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XANTHONES FROM THE HEARTWOOD OF *OCHROCARPOS ODORATUS**

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Abstract—The heartwood of *Ochrocarpos odoratus* (Rafin) Merrill contains seven xanthones; 2-hydroxyxanthone, 3-hydroxy-2-methoxyxanthone, 1,5-dihydroxyxanthone, 2,3,4-trihydroxyxanthone, 1,5,6,-trihydroxyxanthone, and 1,3,5,6- and 1,3,6,7-tetrahydroxyxanthones.

IN CONTINUANCE of our work on extractives from plants of the Guttiferae we have examined the heartwood of *Ochrocarpos odoratus* (subfamily Calophylloideae) obtained from Fiji. Shavings of the heartwood were extracted first with hot $CHCl_3$ and then with hot Me_2CO. Removal of the solvents under reduced pressure gave two solids both of which imparted a green coloration to methanolic ferric chloride solution indicating the presence of phenols.

Column chromatographic purification of the $CHCl_3$ extract gave two fractions the first of which deposited pure 1,5-dihydroxyxanthone (I) identical with an authentic sample.[1] The second fraction, after further purification by chromatography, afforded the new metabolite 3-hydroxy-2-methoxyxanthone (II). This structure was assigned on the basis of spectral information: the UV (Table 1) and IR spectra (see Experimental) were consistent with those of an oxygenated xanthone nucleus and elemental analysis and low resolution mass spectrometry (M^+ at m/e 242) indicated a molecular formula of $C_{14}H_{10}O_4$. The presence of a large $(M - 15)^+$ peak in the mass spectrum[1b] was consistent with the loss of a

TABLE 1. UV SPECTRA OF SOME OXYGENATED XANTHONES IN METHANOL

Xanthone	λ_{max} (nm) (ϵ)				
2,3,4-Trihydroxyxanthone[7] (VIII)	231			317	376
4-Hydroxy-2,3-dimethoxyxanthone[7]	237 (23,400)	256 (32,200)	291 (8600)	308 (9500)	
3,4-Dihydroxy-2-methoxyxanthone[7]	239 (27,400)	255 (26,700)	285 (5600)	333 (12,400)	
2,3,4-Trimethoxyxanthone[7] (VII)	246 (33,100)		278 (9800)	304 (11,200)	
3-Hydroxy-2-methoxyxanthone (II)	240 (32,500)		273 (7100)	309 (11,500)	349 (10,000)
2,3-Dimethoxyxanthone (IX)	242 (32,800)		272 (7900)	305 (11,500)	346 (8400)
2-Hydroxyxanthone (IV)	237 (28,600)	247* (24,200)	299 (3000)		357 (4600)
4-Hydroxyxanthone[5b] (V)	235	250	282	290	353

* Shoulder.

* Part XXIV in the series "Extractives from Guttiferae". For Part XXIII see H. D. LOCKSLEY, J. QUILLINAN and F. SCHEINMANN, *J. Chem. Soc.* C, 1971, in press.

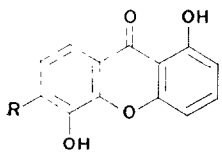
[1] (a) H. D. LOCKSLEY and I. G. MURRAY, *J. Chem. Soc.* C, 392 (1970); (b) B. JACKSON, H. D. LOCKSLEY I. MOORE and F. SCHEINMANN, *J. Chem. Soc.* C, 2579 (1968).

TABLE 2. ^{1}H NMR SPECTRAL RESULTS FOR SOME OXYGENATED XANTHONES

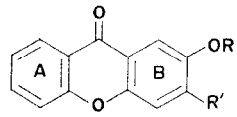
Xanthone	Solvent	Ring position C-1	C-2	C-3	C-4	C-5, C-6, C-7	C-8
3-Hydroxy-2-methoxyxanthone (II)	a	1·98s	OMe 5·76s	OH*	2·50s	1·48–2·28m	1·28br d, J = 7·0 Hz
2-Hydroxyxanthone (IV)	a		OH*	1·76–2·57m			1·48br d, J = 7·0 Hz
2,3,4-Trimethoxyxanthone (VII)	b	2·42s	3 × OMe 5·88s, 5·90s, 6·00s			2·14–2·65m	1·57br d, J = 7·5 Hz
4-Hydroxy-2,3-dimethoxyxanthone[7]	a	2·82s	OMe 6·03s	OMe 5·81s	OH*	1·95–2·75 m	1·80oct
2,3-Dimethoxyxanthone (IX)	b	2·28s	OMe 5·96s	OMe 5·96s	3·05s	2·10–2·65m	1·58br d, J = 7·5 Hz

Chemical shift values are in τ (ppm) using tetramethylsilane as internal reference.
Solvents: a = CF_3CO_2D; b = $CDCl_3$. * = Signals not observed in CF_3CO_2D.
Multiplicities: s = singlet; br d = broad doublet; m = multiplet; oct = octet.

