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THE STRUCTURE OF MANGIFERIN

P. E. NOTT and JOHN C. ROBERTS

Department of Chemistry, University of Nottingham

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Abstract—Further evidence is presented in confirmation of the structure 2-*C*-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-xanthone (I) for mangiferin.

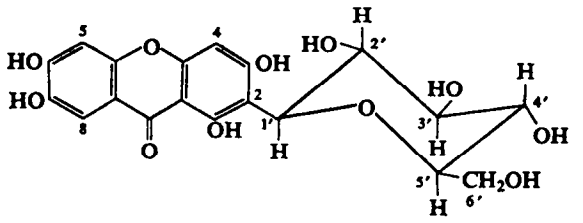
MANGIFERIN[1] is of wide distribution and has been isolated from plants belonging to at least seven different families.[2–9] One of these natural sources, *Aphloia theaeformis* (Flacourtiaceae), yields a metabolite which was originally named aphloiol[9] and an incorrect structure was assigned to it.[10] Later, the identity of aphloiol with mangiferin was established[11] and a recent paper[12] has presented evidence in favour of structure (I) for aphloiol (mangiferin) which accords with suggestions previously made on more limited evidence.[6, 13] In connexion with this structural problem, we now wish to report some of our own evidence (obtained during the last two years) which complements that of Billet *et al.*[12] and confirms their conclusions.

Work published[6, 13, 14] before 1965 left no doubt that mangiferin is a *C*-glucopyranoside of 1,3,6,7-tetrahydroxy-xanthone, but an important unsolved problem concerned the point of attachment of the glucose residue to the xanthone nucleus. Billet *et al.*[12] place this residue, by an argument (based on spectroscopy) which is somewhat tenuous, at position 2. We have confirmed the correctness of this placing by a chemical method.

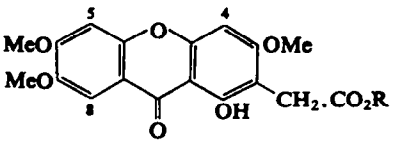
The mangiferin for our work was obtained from "Bitis" wood (*Madhuca utilis*) or, in a somewhat impure state (see below), from Mango bark (*Mangifera indica*). We then re-investigated the products obtained from the alkali-fusion of mangiferin or of its derivatives (cf. Ref. 6). In agreement with the work of Hawthorne *et al.*[6] we found that alkali-fusion of

[1] J. C. ROBERTS, *Chem. Rev.* **61**, 591 (1961).
[2] K. GORTER, *Bull. Jardin Botany Buitenz.* **4**, 260 (1922); *Chem. Abstr.* **17**, 1472 (1923).
[3] W. WIECHOWSKI, *Arch. Exptl. Pathol. Pharmakol.* **97**, 462 (1923); *Chem. Zentr.* **111**, 394 (1923).
[4] P. P. PILLAY and A. LECKSHMI, *Bull. Res. Inst. Univ. Kerala, Trivandrum* **5**, No. 1, 47 (1957); *Chem. Abstr.* **52**, 20423 (1958).
[5] L. HORHAMMER and H. WAGNER In *Recent Developments in the Chemistry of Natural Phenolic Compounds* (Edited by W. D. OLLIS), p. 185. Pergamon Press, Oxford (1961).
[6] B. J. HAWTHORNE, N. F. JANES, F. E. KING and J. W. W. MORGAN, In *Recent Progress in the Chemistry of Natural and Synthetic Colouring Matters and Related Fields* (Edited by T. S. GORE), p. 331. Academic Press, New York (1962).
[7] A. UENO, *J. Pharm. Soc. Japan* **82**, 1482 (1962); *Chem. Abstr.* **59**, 736 (1963).
[8] E. C. BATE-SMITH and J. B. HARBORNE, *Nature* **198**, 1307 (1963).
[9] R.-R. PARIS, *Bull. Sci. Pharmacol.* **49**, 146 (1942).
[10] M. S. ADJANGBA, *Bull. Soc. Chim. France* 376 (1964).
[11] R.-R. PARIS and S. ETCHPARE, *Compt. Rend.* **258**, 5277 (1964).
[12] D. BILLET, J. MASSICOT, C. MERCIER, D. ANKER, A. MATSCHENKO, C. MENTZER, M. CHAIGNEAU, G. VALDENER and H. PACHECO, *Bull. Soc. Chim. France* 3006 (1965).
[13] J. D. RAMANATHAN and T. R. SESHADRI, *Current Sci.* **29**, 131 (1960).
[14] S. ISEDA, *Bull. Chem. Soc. Japan* **30**, 625, 629 (1957); *Chem. Abstr.* **52**, 6329 (1958).

