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PENTAOXYGENATED XANTHONES FROM *BREDEMEYERA FLORIBUNDA*

EDILBERTO R. SILVEIRA,* MARIA JOSÉ C. FALCÃO, ALISIO MENEZES JR, DAVID G. I. KINGSTON† and THOMAS E. GLASS†

Curso de Pós-Graduação em Quimica Orgânica, Laboratório de Produtos Naturais, Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Cx, Postal 12.200, Fortaleza, 60.021, Ceará, Brazil; †Virginia Polytechnic Institute and State University, Department of Chemistry, Blacksburg, VA, 24061, U.S.A.

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Key Word Index—*Bredemeyera floribunda*; Polygalaceae; roots; "raiz-de-cobra"; pentaoxygenated xanthones.

Abstract—A chloroform extract of the roots of *Bredemeyera floribunda* yielded two new pentaoxygenated xanthones, 1,7-dihydroxy-3,4,8-trimethoxyxanthone and 1,3,7-trihydroxy-4,8-dimethoxyxanthone, and the ethanol extract of the resulting marc yielded 1,3,6-trihydroxy-2,7-dimethoxyxanthone. Structure determination of these pentaoxygenated xanthones was accomplished by spectral analysis, mainly NMR, including normal and inverse detection techniques such as HETCOR and HMBC. Chemical derivatization and comparison to literature data were also used.

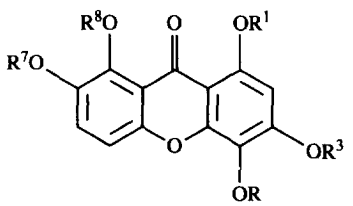
INTRODUCTION

Bredemeyera floribunda Wild. popularly designated as "raiz-de-cobra" (Portuguese = snake's root), belongs to the family Polygalaceae. Some species of the *Polygala* genus, from the same family, have several popular medicinal uses, particularly as expectorants, and are well known to contain polyoxygenated xanthones [1, 2]. An alcoholic solution made from roots of *B. floribunda* is used orally by the peasant people of Ceará, northeast Brazil, to treat snakebite, and a concentrated extract showed antidote activity against both *Bothrops* and *Crotalus* venoms (W. B. Mors, NPPN-UFRJ, personal communication). Rao and co-workers have shown that the ethanol extractives from roots of *B. floribunda* offer protection to gastric lesions induced by ethanol, acetylsalicylic acid and histamine [3]. We now report on the phytochemical investigation of the roots of *B. floribunda*.

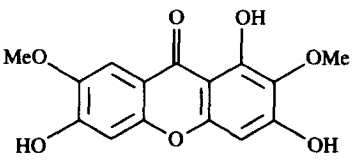
RESULTS AND DISCUSSION

Ground roots of *B. floribunda* were initially extracted with chloroform to yield a viscous residue, after solvent evaporation, designated BFR-C. The marc obtained after chloroform extraction was extracted with ethanol to yield a resinous residue designated BFR-E. Liquid–liquid partitioning and successive column chromatography of BFR-C yielded **1** and **2**. Adsorption chromatography of BFR-E yielded **3**.

Compound **1**, the less polar and major constituent, and **2**, somewhat more polar than **1** and a minor constituent,



	R^1	R^3	R^4	R^7	R^8
1	H	Me	Me	H	Me
2	H	H	Me	H	Me
4	H	Me	Me	Me	Me
5	Me	Me	Me	Me	Me
6	H	H	Me	Me	Me
7	H	Me	H	Me	H
8	H	H	Me	Me	H



had very similar ^1H and ^{13}C NMR spectra (Tables 1 and 2). For both, the ^1H NMR spectra showed a hydrogen exchangeable with D_2O in the far downfield region (δ13.01 and 13.03, respectively) characteristic of a hydroxyl chelated to a carbonyl group. Both showed two

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data for compounds **1**–**5**, and comparison to literature models (**1**, **3**, **4**, **6**–**8**)

H	**1** D*	**1** [6] D	**6** [4] D	**2** A	**3** P	**3** [2] P	**4** C	**4** [5] C	**5** C	**7** [4] D	**8** [9] C + D
2	6.39	6.47	6.50	6.25			6.38	6.40	6.37	6.53	6.41
4					6.77	6.78					
5	7.25	7.23	7.25	7.28	7.16	7.17	7.27	7.05	7.23	6.87	7.06
6	7.36	7.38	7.39	7.41			7.33	7.28	7.26	7.27	7.46
8					7.81	7.81					
OR	3.88	3.79	3.76	3.87	3.76	3.77	3.92	3.94	3.91	3.82	3.93
OR	3.94	3.83	3.80	3.90	3.97	4.00	3.94	to	3.92	3.92	
OR	4.00	3.92	3.90				3.96	4.02	3.99		
OR	5.95†		9.60†				4.01		4.00	11.43†	11.79†
OR	13.01†	13.05†	13.13†	13.02†	14.07†	14.09†	13.17†	13.04†	4.02	11.87†	12.08†

*A, Acetone-d_6; C, chloroform-d; D, DMSO-d_6; M, methanol-d_4 and P, pyridine-d_5.
†Exchangeable with D_2O, R = H.

