Tetrahedron, 1967, Vol. 23, pp. 1363 to 1368. Pergamon Press Ltd. Printed in Northern Ireland

CONSTITUTION OF MANGIFERIN

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(*Received* 15 *August* 1966)

Abstract—Mangiferin, $C_{19}H_{18}O_{11}$, has been isolated from the leaves, heartwood and stem-bark of *Mangifera indica*. Its properties indicate that it is a stable C-glycoside of the xanthone group. Reductive hydrolysis with hydriodic acid and oxidation with ferric chloride yield 1,3,6,7-tetrahydroxyxanthone and glucose respectively indicating that it is a glucoside of the former and oxidation with periodic acid reveals that the sugar is in the pyranose form. The position of linkage has been shown to be 2 by oxidizing mangiferin tri and tetra methyl ethers with periodate to the corresponding α-hydroxyacetaldehydes of trimethoxy and tetramethoxyxanthones and comparing them with synthetic samples obtained from the appropriate 2-allylxanthones. This has been further supported by oxidation of mangiferin tetra methyl ether and 2-allyl-1,3,6,7-tetramethoxyxanthone with permanganate yielding 1,3,6,7-tetramethoxyxanthone-2-carboxylic acid.

IN an earlier note,[1] it was concluded that mangiferin is 1,3,6,7-tetrahydroxyxanthone-2-glucopyranoside (I) and more details were given in later notes.[2-4] Meanwhile, similar results in support of this structure have been mentioned.[5,6] In this paper a detailed statement of our results and the conclusions are presented.

Mangiferin has been isolated in crystalline form from the leaves, heartwood and stem-bark of the plant, the latter being the most convenient and the richest source. The method of isolation involved removal of fatty matter and tannins using light petroleum and acetone respectively and finally extracting the glycoside with 70% aqueous ethanol. Its composition is represented by the formula $C_{19}H_{18}O_{11}$. It forms an octaacetate which is convenient for purification and analysis. Two methyl ethers, tri (II) and tetra (III), can be obtained and they form penta and tetra acetates respectively.

Mangiferin is highly resistant to acidic and enzymatic hydrolysis. It undergoes reductive hydrolysis with hydriodic acid to give 1,3,6,7-tetrahydroxyxanthone (IV), whose structure was established by comparison with a synthetic sample.[7] The nature of the sugar moiety was determined by oxidizing the glycoside with ferric chloride when glucose was obtained. The trimethyl ether consumed 2·2 moles of periodic acid with the liberation of formic acid. The periodate-oxidized trimethyl ether on reduction with sodium borohydride followed by acid hydrolysis gave glycerol. This

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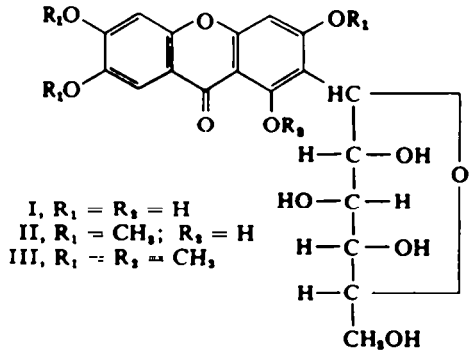
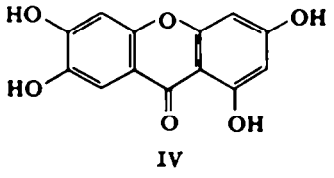
[1] J. D. Ramanathan and T. R. Seshadri, *Curr. Sci.* **29**, 131 (1960).
[2] J. D. Ramanathan, *Bull. Nat. Inst. Sci. India* **23**, 26 (1963).
[3] J. D. Ramanathan and T. R. Seshadri, *Horticult. Adv.* **6**, 180 (1962).
[4] T. R. Seshadri, '*Vidya*', University of Gujarat, 139, March (1963).
[5] B. J. Hawthorne, N. F. Janes, F. E. King, and J. W. W. Morgan, *Recent Progress in the Chemistry of Natural and Synthetic Colouring Matters and Related Compounds* (Edited by T. S. Gore, B. S. Joshi, S. V. Sunthanker and B. D. Tilak) p. 331. Academic Press, New York (1962).
[6] L. Hörhammer and H. Wagner, *Recent Developments in the Chemistry of Natural Phenolic Compounds* (Edited by W. D. Ollis) p. 185, Pergamon Press, London (1961).
[7] P. Yates and G. Stout, *J. Amer. Chem. Soc.* **80**, 1691 (1958).

reaction is given also by C-glycosides.[8] These observations not only support the above conclusion with regard to the presence of glucose as the sugar moiety but also show that the sugar has the pyranose form.