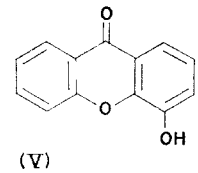
Me radical from the molecular ion. Since the metabolite possessed phenolic properties (green colour with $FeCl_3$) the fourth oxygen was assumed to be present as an OH group. The disposition of these two substituents was made clear from an examination of the NMR spectrum in CF_3CO_2D (Table 2).



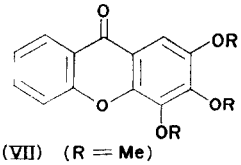
(I) (R = H)
(VI) (R = OH)



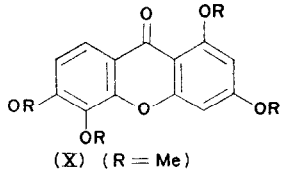
(II) (R = Me, R' = OH)
(III) (R = H, R' = OMe)
(IV) (R = R' = H)
(IX) (R = Me, R' = OMe)



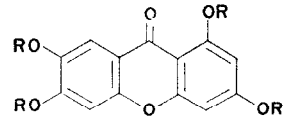
(V)



(VII) (R = Me)
(VIII) (R = H)



(X) (R = Me)
(XII) (R = H)



(XI) (R = Me)
(XIII) (R = H)

A broad doublet observed at low field (τ 1·28) was indicative of an aromatic proton located *ortho* to the xanthone carbonyl function. This signal together with a complex group at τ 1·48–2·28 were similar to those observed for xanthone itself at 60 MHz[2] and were therefore assigned to the four protons of an unsubstituted ring [ring A in (II)] of the xanthone nucleus. Two more signals (τ 1·98 and 2·50), without detectable coupling, were allocated to

[2] D BARRACLOUGH, O. R. GOTTLIEB, H. D. LOCKSLEY, M TAVEIRA MAGALHÃES and F. SCHEINMANN, *J Chem. Soc.* **B**, 603 (1970).

the C-1 and C-4 protons of ring B, respectively,[2] the remaining C-2 and C-3 positions being occupied by the hydroxy- and methoxy- (τ 5·76) substituents. On this analysis two structures (II) and (III) for the metabolite are possible but the latter was eliminated with the observation that the UV spectrum underwent a considerable bathochromic shift on addition of sodium acetate, a result which is compatible only with a *para* disposition of hydroxy and carbonyl functions.[1b,3] Thus the metabolite is reliably formulated as 3-hydroxy-2-methoxyxanthone (II) and this was confirmed by direct comparison with an authentic sample of synthetic origin which became available later.[4]

When filtrates from the trituration of the above fraction were purified by preparative TLC, a yellow solid was obtained. The UV spectrum of this metabolite (Table 1) in MeOH, which showed no change on addition of NaOAc (see Experimental section), the NMR spectrum (Table 2), and the mass spectrum (M^+ at *m/e* 212) together suggested that the structure was either 2- or 4-hydroxyxanthone, (IV) or (V). Direct comparison with an authentic sample[5] showed it was 2-hydroxyxanthone (IV).

The acetone soluble extract of the heartwood was chromatographed over silica gel and two fractions were collected. The first afforded a yellow solid which was shown to be 1,5,6-trihydroxyxanthone (VI) by direct comparison with the authentic compound (from *Calophyllum fragrans* Ridley, Guttiferae).[6] The second fraction contained several components which were only partially resolved by column and preparative TLC, so the mixture was completely methylated with dimethyl sulphate. Four bands, (i)–(iv), were collected following preparative TLC of the reaction mixture.

