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NEW XANTHONES FROM *PSOROSPERMUM FEBRIFUGUM*

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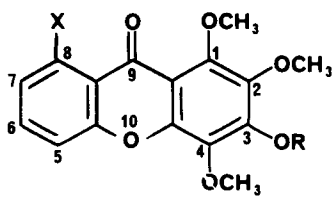
ABSTRACT.—The isolation and structural determination of two new xanthone derivatives, 1,2,4-trimethoxy-3,8-dihydroxyxanthone [**1**], 1,2,4-trimethoxy-3-hydroxyxanthone [**2**] and a known 2-methoxy-3-hydroxyxanthone [**3**] from *Psorospermum febrifugum* var. *ferrugineum* are reported. The structures were elucidated by extensive analysis of ^1H and ^{13}C nmr, ms, and chemical correlations.

In connection with the isolation of bioactive constituents in the roots of *Psorospermum febrifugum* Spach var. *ferrugineum* (Guttiferae), we have recently isolated and elucidated the structures of a new anthrone compound (1) and several novel psorospermin derivatives (2). In this paper, we report the isolation and structural elucidation of two xanthone derivatives.

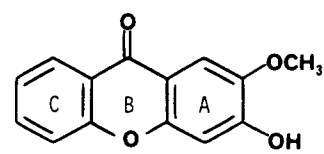
RESULTS AND DISCUSSION

The ground plant was extracted with 95% EtOH after percolation with hexane. The EtOH extract was further separated by several solvent partition and chromatographic steps to yield a fraction (fraction 1, Scheme 1), which upon crystallization from MeOH/Et_2O, yielded a crystalline compound **1** whose hrms indicated the molecular formula $C_{16}H_{14}O_7$. The uv absorption was typical of a polyoxygenated xanthone (3). A bathochromic shift upon adding NaOAc or $AlCl_3$ suggested the presence of two hydroxyl groups at either the 1,8 or 3,6 positions in **1**. This assignment was supported by the presence of two D_2O exchangeable singlets at 6.55 and 13.10 ppm in the ^1H-nmr spectrum. The presence of two hydroxy groups was further confirmed by formation of the diacetate **5** and pentamethyl ether **4**. The high field ^1H-nmr spectrum also indicated typical signals for three vicinal protons at 6.77 (dd, 8.3, 0.7 Hz, H_7), 6.92 (dd, 8.3, 0.7 Hz, H_5), and 7.54 (t, 8.3 Hz, H_6) assigned to the three aromatic protons in ring C. The remaining positions in ring A of the xanthone skeleton were occupied by three methoxyl groups that were observed as singlets at 3.98, 4.03, and 4.05 ppm. The downfield shift of H_5 ($\Delta\delta$ 0.46), H_6, and H_7 ($\Delta\delta$ 0.2) in the ^1H-nmr spectrum of diacetate **5** established that the *peri* hydroxyl was substituted at the 8-position. Based on these data, compound **1** is 3,8-dihydroxy-1,2,4-trimethoxyxanthone. Further confirmation of this is obtained based on the ^{13}C nmr of **1** discussed in a later section.