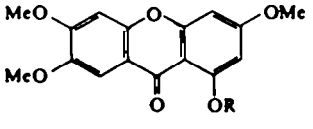


I, $R_1 = R_2 = H$
II, $R_1 = CH_3$; $R_2 = H$
III, $R_1 = R_2 = CH_3$

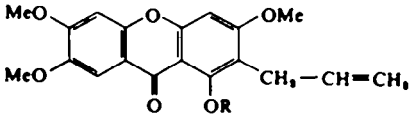


IV

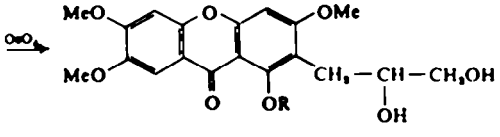
In order to establish the location of the sugar, two parallel series of experiments have been conducted using mangiferin trimethyl and tetramethyl ethers. Treatment of each of these with 2 moles of periodate yielded, as the major fission product, the α-hydroxyacetaldehyde of xanthone methyl ether. Using 1-hydroxy-3,6,7-trimethoxy-xanthone (V), its allyl ether and from it 1-hydroxy-3,6,7-trimethoxy-2-allylxanthone (VI) were prepared. Osmium tetraoxide oxidation of this compound yielded a diol (VII). This underwent periodate fission to yield a xanthone acetaldehyde (VIII) which was further oxidized with one mole of periodate to the hydroxyacetaldehyde (IX),



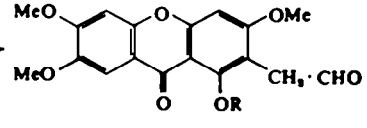
V, R = H



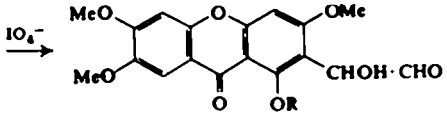
VI, R = H
X, R = Me



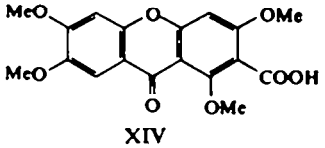
VII, R = H
XI, R = Me



VIII, R = H
XII, R = Me



IX, R = H
XIII, R = Me



XIV

[8] V. K. Bhatia, S. R. Gupta and T. R. Seshadri, *Curr. Sci.* 33, 581 (1964).

identical with the degradation product of mangiferin trimethyl ether. Parallel results have been obtained using mangiferin tetramethyl ether. For the purpose of comparison the synthetic compound XIII was prepared from 1,3,6,7-tetramethoxy-2-allylxanthone (X). These results definitely established the location of the glucose moiety at the 2-position.

The acetaldehyde and hydroxyacetaldehyde derivatives gave 2,4-dinitrophenylhydrazones and osazones respectively. These could be distinguished by their difference in absorption spectra.[9]

In order to obtain further support for the linkage of the glucose unit at the 2-position, mangiferin tetramethyl-ether and 1,3,6,7-tetramethoxy-2-allylxanthone were oxidized using neutral potassium permanganate. Both yield the same 1,3,6,7-tetramethoxyxanthone-2-carboxylic acid (XIV).

About the same time as our work, Haynes carried out similar permanganate oxidations and obtained 1,3,6,7-tetramethoxyxanthone-2-carboxylic acid[10,11] though details have not been reported.