Band (i) furnished 2,3,4-trimethoxyxanthone (VII) having spectral characteristics (see Tables 1 and 2) in accord with those of the authentic compound[4,7] with which it was identical. The natural product prior to methylation was identified, however, as 2,3,4-trihydroxyxanthone (VIII) by direct TLC comparison of the acetone extract against this standard,[4,7] there being no trace of the trimethyl ether (VII) present. The presence of partially methylated 2,3,4-trioxygenated xanthones could not be investigated since these compounds were not available for comparative TLC. Xanthones with this oxygenation pattern occur in Brazilian species of *Kielmeyera* (Guttiferae)[7,8] but as yet have not been found in other genera of this family or in those of any other.

Band (ii) afforded 2,3-dimethoxyxanthone (IX) which was identical with the product of methylation of 3-hydroxy-2-methoxyxanthone (II). Since the dimethyl ether (IX) was absent (TLC comparison) from the original acetone extract it was assumed to have arisen from (II) during methylation.

Work up of bands (iii) and (iv) gave 1,3,5,6- and 1,3,6,7-tetramethoxyxanthones (X) and (XI), respectively, identical with authentic specimens.[9] TLC comparison of the unpurified acetone extract against authentic samples of 1,3,5,6- and 1,3,6,7-tetrahydroxyxanthones[9] (XII) and (XIII), respectively, showed that these were present as metabolites in the heartwood, the ethers having arisen through methylation. Partially methylated derivatives of the

[3] B. Jackson, H. D. Locksley and F. Scheinmann, *J. Chem. Soc.* C, 785 (1967).
[4] J. Quillinan and F. Scheinmann, unpublished results.
[5] (a) F. Lamb, Ph.D. Thesis, University of London, 1957; (b) R. A. Finnegan and P. L. Bachmann, *J. Pharm. Sci.* **54**, 633 (1965).
[6] H. D. Locksley and I. G. Murray, *J. Chem. Soc.* C, 1567 (1969).
[7] O. R. Gottlieb, M. Taveira Magalhães, M. Camey, A. A. Lins Mesquita and D. de Barros Correa, *Tetrahedron* **22**, 1777 (1966).
[8] I. Carpenter, H. D. Locksley and F. Scheinmann, *Phytochem.* **8**, 2013 (1969).
[9] H. D. Locksley, I. Moore and F. Scheinmann, *Tetrahedron* **23**, 2229 (1967).

two tetra-oxygenated xanthones (XII) and (XIII) appeared to be absent but we lacked the authentic samples necessary to confirm this.

Theory[8] predicts that there should be a maximum of 15 "standard" oxygenation patterns for xanthones of higher plants arising from a mixed acetate-shikimate biosynthesis. The isolation of a 2,3-dioxygenated xanthone (II)* now increases to 14 the number of such known 'standard' xanthones.

EXPERIMENTAL

UV spectra (in MeOH) were measured with a Unicam SP800 recording spectrophotometer and IR spectra (Nujol mulls) with either Unicam SP200 or Perkin–Elmer 257 grating instruments. ^{1}H NMR spectra were determined with a Varian Associates A60 instrument using tetramethylsilane as internal reference. Mass spectra were recorded with an A.E.I. MS12 (single focusing) spectrometer: high resolution spectral results were obtained using an A.E.I. MS9 (double focusing) instrument.

Analytical and preparative TLC were carried out with silica gel (Merck Kieselgel G); column chromatography was performed with Hopkin and Williams silica gel MFC

Extraction of the Heartwood of Ochrocarpos odoratus *(Rafin) Merrill*

A trunk section of the above tree from Fiji was supplied by The Tropical Products Institute, London. The outside portion of the heartwood was yellow in appearance whilst the core was red and therefore shavings of both parts were examined separately. However, careful comparison of the extracts obtained from each portion of the heartwood by analytical TLC indicated that the same constituents were present.

Some of the core shavings (1·75 kg) were extracted in a Soxhlet apparatus with hot $CHCl_3$ for 48 hr, and subsequently with hot Me_2CO for 48 hr. An orange solid (31 g) separated from the $CHCl_3$ solution during extraction and this was filtered off. Analytical TLC showed that its composition was the same as that of the mother liquors which, after removal of the solvent, gave a green solid (45·6 g) (4·4% w/w from total $CHCl_3$ extract). The Me_2CO extract, on work up, furnished a red solid (4·1% w/w)

Examination of the Chloroform Extract

The solid (40 g) in Me_2CO was absorbed onto silica gel (100 g) and the dried material chromatographed over a column of silica gel (1·25 kg) prepared with $CHCl_3$. Elution with $CHCl_3$ containing increasing quantities of EtOAc (1–10% v/v) gave several fractions (200 ml) which were combined, on the basis of the constituents revealed by accompanying TLC, to give two fractions.