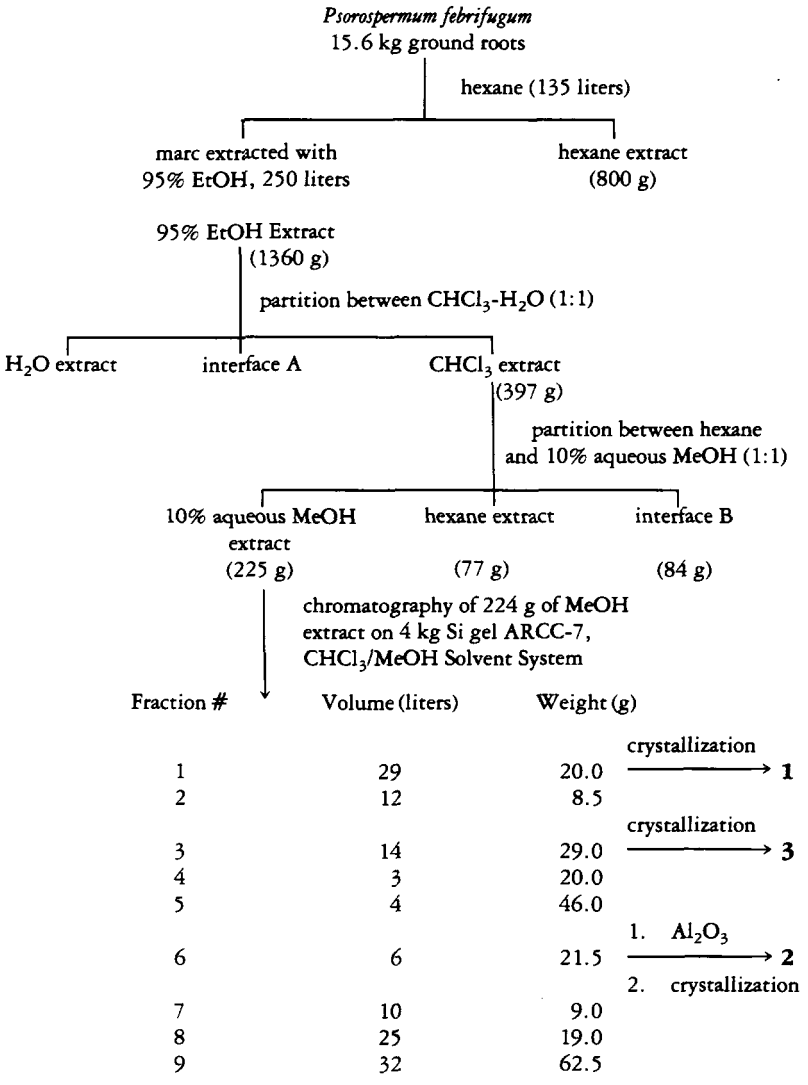
Crystallization of fraction 3 (Scheme 1) from MeOH/Et_2O gave a colorless, crystal-



1 R=H, X=OH
2 R=H, X=H
4 R=CH_3, X=OCH_3
5 R=Ac, X=OAc
6 R=CH_3, X=H
7 R=Ac, X=H



3



SCHEME 1. Isolation of Xanthones **1**, **2**, and **3**

line compound identified as 2-methoxy-3-hydroxyxanthone [**3**] from its physical properties and spectral analysis (4).

Fraction 6 (Scheme 1) was further separated by solvent partition, chromatography on an Al_2O_3 column, acid-base partition, and, finally, by crystallization from MeOH to yield **2** as a yellow crystalline compound whose uv absorption was again typical of a substituted xanthone. A bathochromic shift upon adding NaOAc suggested the presence of a 3-hydroxyl group, which was supported by the observation of a D_2O exhangeable signal at 6.44 (s) ppm and also by formation of a monoacetate **7** and tetramethyl ether derivative **6**. Four interrelated aromatic proton signals at 7.51 (dd, 8.3 and 1.0 Hz, H_5), 7.69 (ddd, 8.3, 7.1 and 1.6 Hz, H_6), 7.37 (ddd, 8.0, 7.1 and 1.0 Hz, H_7), and 8.30 (dd, 8.0 and 1.6 Hz, H_8) ppm could be attributed to the proton resonances of the C-ring. The methyl proton signals at 3.98 (s), 4.00 (s), and 4.04 (s) were assigned to the methoxy proton resonances attached to ring A. Based on analysis of this data, compound **2** is 1,2,4-trimethoxy-3-hydroxyxanthone.

Prior studies on the ^{13}C nmr of xanthonoids were reviewed by Sultanbawa (5,6).