EXPERIMENTAL

Isolation of mangiferin. The fresh air-dried bark (1·2 kg) of *Mangifera indica* obtained from Delhi garden was successively extracted with boiling light petroleum (2 × 2 l.; 6 hr each time) to remove fatty matter, with cold acetone (6 × 2 l.; 24 hr each time) to remove tannins and finally with 70% EtOH (3 × 1 l.; 6 hr each time). The combined alcohol extract was concentrated under reduced press when mangiferin separated as a yellow amorphous powder (32 g). Adopting a similar type of extraction the heartwood (1 kg) yielded mangiferin (1 g). Recrystallization from 70% EtOHaq gave pale yellow needles, m.p. 270–272° (dec). (Found: C, 54·3; H, 4·5. $C_{19}H_{18}O_{11}$ requires: C, 54·0; H, 4·3%.) IR (KBr) 3413 (s), 2924 (m), 2336 (w), 1645 (s), 1613 (s), 1486 (s), 1399 (m), 1295 (m), 1250 (s), 1190 (m), 1134 (w), 1093 (s), 1075 (m), 1053 (m), 1031 (m), 982 (m), 893 (w), 877 (w), 832 (m), 820 (m) and 800 (m) cm^{-1}. UV (60% ethanol: 239 (4·35), 257 (4·52), 314 (4·15) and 360 mμ (4·10); $[\alpha]_D^{28}$ +32° (EtOHaq). In circular paper chromatography in 60% AcOH it gave a yellow ring when sprayed with ammonia, R_f 0·60 (34°). It is soluble in EtOHaq. Na_2CO_3aq and NaOHaq giving deep yellow solns. In alcoholic soln $FeCl_3$ gave a deep green colour, Mg/HCl an orange red colour and Zn/HCl (boiling) red colour. With HCl and alc. $FeCl_3$ it produced a greenish yellow ppt immediately. It gave a positive Molisch's test.

Acetylation

Mangiferin (2 g) was acetylated using Ac_2O (15 ml) and pyridine (1 ml). The acetate was passed through a column of alumina using acetone as the eluant and then on recrystallization with AcOEt–light petroleum it separated as a white amorphous powder, m.p. 141–143° (sintering at 127°). It gave no colour with alc. $FeCl_3$. (Found: C, 54·6; H. 4·6. $C_{36}H_{37}O_{21}$ requires: C, 55·0; H, 4·5%.)

The above acetate (1 g) in EtOH (25 ml) was deacetylated (refluxing) using conc H_2SO_4 (1 ml). The pale yellow compound, that separated, on recrystallization with 60% EtOH gave yellow needles, m.p. and mixed m.p. with mangiferin, 270–272° (dec).

Methylation

1. *Using dimethyl sulphate.* Mangiferin (1 g) was refluxed with Me_2SO_4 (8 ml, excess) in acetone (200 ml) and K_2CO_3 till a test sample gave no colour with alc. $FeCl_3$. The methyl ether was purified by passing through a column of alumina using AcOEt as the eluant. It could not be crystallized (semi-solid) and was highly soluble in all organic solvents. IR ($CHCl_3$); 2874 (s), 2392 (w), 1645 (m), 1453 (m), 1387 (m), 1366 (m), 926 (m), 901 (w) and 848 (w) cm^{-1}.

[9] A. C. Jain and T. R. Seshadri, *Curr. Sci.* 35, 323 (1966).
[10] L. J. Hzynes, *Bull. Nat. Inst. Sci. India* 31, 198 (1965).
[11] T. R. Seshadri, *Bull. Nat. Inst. Sci. India* 31, 200 (1965).

2. *With diazomethane.* Mangiferin (0·5 g), in a mixture of dioxan (50 ml), EtOH (75 ml) and water (4 ml), was methylated using diazomethane in ether (50 ml). After 24 hr more diazomethane in ether was added and after 48 hr ether was removed under ordinary press and the remaining solvents under diminished press. The residue, after drying over KOH in vacuum, was taken up in MeOH. It could be separated into two fractions, the first fraction giving a deep green colour with alc. $FeCl_3$, m.p. 193–195° (dec) shrinking at 170°); $[\alpha]_D^{29}$ +19·2° (EtOH). (Found: C, 53·8; 53·6; H, 5·7, 5·5; OCH_3, 19·5. $C_{38}H_{34}O_{11}\cdot 1\frac{1}{2}\ H_2O$ requires: C, 53·7; H, 5·5; 3 OCH_3, 20·5%.) The second fraction, m.p. 230–231° (dec), gave a negative ferric reaction. (Found: C, 57·1; H, 5·5; OCH_3 22·9. $C_{32}H_{28}O_{11}$ requires: C, 57·7; H, 5·4; 4 OCH_3 25·9%.)