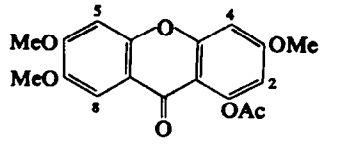
mangiferin itself yielded mangific acid, the constitution of which remains ambiguous. However, we further found that alkali-fusion of tri-*O*-methylmangiferin (or, curiously, of somewhat impure mangiferin—see below) gave a different acid, which, with diazomethane, yielded a crystalline methyl ester, $C_{19}H_{18}O_8$. Proton magnetic resonance (PMR) data and other spectroscopic evidence indicated structure (II; R=Me) for this compound. This structural assignment was confirmed by a comparison of the parent acid with a synthetic



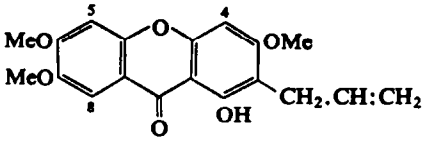
(I)



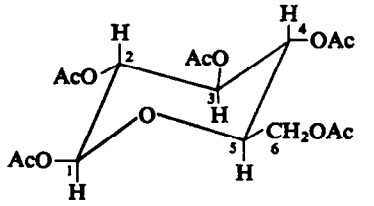
(II)



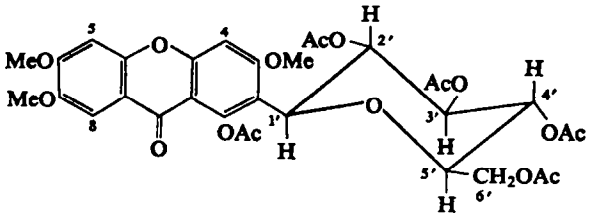
(III)



(IV)



(VI)



(V)

specimen prepared in the following way. 1-Hydroxy-3,6,7-trimethoxy-xanthone was converted into its 1-*O*-allyl derivative (III; O.CH_2.CH=CH_2 for OAc) which was re-arranged to 2-allyl-1-hydroxy-3,6,7-trimethoxy-xanthone (IV). Oxidation of the acetate of this latter compound with the Lemieux–Rudloff reagent [15] gave a product which, by removal of the acetate group, yielded the acid (II; R=H), identical in all respects with the product obtained from the alkali-fusion as described above. The attachment of the *C*-glucosyl residue of mangiferin to the 2-position of the xanthone nucleus is therefore confirmed.

[15] R. U. LEMIEUX and E. VON RUDLOFF, *Can. J. Chem.* 33, 1701 (1955).

We include below the results of a PMR investigation of penta-*O*-acetyl-tri-*O*-methylmangiferin (V). The interpretation of this spectrum confirms the conclusions which the French workers[12] have obtained (especially in respect of the β-nature of the glucosidic link) by the employment of other mangiferin derivatives.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus. Unless otherwise specified, u.v. spectra were recorded on a Unicam spectrophotometer (S.P. 800) and i.r. spectra were determined (on compounds in potassium bromide discs) with a Unicam spectrophotometer (S.P. 200). PMR spectra were recorded on a Perkin-Elmer Model R 10, 60 Mc/sec spectrometer, tetramethylsilane (unless otherwise stated) being used as internal reference; in the sequel, figures in parentheses (following the statement of the nature of the signal) indicate intensities.