Table 2. ^{13}C NMR data for compounds **1**–**5** and comparison to literature models **6** and **7**

C	**1** P*	**1** D	**1** C + M	**2** A	**3** D	**3** [2] P	**4** C	**5** C	**6** [4] D	**7** [4] D
1	159.9	159.3	159.4	159.8	153.4	153.8	159.5	157.3	159.2	157.3
2	95.0	94.6	94.3	98.4	130.5	131.7	94.3	91.0	94.6	95.0
3	159.7	158.4	158.7	158.8	152.4	155.2	159.3	156.8	158.4	160.0
4	128.5	127.5	127.9	127.8	94.0	94.8	127.9	129.5	127.5	129.7
4a	148.4	148.3	148.8	150.0	157.9	159.4	150.8	151.1	145.3	147.0
4b	150.4	149.4	150.4	151.0	154.6	156.3	148.6	148.5	149.4	147.9
5	114.0	113.4	113.9	114.4	102.7	103.8	115.4	112.3	113.4	106.1
6	125.4	124.6	123.6	124.4	151.9	153.4	120.3	118.5	124.4	124.1
7	148.4	147.0	146.0	147.8	146.0	146.8	149.2	149.9	137.6	140.4
8	146.3	145.4	144.7	146.1	104.5	105.3	148.7	149.3	145.3	147.9
8a	115.6	114.6	114.4	116.3	111.1	112.2	113.0	118.0	113.4	106.1
8b	103.8	102.2	103.3	103.4	101.8	103.1	103.4	107.8	102.7	101.4
C=O	181.7	180.8	181.0	181.6	179.1	180.4	181.3	175.5	180.6	184.0
OMe	61.6	61.0	62.1	62.2	60.0	60.3	61.7	61.6	61.0	56.7
OMe	61.3	60.9	61.5	61.7	55.9	55.9	61.6	61.5	60.9	56.7
OMe	56.3	56.4	56.0	—	—	—	56.9	56.8	56.4	—
OMe	—	—	—	—	—	—	56.2	56.3	—	—
OMe	—	—	—	—	—	—	—	56.1	—	—

*Abbreviations: see Table 1.

pairs of doublets centred at δ7.36 (1H, J = 9.0 Hz) for **1** and δ7.41 for **2**, and δ7.25 (1H, J = 9.0 Hz) for **1** and δ7.28 for **2**. Both showed a singlet at δ6.39 (1H) and δ6.25 (1H) for **1** and **2**, respectively. Besides the other D_2O exchangeable hydrogens, the major difference between **1** and **2** was that the latter had only two methoxy groups whereas the former presented three methoxy absorptions.

The ^{13}C NMR spectra of both compounds were also very similar (Table 1). They showed 13 sp^2 carbons with very similar chemical shifts, and as was expected three and two methoxy absorptions for **1** and **2**, respectively. The methoxy group absorptions were very informative, since both compounds exhibited two methoxy absorptions around δ61.0, indicative of the steric crowding around both groups. In addition, **1** showed a signal for one unhindered aromatic methoxy group at δ56.4.

Compound **1**, showed a molecular ion (HR-MS) at m/z 318.0740 ($C_{16}H_{14}O_7$ requires 318.0739), whereas **2**, showed a molecular ion (HR-MS) at m/z 304.0588 ($C_{15}H_{12}O_7$ requires 304.0583), as was expected 14 amu lower than that of **1**.