The trimethylether of mangiferin was acetylated using Ac_2O and pyridine. Recrystallization of the methyl ether acetate from AcOEt–light petroleum gave white needles, m.p. 176–178°. (Found: C, 56·6; H, 5·3. $C_{39}H_{38}O_{16}$ requires: C, 56·9; H, 5·2%.)

Acetylation of mangiferin tetramethyl ether by the above method and recrystallization from AcOEt–light petroleum afforded colourless needles, m.p. 184–185°. (Found: C, 57·6; H, 5·5. $C_{31}H_{34}O_{16}$ requires: C, 57·6; H, 5·3%.)

Action of hydriodic acid

To the suspension of mangiferin (2·5 g) in phenol (15 g) HI (25 ml, *d*, 1·7) was added gradually cooling the flask during the addition. The mixture was gently refluxed (135–137°) for 7 hr and poured into an aqueous soln of $NaHSO_3$. Dark brown product (1 g) that separated gave green colour with alc. $FeCl_3$.

The above product was acetylated using Ac_2O (4 ml) and pyridine (0·5 ml). Recrystallization of the acetate from EtOH afforded colourless wooly needles, m.p. 197–198° undepressed on admixture with an authentic sample of 1,3,6,7-tetraacetoxyxanthone.[7] (Found: C, 59·1; H, 4·0. $C_{31}H_{16}O_{16}$ requires: C, 58·8; H, 3·7%.)

Ferric chloride oxidation

Mangiferin (0·5 g) and $FeCl_3$ (2·5 g in 8 ml water) were heated under reflux at 115° for 15 min and then at 125° for 6 hr. The mixture was diluted with water (20 ml) and the dark coloured complex filtered off. The pale yellow filtrate, after treatment with resins IRC–120 (H^+) and IRA–400 (OH^-) to remove Fe^{3+} and Cl^- ions, was evaporated to a small volume. The syrupy product, so obtained, was identical with glucose in circular chromatography (R_f 0·56, 34°) using phenol saturated with water as the irragating solvent and as developer aniline hydrogen phthalate. It also formed an osazone (time of formation, 4 min), m.p. 208–210°.

Periodic acid oxidations of mangiferin trimethyl ether

(i) *Oxidation and identification of formic acid.* Periodic acid (0·5M; 1 ml) was added to a soln of the methyl ether (23·8 gm) in peroxide free dioxan (2 ml) and a little water, the soln made up to 25 ml and kept at 0°. At regular intervals an aliquot (2 ml) was taken for titration. Blank titration was also carried out side by side. One mole of the methyl ether consumed 2·2 moles of periodic acid in 24 hr. The remaining soln was then distilled collecting about 3 ml of the distillate. It showed an acidic character and gave the chromotropic acid colour reaction only after prereducing the acid with Mg and HCl. This confirmed the presence of formic acid in the distillate. Colour reaction with an authentic sample of formic acid under identical conditions gave the same result.

(ii) *Oxidation followed by borohydride reduction.* To a soln of the methyl ether (3·15 mg) in water (3 ml) was added a soln of peroidic acid in water and the mixture kept at room temp for 4 hr. The soln was carefully neutralized with Na_2CO_3aq, $NaBH_4$ (3·15 mg) in water (0·2 ml) added, the soln left at room temp overnight and then heated with 1N HCl (0·4 ml) at 100° for 15 min. Circular chromatography of this soln with AcOEt:pyridine:water (10:4:3) as solvent and as spray a mixture of 2% sodium metaperiodate (4 parts) and alkaline $KMnO_4$ (1% $KMnO_4$ in 2% Na_2CO_3aq, 1 part) gave a yellow ring on a pink back-ground R_f 0·83 (20°). An authentic sample of glycerol and its mixture with the above soln also gave a single ring R_f 0·83.

(iii) *Oxidation to the corresponding formyl compound:* A soln of $NaIO_4$ (3·2 g in 100 ml water) was added to the soln of the methyl ether (0·86 g in 100 ml water), shaken and kept in a dark place. After 72 hr a fluffy white product separated and the soln was slightly acidic. It was made more acidic

with dil HCl (for breaking the $NaIO_4$ complex) and thoroughly extracted with ether. The ethereal extract was dried and evaporated. The residue after purification by passing through a short column of alumina (EtOH) and recrystallization from EtOH gave colourless prisms, m.p. 212°. (Found: C, 59·7; H, 4·4. $C_{18}H_{16}O_8$ requires: C, 60·0; H, 4·4%.) It gave an osazone which crystallized from EtOH as orange prisms, m.p. 242°; UV 340 and 400 mμ.