(a) *Isolation of* 1,5-*dihydroxyxanthone* (I). Fraction 1 (eluted with EtOAc–$CHCl_3$, 2:23) deposited yellow crystals after standing overnight. Filtration afforded pure 1,5-dihydroxyxanthone (I) as bright yellow plates (725 mg), R_f 0·6 (EtOAc–$CHCl_3$, 1:9), m.p. 267–269° (lit.,[1b] 266–267°), identical with an authentic specimen (IR spectrum and mixed m.p.)

(b) *Isolation of* 3-*hydroxy*-2-*methoxyxanthone* (II). Fraction 2 (eluted with EtOAc–$CHCl_3$, 1:9) was evaporated to give a yellow solid (9·7 g) which was dissolved in hot Me_2CO–MeOH (1:1, v/v) and adsorbed onto silica (50 g). The dried material was eluted from a column of silica gel (Kieselgel 0·05–0·2 mm) prepared in benzene, with Me_2CO–PhH mixtures. The fraction eluted with the above solvent mixture (ratio, 2:23) when taken to dryness gave a yellow solid (2·53 g) which was triturated with cold acetone three times. Recrystallization of the residue from MeOH (charcoal) afforded 3-*hydroxy*-2-*methoxyxanthone* (II) as a colorless microcrystalline solid (70 mg), R_f 0·4 in EtOAc–light petroleum (b.p. 60–80°), 7:13, m.p. 225–230°. ν_{max} 3110 (free OH), 1625 (C=O) and 1145 (C—O—C stretch) cm^{-1} λ_{max} (ϵ) (see Table 1). Spectrum with added NaOAc, 231 (37,000), 267sh (8500), 277sh (6400), 360 (21,200): with added NaOH, 231 (38,400), 267 (10,600), 277sh (8300) and 360 (24,600) nm. ^{1}H NMR spectral results, see Table 2. [Found: C, 69·65; H, 4·3, *M* (mass spectrometry), 242. $C_{14}H_{10}O_4$ requires: C, 69·45; H, 4·2%; *M*, 242.]

(c) *Isolation of* 2-*hydroxyxanthone* (IV). The combined filtrates from the trituration of fraction 2 above were evaporated to give a yellow solid (800 mg) which was purified by preparative TLC using EtOAc–light petroleum (b.p. 60–80°), 2:3. 2-Hydroxyxanthone (IV) was obtained as yellow needles (61 mg) from MeOH (with charcoal), R_f 0·65 (EtOAc–light petroleum (b.p. 60–80°), 7·13), m.p. 231° (lit.[5a] 237°) indistinguishable (IR spectrum and undepressed mixed m.p.) from an authentic sample.

Examination of the Acetone Extract

The extract (25 g) was chromatographed over a column of silica gel (1 kg) prepared with Me_2CO–$CHCl_3$ (1:9) and eluted by mixtures of these two solvents containing increasing proportions of Me_2CO. Combinations of eluate fractions on the basis of constituents revealed by TLC ultimately gave two fractions.

* In accordance with the system of numbering adopted earlier for xanthones of higher plant origin,[8] a 2,3-dioxygenated xanthone becomes a 6,7-dioxygenated xanthone. In this way we make the assumption that the two oxygens originate from shikimic acid.

(a) *Isolation of* 1,5,6-*trihydroxyxanthone* (VI). Fraction 1 (eluted with Me_2CO–$CHCl_3$, 1:3), after standing overnight, deposited the title compound (VI) as small yellow plates (330 mg). The xanthone (VI) had R_f 0·6 (HOAc–$CHCl_3$, 3:17), m.p. 282–286° (lit.[10] 285–286°) and was identical (IR spectrum and undepressed mixed m.p.) with an authentic specimen.