At this point, a pentaoxygenated xanthone pattern fitted all of the spectrometric data. Since one hydroxy group was chelated and two methoxy groups were sterically compressed, and since the coupled hydrogen system was not at the C-7, C-8 position (based on arguments developed below), only structures **1** and **6** were possible for **1**. Structure **6** was excluded based on the lack of any change in the UV spectrum in the presence of sodium acetate and ^{13}C NMR comparison to the literature data (see Table 1) [4]. Thus, **1** had to be 1,7-dihydroxy-3,4,8-trimethoxyxanthone (**1**). Methylation of **1** with excess

diazomethane yielded the methoxy derivative, **4**, with similar physical and spectroscopic properties to the literature data for this compound [5]. As was expected, the extra methoxy of **4** absorbed at δ56.0 in the ^{13}C NMR spectrum, compatible with the facile methylation of the non-chelated hydroxy at C-7. Meanwhile, permethylation of **1** with Me_2SO_4 in dried acetone yielded the expected pentamethoxy derivate **5**.

COSY, HETCOR and HMBC experiments (Fig. 1(a)) allowed the NMR assignments suggested in Tables 1 and 2.

As was stated earlier, the only difference between **1** and **2** was the lack of the methoxy group at δ56.4 for **2**, compared to **1**. The presence of signals for two sterically compressed methoxy groups at δ62.2 and 61.7 in **2** was indicative of the presence of methoxy substituents at C-4 and C-8, and thus **2** had to be the C-3 desmethyl analogue of **1**, or 1,3,7-trihydroxy-4,8-dimethoxyxanthone (**2**).

A literature survey revealed that Hong-fa and Jing-ye claimed to have isolated 1,7-dihydroxy-3,4,8-trimethoxyxanthone (**1**) from *Swertia mussotii* [6]. Unfortunately, there was no ^{13}C data for comparison, but their ^{1}H NMR data was not compatible with our data (see Table 1), obtained in the same solvent (DMSO-d_6). Later, Lin *et al.* [4] isolated, from *Tripterospermum lanceoletum*, a compound they named methyllanceolin, 1,3-dihydroxy-4,7,8-trimethoxyxanthone (**6**), whose ^{1}H NMR data (Table 2) is identical with the xanthone from *S. mussotii*. Moreover, the melting points are comparable (218–220° for **6** and 217.3° for the *S. mussotii* xanthone) and the UV data are identical [4, 6]. These data lead to the conclusion that the xanthone from *S. mussotii* is **6** and not **1**. Thus **1** and **3**, to the best of our knowledge, are now being reported for the first time in the literature. The 1,3,4 oxygenation pattern has already been observed for the Polygalaceae family [7].

Compound **3** (mp 229–230°), the most polar of all three xanthones, showed a $[M]^+$ at *m/z* 304 ($C_{15}H_{12}O_7$) and was, therefore, an isomer of 2. Its ^{13}C NMR spectrum showed that it had only one sterically hindered methoxy group with a signal at δ60.3. It also showed a chelated hydroxy group at δ13.20 in its ^{1}H NMR spectrum, but unlike **1** and **2** its aromatic protons appeared as three singlets at δ7.42, 6.84, and 6.42, in DMSO-d_6; the proton singlet at δ6.25, common to both **1** and **2**, was no longer present in **3**. The disappearance of the *ortho* coupled system was further evidence that **3** had an oxidation pattern which was different from that of **1** and **2**. HETCOR and HMBC experiments (Fig. 1(b)) were useful tools for structure determination of **3** as the 1,3,6-trihydroxy-2,7-dimethoxyxanthone (**3**), and the suggested NMR assignments on Tables 1 and 2. This compound has previously been isolated from *Polygala tenuifolia* [2], and a comparison of its ^{1}H and ^{13}C NMR data (Tables 1 and 2) with those of onjixanthone II (lit. 231–233° [2]) indicate the two to be identical. This xanthone has been shown to be an active ingredient of *P. tenuifolia*, causing inhibition of aldose reductase, useful in the treatment of complications of diabetes [8].

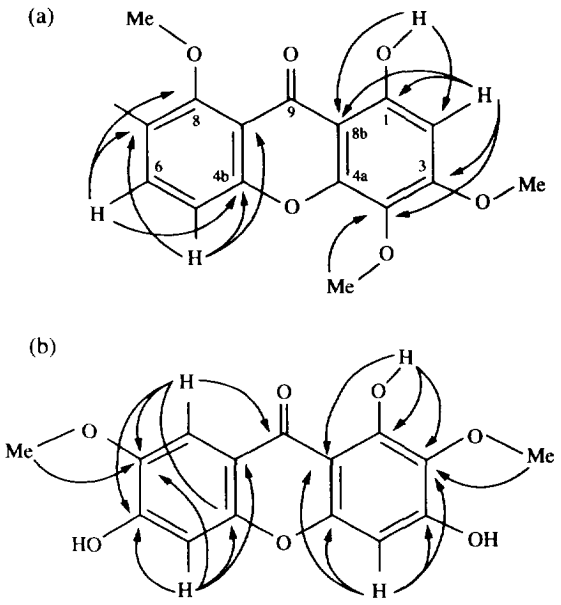


Fig. 1. ^{1}H, ^{13}C-long-range correlation observed through inverse-detected NMR experiment (HMBC) for: BFR-1(**1**) Fig. 1(a) and BFR-3(**3**) Fig. 1(b).