We have extended this study to include the xanthones isolated from *P. febrifugum*. The chemical shifts were assigned with the aid of ^{13}C nmr (proton fully coupled and proton noise decoupled) and by the application of known chemical shift rules, substituent effects, and comparison of the results among compounds possessing identical structural features. These data provide a basis for further structural work among these compounds.

The hydroxy carbons (C_3 and C_8) in both compounds **1** and **3** were readily identified as singlets in their proton fully coupled spectra. All the carbons (C_1, C_2, C_4) bearing $-OCH_3$ groups were observed as quartets due to long range coupling with methyl protons. Two of four quaternary carbons (C_{9a} and C_{4a}) appeared as singlets whereas C_{8a} and C_{10a} were coupled with the *meta* aromatic protons. In compound **1**, C-9 showed a downfield shift of 7 ppm compared to **2** and **3**, which suggests chelation with an adjacent hydroxy group. The multiplicity (dd, due to long range coupling with H_7 and H_6) and the chemical shift of C_8 in the gated decoupled spectrum of **1** further confirmed the position of the chelated hydroxy group at C_8. The rest of the assignments are in excellent agreement with related xanthones (7).

EXPERIMENTAL

PLANT MATERIAL.—*P. febrifugum* var. *ferrugineum* roots were collected in Tanzania by Mr. Leonard Mwasumbi of the University of Dar-es-Salaam. Specimens of the plant collected by the same person from the same location had previously been authenticated at the Economic Botany Laboratory, Beltsville Agricultural Research Center, Beltsville, Maryland, where a voucher specimen is on deposit.

GENERAL EXPERIMENTAL PROCEDURES.—All melting points are uncorrected and were obtained on a Laboratory Devices Mel-Temp apparatus. Column chromatography was performed on ARCC-7 Si gel (Mallinckrodt, Inc., Paris, Kentucky). Ir spectra were obtained in KBr on a Beckman IR-33 spectrophotometer. Uv spectra were recorded in the solvents indicated on either a Cary 17 or Perkin-Elmer-Coleman 124 double beam spectrophotometer. Electron impact and chemical ionization mass spectra were obtained on a Finnigan model 4023 mass spectrometer, and high resolution accurate mass measurements were made on a Kratos MS 50 mass spectrometer. ^1H-nmr spectra were obtained in the solvent indicated on either a Varian XL-200 or the Nicolet 470 MHz spectrometer at the Purdue University Biological Magnetic Resonance Laboratory (NIH Grant RR1077). Chemical shifts are given as ppm with reference to TMS or to solvent, and *J*-values are reported in Hz. ^{13}C-nmr spectra were recorded on a Nicolet-200 spectrometer operating at 50.31 MHz in the FT mode in DMSO-d_6. Chemical shifts are in ppm downfield from TMS.

EXTRACTION AND FRACTIONATION.—The large, woody roots were coarsely chipped, defatted by percolation with hexane, dried, then finely ground. The powdered plant material (15 kg) was defatted with hexane, then extracted with 95% EtOH by slow percolation at room temperature. Solvent was removed in vacuo at 40°, and the residue was partitioned between $CHCl_3$ and H_2O. Following removal of solvent the $CHCl_3$ solubles were partitioned between hexane and 10% aqueous MeOH.

The MeOH extract was chromatographed over 4 kg of ARCC-7 silica using a gradient of increasing MeOH in $CHCl_3$. Nine fractions were collected based on tlc.

Fraction 1, (20 g, eluted by 1% MeOH in $CHCl_3$), was partitioned between 20% aqueous MeOH and hexane. From the methanolic phase, following extraction with $CHCl_3$ and crystallization from MeOH/Et_2O, **1** (100 mg) was obtained.

Fraction 3, (29 g, eluted by 1% MeOH in $CHCl_3$), showing a major blue fluorescent spot, was partitioned between 20% aqueous MeOH and toluene-hexane (1:1). From the methanolic phase, following extraction with $CHCl_3$ and crystallization from MeOH, **3** (180 mg) was obtained.