(b) Fraction 2 (eluted with Me_2CO–$CHCl_3$, 3:7), when evaporated to dryness furnished a yellow solid (4·77 g) containing several components (analytical TLC). By elution from a second column of silica gel with Me_2CO–$CHCl_3$ (1:3) another solid (1·75 g) was obtained which could not be purified further.

The mixture in dry Me_2CO was therefore treated with an excess of Me_2SO_4 (5 ml) and K_2CO_3 (10 g) and the reactants heated for 5 hr under reflux. Work-up in the usual manner gave a yellow gum (2 g) which was purified by preparative TLC in EtOAc–PhH (3:1). Four bands, (i)–(iv), were collected, the constituents being obtained from these by elution with Me_2CO.

(i) *Isolation of* 2,3,4-*trimethoxyxanthone* (VII). Band (i), a yellow solid (219 mg), was further purified by preparative TLC in EtOAc–light petroleum (b.p. 60–80°), 1:3. The solid was treated in Me_2CO with charcoal, re-isolated and finally recrystallized from Me_2CO–light petroleum (b.p. 60–80°) to give 2,3,4-trimethoxyxanthone (VII) as colourless needles (80 mg), R_f 0·8 (Me_2CO–PhH, 1:9), m.p. 142–145° (lit.[7] 153–155°) identical (IR spectrum and undepressed mixed m.p.). with an authentic sample prepared in this laboratory.[4]

(ii) *Isolation of* 2,3-*dimethoxyxanthone* (IX). Band (ii), a yellow solid, yielded the title compound (IX) (50 mg). Final purification was effected by preparative TLC (EtOAc–PhH, 3:17) followed by treatment with charcoal in hot Me_2CO. 2,3-*Dimethoxyxanthone* (IX) was obtained as a white amorphous solid from Me_2CO–light petroleum (b.p. 60–80°), R_f 0·65 (Me_2CO–PhH, 1:9) m.p. 154–155°. ν_{max} 1650 (xanthone C=O) and 1135 (C—O—C stretch) cm^{-1}. [Found: *M* (mass spectrometry), 256·0739. $C_{15}H_{12}O_4$ requires: 256·0736.]

(iii) *Isolation of* 1,3,5,6-*tetramethoxyxanthone* (X). Band (iii), a white solid (160 mg), was further purified by preparative TLC (R_f 0·45 in EtOAc–PhH, 4:1). 1,3,5,6-Tetramethoxyxanthone (X) crystallized from Me_2CO–light petroleum (b.p. 60–80°) as colourless needles m.p. 132–133° (lit.[11] 149–152°) identical (IR spectrum and co-chromatography) with an authentic specimen.

(iv) *Isolation of* 1,3,6,7-*tetramethoxyxanthone* (XI). Band (iv), a white solid (22 mg), was purified as for band (iii). The title compound (XI) was obtained as a white amorphous powder, R_f 0·35 (EtOAc–PhH, 4:1), m.p. 185–190° (lit.[12] 206–208°) identical (IR spectrum and undepressed mixed m.p.) with an authentic specimen.

(c) *Detection of* 2,3,4-*trihydroxy-*, 1,3,5,6-*tetrahydroxy-*, *and* 1,3,6,7-*tetrahydroxy-xanthones* (VIII), (XII), *and* (XIII). Co-chromatography of authentic samples of the above xanthones; (VIII),[4] R_f 0·45 (Me_2CO–$CHCl_3$, 1:3); (XII) and (XIII),[9] R_f 0·3 (HOAc–$CHCl_3$, 1:3); with the unpurified acetone extract indicated that the three xanthones were present in the heartwood. 2,3-Dimethoxy-, 2,3,4-trimethoxy-, 1,3,5,6-tetramethoxy-, and 1,3,6,7-tetramethoxyxanthones, (IX), (VII), (X), and (XI), respectively, were shown to be absent by use of the same technique.

Acknowledgements—We thank the Tropical Products Institute, London, for supplies of timber and the S.R.C. for a Research Studentship (to I.G.M.).

[10] H. D. Locksley, I. Moore and F. Scheinmann, *J. Chem. Soc.* C, 430 (1966).
[11] B. Jackson, H. D. Locksley and F. Scheinmann, *J. Chem. Soc.* C, 178 (1966).
[12] A. Jefferson and F. Scheinmann, *Nature, Lond.* **207**, 1193 (1965); *idem. J. Chem. Soc.* C, 175 (1966).