The silica used for thin-layer chromatography (TLC) was "Kieselgel H nach Stahl" (Merck); the polyamide was "Polyamidpulver nach Stahl" (Merck). The silica used for column chromatography was grade "M.F.C." (Hopkin Williams).

Mangiferin. This was obtained by the following methods: (*a*) Powdered Bitis wood (*Madhuca utilis*) was extracted by the method of Hawthorne *et al.*[6] The extract crystallized from 60% ethanol to give mangiferin as pale-yellow prisms, m.p. 271° (decomp.) (lit.,[2] 271°). TLC on polyamide, using ethanol (99%) as mobile phase, showed a single apricot-coloured fluorescent (u.v.) spot, R_f 0·4.

(*b*) Powdered mango bark (*Mangifera indica*) was extracted by the method described above for Bitis wood or by Iseda's method.[14] Crystallization of the extract gave prisms, m.p. 270° (decomp.). TLC (as above) showed two apricot-coloured fluorescent spots (R_f 0·4 and 0·6). Attempts to separate the major (R_f 0·4) and minor (R_f 0·6) constituents by fractional crystallization or by chromatography were abortive. An ethanolic solution of the impure mangiferin ($\gtrsim$20 mg), chromatographed on a polyamide column (30 × 5 cm), yielded two bands, but elution and isolation of the products showed that they were grossly contaminated with material which had been dissolved from the adsorbent. No other adsorbent appeared to be effective.

Tri-O-methylmangiferin. To a solution of mangiferin (1·05 g) in dimethylformamide (100 ml) was added an excess of diazomethane (from 10 g of *N*-nitrosomethylurea) in ether (100 ml). The mixture was kept at 0° for 16 hr and the excess of reagent and the solvents were then removed *in vacuo*. Precipitation of the product from dimethylformamide-acetone gave tri-*O*-methylmangiferin (730 mg), m.p. 296–297° (decomp.) (lit.,[6] 298–299°).

Alkali-fusions. (*a*) Pure mangiferin (1·46 g) was fused with alkali (under the conditions described by Hawthorne *et al.*[6]) and the product was methylated with an excess of diazomethane (from 10 g of *N*-nitrosomethylurea). The crude ester (553 mg) was extracted with boiling chloroform and the residue (148 mg) was crystallized from pyridine–methanol and then from pyridine to give methyl tri-*O*-methylmangifate as yellow prisms (*ca.* 70 mg), m.p. 231–233° (lit.,[6] 231–232°) (Found: C, 59·9; H, 5·4. Calc. for $C_{21}H_{22}O_9$: C, 60·3; H, 5·3%), $\lambda_{max}^{CHCl_3}$ 245, 263, 318, and 360 mμ (10^{-3} ϵ 34·3, 45·4, 21·8, and 11·6), ν_{max} included bands at 3400, 1745, 1655, and 1615 cm.$^{-1}$ The PMR spectrum (solvent, trifluoro-acetic acid) showed (i) a singlet (1) at τ 2·29, (ii) a singlet (1) at τ 2·71, (iii) a singlet (1) at τ 2·99, (iv) a deformed triplet (1) at τ 5·46, (v) three singlets (3 × 3) at τ 5·80–5·85, (vi) a singlet (3) at τ 6·08, (vii) a multiplet (2) at τ 6·95, and (viii) a multiplet (2) at τ 7·75.