Synthesis of the formyl compound

1-*Allyloxy*-3,6,7-*trimethoxyxanthone*. 1-Hhydroxy-3,6,7-trimethoxyxanthone (0·9 g) was allylated using allyl bromide (2·5 ml, excess) in presence of K_2CO_3 and acetone till there was no colour with alc. $FeCl_3$. Its crystallized from MeOH as colourless needles (0·7 g), m.p. 159–160°. (Found: C, 66·5; H, 5·4; $C_{19}H_{18}O_6$ requires: C, 66·6; H. 5·3%.)

2-*Allyl*-1-*hydroxy*-3,6,7-*trimethoxyxanthone* (VI). 1-Allyloxy-3,6,7-trimethoxyxanthone (0·6 g) was refluxed in dimethylaniline (6 ml) for 4 hr, cooled and 2N HCl (15 ml) added. The white solid that separated recrystallized from AcOH as colourless needles (0·4 g), m.p. 184–185°. (Found C, 66·2; H, 5·5. $C_{19}H_{18}O_6$ requires: 66·6; H, 5·3%.) It gave a green colour with alc. $FeCl_3$.

Osmium tetraoxide oxidation of 2-*allyl*-1-*hydroxy*-3,6,7-*trimethoxyxanthone to the corresponding diol* (VII)

Osmium tetroxide (0·15 g, 1 mole) was added to a soln of 2-allyl 1-hydroxy-3,6,7-trimethoxyxanthone (0·195 g) in dry pyridine (2·3 ml) kept stirring for 3 hr and the chocolate brown complex that separated was decomposed by adding with stirring a soln of $NaHSO_3$ (0·27 g) in water (4·5 ml) and pyridine (3 ml). The ratio of $NaHSO_3$: water : pyridine used was kept at 2:3:35. The clear red soln (obtained in about 30 min) was extracted with chf (4 × 150 ml), the chf extract washed with dil HCl and finally with water and the solvent removed completely. The residue crystallized from glacial AcOH as pale yellow needles (0·12 g) m.p. 215–217°. (Found: C, 60·7; H, 5·6; $C_{19}H_{20}O_8$ requires: C, 60·6; H, 5·4%.) It gave a green colour with alc. $FeCl_3$.

Oxidation of the diol VII *to aldehyde* VIII

A soln of KIO_4 (1M, 0·12 g in 40 ml water) was added to the soln of the diol (0·17 g) in glacial AcOH (30 ml) in 30 min, kept stirred at 160° for 4 hr and after allowing it to stand at the same temp overnight, the AcOH was carefully neutralized with solid Na_2CO_3. The product that separated recrystallized from glacial AcOH containing a little water, as pale yellow needles (0·1 g), m.p. 202–203°. (Found: C, 62·6; H, 4·2; $C_{18}H_{16}O_7$ requires: C, 62·8; H, 4·5%.) It gave a green colour with alc. $FeCl_3$ and a yellow 2,4-dinitrophenylhydrazone; UV: 257 and 347 mμ.

Periodic acid oxidation of the aldehyde VIII *to the corresponding hydroxy derivative* IX

A soln of HIO_4 in water (32 mg in 2 ml, 1 mole) was added in 20 min to the soln of the aldehyde (50 mg) in a mixture of glacial AcOH (20 ml) and THF (5 ml) keeping the temp of the reaction mixture at 10° and then allowed to stand at room temp for 24 hr. After neutralizing the AcOH with Na_2CO_3, the ppt recrystallized from alcohol as colourless thick prisms, m.p. 212–214° undepressed on admixture with the sample from mangiferin trimethyl ether. (Found: C, 58·6; H, 5·0. $C_{18}H_{16}O_8$ requires: C, 58·5; H, 4·6%.) It gave a green colour with alc. $FeCl_3$ and an osazone which recrystallized from EtOH as orange prisms, m.p. 242–244°, undepressed on admixture with the one obtained from periodate-oxidized mangiferin trimethyl ether. Their UV (340 and 400 mμ) and IR (Nujol) spectra were identical.