Fraction 6 (21.5 g, eluted by 2% MeOH in $CHCl_3$) was treated in the same way as Fraction 3. Examination of the liquors remaining after crystallization from MeOH revealed the presence of two major blue fluorescent compounds that were identical with major constituents of the interfacial Fraction B with which they were pooled. An aliquot of this material, 11.4 g, was chromatographed on 270 g of Al_2O_3, eluted stepwise by $CHCl_3$, $CHCl_3$-EtOAc (1:1), EtOAc, EtOAc-MeOH (1:1), and MeOH.

The third fraction, eluted by $CHCl_3$-EtOAc (1:1), was shaken with aqueous 5% $KHCO_3$ solution, and the aqueous layer was acidified and extracted with $CHCl_3$. Compound **2** (85 mg) was obtained as yellow needles from MeOH.

1,2,4-TRIMETHOXY-3,8-DIHYDROXYXANTHONE [**1**].—Crystallized from MeOH/Et_2O as yellow needles, mp 182-184°; uv (MeOH) λ max (log ε) 355 (4.33), 310 (4.49), 238 (4.92) nm, with NaOAc,

366 (4.78), 263 (4.63), 222 (5.13) nm, with $AlCl_3$, 419 (3.84), 355 (4.24), 281 (4.39), 263 (4.47) nm (no change upon addition of HCl); ir (KBr) ν max (cm^{-1}) 3300, 1680, 1580; ^1H nmr (470 MHz, $CDCl_3$) δ 3.98 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 6.55 (1H, s, 3-OH), 6.77 (1H, dd, 8.3, 0.7 Hz, H_7), 6.92 (1H, dd, 8.3, 0.7 Hz, H_5), 7.54 (1H, t, 8.3 Hz, H_6), 13.10 (1H, s, 8-OH); ^{13}C nmr (50 MHz, DMSO-d_6) 130.0 (C-1), 148.4 (C-2), 151.5 (C-3), 139.0 (C-4), 111.7 (C-5), 132.0 (C-6), 104.0 (C-7), 162.0 (C-8), 181.5 (C-9), 147.2 (C-4a), 107.0 (C-8a), 108.7 (C-9a), 154.8 (C-10a), 62.0, 61.8, 61.7 (OCH_3); eims *m/z* (rel. int., %) 318 (M^+, 89), 303 (M^+-CH_3, 100), 275 (45), 260 (45), 189 (20), 137 (11), 120 (6); ei-hrms, obsd. 318.0747 (M+) calcd. for $C_{16}H_{14}O_7$, 318.0739.

1,2,3,4,8-PENTAMETHOXYXANTHONE [**4**].—A solution of compound **1** (15 mg, 0.04 mmol) and K_2CO_3 (15 mg, 0.18 mmol) in 8.0 ml of Me_2CO was treated with 3 drops of iodomethane at room temperature overnight. The reaction mixture was filtered, the residue was washed with 2×5.0 ml of Me_2CO, and the filtrate was evaporated under reduced pressure. The residue thus obtained was purified by Si gel thick-layer chromatography (5% MeOH in $CHCl_3$) to give the methyl ether as a colorless crystalline solid (10 mg, 63%), mp 110-112°; uv (MeOH) λ max (log ϵ) 350 (3.50), 305 (3.82), 249 (4.26), 201 (4.24) nm; ^1H nmr (80 MHz, $CDCl_3$) δ 3.92 (3H, s, OCH_3), 3.99 (6H, s, 2×OCH_3), 4.02 (3H, s, OCH_3), 4.09 (3H, s, OCH_3), 6.77 (1H, dd, 8.3, 1.0 Hz, H_7), 7.04 (1H, dd, 8.3, 1.0 Hz, H_5), 7.54 (1H, t, 8.3 Hz, H_6); cims (CH_4) *m/z* (rel. int., %), 347 ($M+H^+$, 100), ei-hrms, obsd. 346.1049 (M^+) calcd. for $C_{18}H_{18}O_7$, 346.1052.