(*b*) Somewhat impure mangiferin (0·5 g; *ex* mango bark) was fused with alkali (under the conditions given above) and the product was methylated with excess of diazomethane (from 3 g of *N*-nitrosomethylurea). A solution of the crude ester in chloroform was chromatographed on a column (20 × 5 cm) of silica. Chloroform eluted a dark-brown fluorescent (u.v.) band. Removal of the solvent from the eluate and repeated crystallization of the residue from benzene yielded *methyl* 1-*hydroxy*-3,6,7-*trimethoxy*-2-*xanthonyl-acetate* (II; R = Me) as pale-yellow prisms (44 mg), m.p. 195–197° (Found: C, 60·8; H, 4·7. $C_{19}H_{18}O_8$ required: C, 61·0; H, 4·9%), $\lambda_{max}^{CHCl_3}$ 244, 268, 316, and 358 mμ (10^{-3} ε 25·8, 36·8, 16·4, and 9·6), ν_{max} included bands at 1730, 1645, and 1610 cm.$^{-1}$ The PMR spectrum ($CDCl_3$) showed (i) a singlet (1) at τ 2·53, (ii) a singlet (1) at τ 3·21, (iii) a singlet (1) at τ 3·67, (iv) three singlets (3 × 3) at τ 6·01–6·12, and (v) a singlet (5) at τ 6·30 (CH_2 and ester OCH_3). [The spectrum of methyl phenylacetate (run under similar conditions) showed, *inter alia*, a singlet (2) at τ 6·49 (CH_2) and also a singlet (3) at τ 6·47 (ester OCH_3).] The xanthone ester gave a positive Gibbs test[16] (λ_{max} 688 mμ). The ester (100 mg) was warmed with 2 *N*-sodium hydroxide (20 ml) for 1 hr on the steam-bath and the cooled reaction mixture was extracted with chloroform. From the aqueous residue a bicarbonate-soluble fraction was isolated. A solution of this material in chloroform was chromatographed on a column (15 × 5 cm) of silica. Chloroform–acetone (1:1, by vol.) eluted a brown fluorescent band which was collected. Removal of the solvents from this eluate and two crystallizations of the residue from dimethylformamide–methanol yielded 1-*hydroxy*-3,6,7-*trimethoxy*-2-*xanthonyl-acetic acid* (II; R = H) as yellow prisms (8 mg), m.p. 256–257° (decomp.) (Found: C, 60·3; H, 4·8. $C_{18}H_{16}O_8$ required: C, 60·0; H, 4·5%), $\lambda_{max}^{CHCl_3}$ 244, 268, 316, and 358 mμ (10^{-3} ε 21·8, 32·8, 15·5, and 9·1), ν_{max} included bands at 2950, 1700, 1650, and 1610 cm.$^{-1}$

(*c*) Tri-*O*-methylmangiferin (1·26 g) was fused with alkali (as above) and the product was methylated with an excess of an ethereal solution of diazomethane (from 10 g of *N*-nitrosomethylurea). A benzene solution of the crude ester (163 mg) was chromatographed on a column (20 × 5 cm) of silica. Ethyl acetate–benzene (1:1, by vol.) eluted a dark-brown fluorescent band. Removal of the solvents from the eluate and repeated crystallization of the residue (88 mg) from benzene gave a pure product (12 mg of pale-yellow prisms) which was identical (m.p., mixed m.p., i.r. spectrum, and TL chromatographic properties) with methyl 1-hydroxy-3,6,7-trimethoxy-2-xanthonyl-acetate (see above).

Penta-O-*acetyl*-*tri*-O-*methylmangiferin* (*V*). Tri-*O*-methylmangiferin (1 g) was heated under reflux for 3 hr with dry pyridine (5 ml) and distilled acetic anhydride (20 ml). An ethyl acetate solution of the crude product (1·41 g), isolated in the usual way, was chromatographed on a column (10 × 5 cm) of silica. Continued percolation of ethyl acetate through the column eluted a white fluorescent band. The solvent was removed from the eluate and the residue was repeatedly crystallized from methanol and then from acetone to give the required product as long prisms (115 mg), m.p. 215–216° (lit.,[6] 218–219°) (Found: C, 56·6; H, 5·4, Calc. for $C_{32}H_{34}O_{16}$: C, 56·9; H, 5·1%), λ_{max}^{EtOH} (Unicam, S.P. 700) 209, 230, 245 (infl.), 251 (infl.), 270 (infl.), 307, and 342 mμ (10^{-3} ε 22·3, 22·6, 29·8, 33·8, 13·7, 16·4, and 9·4), ν_{max} (Unicam S.P. 100) included bands at 1781, 1757, 1653, 1624, and 1615 cm.$^{-1}$ This compound gave a positive Gibbs test[16] (λ_{max} 684 mμ).