Periodate oxidation of mangiferin tetramethyl ether to the corresponding formyl compound

Mangiferin tetramethyl ether (0·4 g) in water (100 ml) and pyridine (10 ml) was oxidized using $NaIO_4$ (1·6 g in 100 ml water) and the reaction worked up as mentioned in the case of mangiferin trimethyl ether. The product recrystallized from EtOH as colourless needles, m.p. 245°. (Found: C, 61·6; H, 4·8 $C_{19}H_{18}O_8$ requires: C, 61·0; H, 4·8%.) It gave no ferric reaction and gave an orange-red osazone; UV: 260 and 380 mμ.

Synthesis of the formyl compound

1,3,6,7-*Tetramethoxy-2-allylxanthone.* 1-Hydroxy-3,6,7-tetramethoxy-2-allylxanthone was refluxed with Me_2SO_4 (excess), K_2CO_3 and acetone (120 hr). Recrystallization of the product from AcOH gave colourless needles, m.p. 147–148°. (Found: C, 67·6; H, 6·0. $C_{20}H_{20}O_6$ requires: C, 67·4; H, 5·6%.)

Osmium tetraoxide oxidation of 1,3,6,7-*tetramethoxy-2-allylxanthone to the corresponding diol* XI

The tetramethoxy-2-allylxanthone (0·4 g) in dry pyridine (5 ml) was oxidized using OsO_4 (0·3 g) as mentioned in the case of the trimethoxy compound. The diol crystallized from aqueous AcOH as colourless needles (0·26 g), m.p. 185–186°. (Found: C, 61·7; H, 5·7; $C_{20}H_{22}O_8$ requires: C, 61·5; H, 5·6%.)

Oxidation of the diol XI *to the aldehyde* XII

The diol (0·2 g) in glacial AcOH (35 ml) was oxidized using $NaIO_4$ (0·11 g) in water (40 ml). The product recrystallized from aqueous AcOH (charcoal) as light gray needles (0·12 g), m.p. 172°. (Found: C, 64·3; H, 5·2. $C_{19}H_{16}O_7$ requires: C, 63·8; H, 5·0%.) It gave a 2,4-dinitrophenylhydrazone, UV: 264 and 356 mμ.

Periodate oxidation of the aldehyde XII *to the corresponding hydroxy derivative* XIII

The aldehyde (0·1 g) in a mixture of glacial AcOH (40 ml) and THF (10 ml) was oxidized using $NaIO_4$ (0·065 g, 1 mole) in water (5 ml). The product crystallized from EtOH as colourless needles, m.p. 245° undepressed on admixture with the sample obtained after periodate oxidation of mangiferin tetramethyl ether. (Found: C, 61·5; H, 5·2; $C_{19}H_{18}O_8$ requires: C, 61·0; H, 4·8%.) It gave an orange–red osazone; UV: 260 and 380 mμ. UV spectra of this osazone and the one from periodate-oxidized mangiferin tetramethyl ether were superimposable.

Oxidation of mangiferin tertamethyl ether with neutral potassium permanganate

The methyl ether (0·5 g) in acetone (80 ml) and water (4 ml) was refluxed for 6 hr with $KMnO_4$ (4 g) and $MgSO_4$ (2 g). SO_2 was passed into the soln till it became colourless and the white product that separated was filtered off. It was purified by dissolving in $NaHCO_3$aq, acidifying the clear soln. and then extracting with ether. Recrystallization from AcOH gave colourless needles, m.p. 255–257° (Found: C, 58·7; H, 58·4; H, 4·6, 4·8; $C_{18}H_{16}O_8$, $\frac{1}{2}H_2O$ requires: C, 58·5; H, 4·6%.)

Oxidation of 1,3,6,7-*tetramethoxy-2-allylxanthone to the corresponding carboxylic acid*

2-Allyltetramethoxyxanthone (50 mg) in acetone (10 ml) and water (0·5 ml) was oxidized with $KMnO_4$ (0·4 g) containing $MgSO_4$ (0·2 g). The colourless product that separated after passing SO_2 recrystallized from AcOH to give colourless needles, m.p. 256–257° undepressed on admixture with the sample of the acid obtained from mangiferin tetramethyl ether. (Found: C, 58·0; H, 4·9; $C_{18}H_{16}O_8$ $\frac{1}{2}H_2O$ requires: C, 58·5; H, 4·6%.)