1,2,4-TRIMETHOXY-3,8-DIACETOXYXANTHONE [**5**].—A solution of compound **1** (7.0 mg, 0.02 mmol) in 0.5 ml of pyridine was treated with 0.5 ml of Ac_2O overnight at room temperature. After usual workup the acetate **5** crystallized as white needles (7.0 mg, 80%) from MeOH, mp 128-130°; ^1H nmr (80 MHz, $CDCl_3$) δ 2.42 (3H, s, $OCOCH_3$), 2.48 (3H, s, $OCOCH_3$), 3.90 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 6.97 (1H, dd, 7.6, 1.4 Hz, H_7), 7.38 (1H, dd, 8.5, 1.4 Hz, H_5), 7.69 (1H, dd, 8.5, 7.6 Hz, H_6); cims (CH_4) *m/z* (rel. int., %) 403 ($M+H^+$, 100), 389 (8), 361 (35).

2-METHOXY-3-HYDROXYXANTHONE [**3**].—Large yellow prisms from MeOH, mp 228-230° (lit. 225-230°) (4); uv (MeOH) λ max (log ϵ) 350 (3.93), 3.15 (4.12), 270 (sh), 237 (4.59) nm, with NaOAc 370 (4.4), 379 (sh), 230 (4.73) nm; ir (KBr) ν max 3290, 1600, 1575, 1115 cm^{-1}; ^1H nmr (470 MHz, $CDCl_3$) δ 4.01 (3H, s, 2-OCH_3), 7.00 (1H, s, H_4), 7.35 (1H, ddd, 8.0, 7.1, 1.1 Hz, H_7), 7.45 (1H, dd, 8.4, 1.1, 0.5 Hz, H_5), 7.67 (1H, ddd, 8.4, 7.4, 1.8 Hz, H_6), 7.68 (1H, s, H_1), 8.30 (1H, ddd, 8.0, 1.8, 0.5, H_8); ^{13}C nmr (50 MHz, DMSO-d_6) δ 105.3 (C-1), 146.1 (C-2), 152.0 (C-3), 102.8 (C-4), 117.8 (C-5), 134.4 (C-6), 124.0 (C-7), 125.7 (C-8), 174.5 (C-9), 154.5 (C-4a), 120.7 (C-8a), 113.2 (C-9a), 155.4 (C-10a), 55.8 (2-OCH_3); cims (CH_4) *m/z* (rel. int., %) 243 ($M+H^+$, 100), 229 (1); ei-hrms, obsd. 242.0580 (M^+) calcd. for $C_{14}H_{10}O_4$, 242.0579.

1,2,4-TRIMETHOXY-3-HYDROXYXANTHONE [**2**].—Crystallized from MeOH as yellow needles, mp 166-167°; uv (MeOH) λ max (log ϵ) 350 (3.99), 304 (4.3), 279 (4.2), 238 (4.66) nm, with NaOAc, 360 (4.4), 275 (sh), 235 (4.68) nm; ^1H nmr (470 MHz, $CDCl_3$) δ 3.98 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 7.37 (1H, ddd, 8.0, 7.1, 1.0 Hz, H_7), 7.51 (1H, dd, 8.3, 1.0 Hz, H_5), 7.69 (1H, ddd, 8.3, 7.1, 1.6 Hz, H_6), 8.30 (1H, dd, 8.0, 1.6 Hz, H_8); ^{13}C nmr (50 MHz, DMSO-d_6) δ 132.3 (C-1), 148.6 (C-2), 150.5 (C-3), 138.7 (C-4), 117.5 (C-5), 134.5 (C-6), 124.0 (C-7), 125.8 (C-8), 173.9 (C-9), 147.3 (C-4a), 121.5 (C-8a), 108.5 (C-9a), 154.3 (C-10a), 61.6, 61.8, 61.7 (OCH_3); cims (CH_4) *m/z* (rel. int., %) 303 ($M+H^+$, 100), 289 (1), 273 (2), ei-hrms, obsd. 303.0868 ($M+H^+$) calcd. for $C_{16}H_{14}O_6+H^+$, 303.0868.