1,3,6,7-*Tetrahydroxyxanthone*. This was prepared either by the method of Tanase (see Ref. 14) or by the method of Ueno.[7]

1-*Hydroxy*-3,6,7-*trimethoxyxanthone*. To a solution of the foregoing xanthone (832 mg)

[16] F. E. KING, T. J. KING and L. C. MANNING, *J. Chem. Soc.* 563 (1957).

TABLE 1. PROTON MAGNETIC RESONANCE SPECTRA*

(Solvent, $CDCl_3$; internal reference, tetramethylsilane)

τ Scale; J in c/s

Compound	*H*-8	*H*-5	*H*-4	*J*	*H*-2	*J*	O.C*H*$_3$-3+6	O.C*H*$_3$-7	OC.C*H*$_3$-1
V	2·65 (s1)	3·30 (s2)		—	—	—	6·05 (s6)	6·10 (s3)	7·46 (s3)
III	2·55 (s1)	3·37 (s1)	3·43 (d1)	~3	3·51 (d1)	~3	6·09 (s6)	6·20 (s3)	7·53 (s3)

Compound	*H*-1'‡	*J*	*H*-2'-3'-4'	*H*-5'/2 × *H*-6'	OC.C*H*$_3$-1'	OC.C*H*$_3$-2'	OC.C*H*$_3$-3'-4'	OC.C*H*$_3$-6'
V	4·12 (d1)	~9	4·3–5·5 (m3)	5·6–>6·0 (m3)	—	8·18 (s3)	7·93 (s6)	7·97 (s3)
VI†	4·26 (d1)	~8	4·6–5·2 (m3)	5·8–6·5 (m3)		Five closely spaced singlets 7·90–8·00 (15)		

* The nature and intensity of the signals are shown in parenthesis; s = singlet, d = doublet, m = multiplet.

† When compound VI is being considered the primes on the figures at the top of the columns should be removed.

‡ The coupling constant for this proton in (V) confirms that the sugar moiety is attached by a β-glucosidic linkage.[17]

[17] R. U. LEMIEUX, R. K. KULLNIG, H. J. BERNSTEIN and W. G. SCHNEIDER, *J. Am. Chem. Soc.* **80**, 6098 (1958).

in methanol (25 ml) was added a solution of an excess of diazomethane (from 10 g of *N*-nitrosomethylurea) in ether (150 ml). The mixture was kept for 16 hr at 0° and the solvents and the excess of diazomethane were then removed to give crude 1-hydroxy-3,6,7-trimethoxyxanthone (831 mg), m.p. 215–218°. A small portion (50 mg) was sublimed and the sublimate was crystallized from 30% aqueous ethanol to give yellow prisms (23 mg), m.p. 217–219° (lit.,[14] 218–219°).

1-*Acetoxy*-3,6,7-*trimethoxyxanthone* (*III*). The foregoing xanthone was acetylated (pyridine/acetic anhydride) to yield a product which crystallized from ethanol in colourless prisms, m.p. 216–217·5° (lit.,[14] 216°).

1-*Allyloxy*-3,6,7-*trimethoxyxanthone*. A solution of crude 1-hydroxy-3,6,7-trimethoxyxanthone (780 mg) and of allyl bromide (3 ml) in acetone (20 ml), together with anhydrous K_2CO_3 (5 g), was heated under reflux for 24 hr. The solids were filtered off and the acetone was removed from the filtrate to give a residue which crystallized from ethanol as colourless needles (729 mg), m.p. 149·5–150·5°. A further crystallization from ethanol yielded the pure *xanthone*, m.p. 152–153° (Found: C, 66·6; H, 5·5. $C_{19}H_{18}O_6$ required: C, 66·7; H, 5·3%).

