9 Hz, 5-H), 3·37(1H, s, 4-H), and 6·07, 6·15, and 6·25(9H, s, 2-, 3-, and 7-OMe).

1,8-Dihydroxy-2,3,7-trimethoxyxanthone (20 mg) and acetic anhydride–pyridine 1 : 1 (2 ml) were left overnight at room temperature and poured into ice–water. The precipitated solid was washed with dilute HCl and water and dried to give needles of 1,8-*diacetoxy*-2,3,7-*trimethoxyxanthone*, m.p. 150—152° (from ethanol), R_F 0·4 ($CHCl_3$), τ ($CDCl_3$, 100 MHz), 2·68(1H, d, *J* 9 Hz, 6-H), 2·87(1H, d, *J* 9 Hz, 5-H), 3·23(1H, s, 4-H), 6·03, 6·10, and 6·16(9H, s, 2-, 3-, and 7-OMe), and 7·49 and 7·66(6H, s, 1- and 8-OAc).

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Separation of Fraction (c).—*Isolation of jacareubin.* The residue (5 g) was separated on acid-washed silica gel (300 g). On elution with chloroform–methanol (99 : 1) jacareubin (20 mg) was obtained, m.p. 252—256° (lit.,[35] 254—256°) [identical with an authentic sample (mixed m.p., i.r., and n.m.r. spectra)].

Further elution with chloroform–methanol (97 : 3) gave a light brown solid, m.p. 280° (decomp.) (not characterised).

With chloroform–methanol (19 : 1) a steroid, m.p. 260°, was obtained. It gave a positive Libermann–Burchardt test, $\nu_{max.}$ (Nujol) 3419, 2924, 1374, 1167, 1107, 1076, 1019, 800, and 725 cm^{-1}. It gave an acetate, m.p. 160—162°, $[\alpha]_D^{27}$ −6·3° (*c* 1·2, $CHCl_3$).

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