1,2,3,4-TETRAMETHOXYXANTHONE [**6**].—A solution of compound **2** (10 mg, 0.033 mmol) and K_2CO_3 (20 mg, 0.14 mmol) in 6.0 ml of dry Me_2CO was treated with two drops of iodomethane at room temperature overnight. The reaction mixture was filtered, the residue washed with Me_2CO (2×5.0 ml), and the combined filtrate was evaporated under vacuo. The residue thus obtained was purified by Si gel thick-layer chromatography (5% MeOH in $CHCl_3$) to yield the tetramethylether as colorless needles (10 mg, 95%), mp 82-84°; uv (MeOH) λ max (log ϵ) 348 (4.01), 285 (4.32), 247 (4.80), 210 (4.62) nm; ^1H nmr (200 MHz, $CDCl_3$) δ 3.95 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 4.13 (3H, s, OCH_3), 7.37 (1H, dd, 8.3, 7.2, 1.0 Hz, H_7), 7.51 (1H, dd, 8.7, 1.0 Hz, H_5), 7.69 (1H, ddd, 8.7, 7.2, 1.5 Hz, H_6), 8.30 (1H, dd, 8.3, 1.5 Hz, H_8); cims (CH_4) *m/z* (rel. int., %) 317 ($M+H^+$, 100), ei-hrms, obsd. 316.0935 (M^+) calcd. for $C_{17}H_{16}O_6$, 316.0947.

1,2,4-TRIMETHOXY-3-ACETOXYXANTHONE [**7**].—A solution of compound **2** (7.0 mg, 0.02 mmol) in 0.2 ml of pyridine and 0.2 ml of Ac_2O was reacted overnight at room temperature. After workup the acetate **7** crystallized from MeOH as white needles (7.0 mg, 88%), mp 132-134°; uv (MeOH) λ max (log ϵ) 348 (3.41), 275 (3.67), 248 (4.20), 203 (4.20) nm; ^1H nmr (470 MHz, $CDCl_3$) δ 2.44 (3H, s, $OCOCH_3$), 3.92 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 7.37 (1H, ddd, 8.0, 7.1,

1.0 Hz, H_7), 7.51 (1H, dd, 8.4, 1.0 Hz, H_5), 7.70 (1H, ddd, 8.4, 7.1, 1.6 Hz, H_6), 8.30 (1H, dd, 8.0, 1.6 Hz, H_8); cims (CH_4) *m/z* (rel. int., %), 345 ($M+H^+$, 100), 303 (M^+-COCH_2, 2.5).

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LITERATURE CITED

1. A. Amonkar, C. Chang, and J.M. Cassady, *Experientia*, **37**, 1138 (1981).
2. A.M. Habib, D.K. Ho, S. Masuda, T. McCloud, K.S. Reddy, M. Aboushoer, A. McKenzie, S.R. Byrn, C. Chang, and J.M. Cassady, *J. Org. Chem.*, (in press).
3. M.L. Cardona, M.I. Fernandez, J.R. Pedro, E. Seoane, and R. Vidal, *J. Nat. Prod.*, **49**, 95 (1986).
4. H.D. Locksley and I.G. Murray, *Phytochemistry*, **10**, 3179 (1971).
5. M.U.S. Sultanbawa, *Tetrahedron*, **36**, 1465 (1980).
6. P.W. Westerman, S.P. Gunasekera, M.U.S. Sultanbawa, and R. Kazlanskas, *Org. Magn. Reson.*, **9**, 631 (1977).
7. J.F. Castelao, Jr., O.R. Gottlieb, R.A. DeLima, H.A.L. Mesquito, H.E. Gottlieb, and E. Wenkert, *Phytochemistry*, **16**, 735 (1977).

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