2-*Allyl*-1-*hydroxy*-3,6,7-*trimethoxyxanthone* (*IV*). The crude foregoing xanthone (710 mg) and dimethylaniline (50 ml) were heated under reflux for 3 hr. The dimethylaniline was distilled off, *in vacuo*, to leave a residue which crystallized from ethanol as pale yellow prisms (466 mg), m.p. 183–185°. A further crystallization from ethanol gave the pure *xanthone*, m.p. 184–185° (after changing from prisms to needles at 168–170°) (Found: C, 66·9; H, 5·6. $C_{19}H_{18}O_6$ required: C, 66·7; H, 5·3%), λ_{max}^{EtOH} 209, 243, 261, 317, and 358 mμ (10^{-3} ε 21·3, 26·9, 33·6, 17·2, 9·0), ν_{max} included bands at 1645, 1608, 995, and 920 cm.$^{-1}$ This compound gave a positive Gibbs test[16] (λ_{max}, 690 mμ).

1-*Acetoxy*-2-*allyl*-3,6,7-*trimethoxyxanthone*. To a solution of the foregoing crude xanthone (450 mg) in acetic anhydride (10 ml) was added aqueous perchloric acid (60%: 5 drops). The mixture, having been kept at room temperature for 1½ hr, was poured into water. The product was collected and was crystallized from ethanol to give colourless needles (435 mg), m.p. 185–188°. Two further crystallizations from ethanol gave the pure *xanthone*, m.p. 187–188·5° (Found: C, 65·7; H, 5·1. $C_{21}H_{20}O_7$ required: C, 65·6; H, 5·2%), λ_{max}^{EtOH} 210, 248, 270 (infl.), 308, and 339 (infl.) mμ, (10^{-3} ε 26·2, 47·2, 13·3, 22·9, and 11·8), ν_{max} included bands at 1756, 1645, 1630, 1600, 995, and 920 cm.$^{-1}$

1-*Hydroxy*-3,6,7-*trimethoxy*-2-*xanthonyl-acetic acid* (*II*; *R* = *H*). A solution of the foregoing compound (100 mg) in pyridine (10 ml) was added to a solution of $NaIO_4$ (1·65 g), $KMnO_4$ (20 mg), and K_2CO_3 (1·0 g) in water (100 ml).[15] To this mixture, which had been kept at room temperature for 16 hr, was added $Na_2S_2O_5$ (0·50 g) and sufficient 20 N-H_2SO_4 to give a strongly acid reaction. The solution was extracted with chloroform and the bicarbonate soluble fraction was isolated. A chloroform solution of this crude acid (88 mg) was chromatographed on a column (10 × 4 cm) of silica. Continued percolation of chloroform through the column eluted a white fluorescent band. Evaporation of the solvent gave a residue (72 mg) which crystallized from chloroform–methanol to yield the acetyl derivative (m.p. 315–317°; 55 mg) of the required acid. A solution of this derivative (55 mg) in aqueous sodium hydroxide (5%; 10 ml) was kept at room temperature for 2 hr. The solution was then filtered through a pad of diatomite and the filtrate was acidified (20 N-H_2SO_4). The product was extracted into chloroform and the solution was washed with water. Removal of the chloroform gave a residue (32 mg) which was crystallized from dimethylformamide/methanol and then from dimethylformamide to yield the *acid* as yellow prisms (11 mg), m.p. 256–257° (decomp.) (Found: C, 59·9; H, 4·5. Calc. for $C_{18}H_{16}O_8$: C, 60·0; H, 4·5%). This compound

was identical (m.p., mixed m.p., i.r. spectrum, and TL chromatographic properties) with the acid which had been obtained in the alkaline fusion of mangiferin (*ex* mango bark).

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