EXPERIMENTAL

General. Mps: uncorr.; ^{1}H NMR: 200 and 400 MHz; ^{13}C NMR: 50 and 100 MHz; EIMS: 70 eV.

Plant material. Bredemeyera floribunda plants were collected in Vicçosa-Ce, Brazil, and identified by Dr Afrânio G. Fernandes (Universidade Federal do Ceará). A voucher specimen (#15.844) representing the collection was deposited at the Herbário Prisco Bezerra of the Departamento de Biologia, Universidade Federal de Ceará, Brazil.

Extraction and isolation of constituents. After removal of the aerial part, the roots (3.8 kg) were dried, ground and extracted with $CHCl_3$ to yield 76.0 g of a brown viscous extract, designated BFR-C, after solv. evapn. The marc obtained after $CHCl_3$ extraction was extracted with EtOH to yield 119.9 g of a vitreous brown resin designated BFR-E. Hexane–MeOH liquid partition of BFR-C yielded 33.5 g of the hexane solubles denominated BFRC-H and 35.4 g of the MeOH solubles denominated BFRC-M. BFRC-M was adsorbed on 80 g of silica gel and was coarsely chromatographed over a small layer of silica gel by elution with hexane (BFRCM-H 0.9 g), $CHCl_3$ (BFRCM-C, 12.1 g), EtOAc (BFRCM-A, 4.1 g), and finally MeOH (BFRCM-M, 16.6 g).

1,7-*Dihydroxy*-3,4,8-*trimethoxyxanthone* (**1**). Successive CC over silica gel of BFRCM-C yielded 174.6 mg of a yellow amorphous solid, homogeneous by TLC, designated **1**, mp 213–214°, HR-EI-MS *m/z* 318.0740 (Calc. for $C_{16}H_{14}O_7$, 318.0739); EI-MS *m/z* (rel. int.): 318 (65), 303 (100), 288 (45), 285 (33), 271 (20), 259 (22); ^{1}H NMR (200 MHz, DMSO-d_6): Table 1; ^{13}C NMR (100 MHz, $CDCl_3$ + MeOD; 50 MHz, DMSO-d_6 or pyridine-d_5): Table 2. UV λ^{MeOH} nm (log ε): 237 (4.44), 266 (4.58), 320

(4.04), 385 (3.68); $\lambda^{MeOH+NaOAc}$: 237, 267, 322, 387; $\lambda^{MeOH+AlCl_3}$; 238, 282, 323, 365; $\lambda^{MeOH+NaOH}$: 250, 275, 310 sh, 345.

1-*Hydroxy*-3,4,7,8-*tetramethoxyxanthone* (**4**). Compound **1** (60 mg) was treated with excess CH_2N_2 soln in Et_2O for two days. The reaction mixture, after evapn of the solvent, was chromatographed over a small layer of silica gel to yield 50 mg of **4**, mp 193–194° (lit. 192–194°) (5); EI-MS *m/z* (rel. int.): 332 (35), 317 (65), 299 (18), 273 (20), 259 (42), 77 (90), 69 (100); ^{1}H NMR (200 MHz, $CDCl_3$): Table 1; ^{13}C NMR (50 MHz, $CDCl_3$): Table 2.

1,3,4,7,8-*Pentamethoxyxanthone* (**5**). Compound **1** (70 mg) was refluxed with freshly distilled Me_2SO_4 in dry Me_2CO, in the presence of K_2CO_3. Five hours later, after complete disappearance of the starting material (TLC), the reaction mixture was worked-up and chromatographed over silica gel to yield two products, one identical by TLC to **4** and the other (**5**) (31.3 mg), less polar than **4**, mp 185–188°; EI-MS *m/z* (rel. int.): 346 (30), 331 (28), 317 (15), 301 (13), 273 (40), 167 (11), 137 (11), 77 (100), 69 (80). ^{1}H NMR (200 MHz, $CDCl_3$): Table 1; ^{13}C NMR (50 MHz, $CDCl_3$): Table 2.

1,3,7-*Trihydroxy*-4,8-*dimethoxyxanthone* (**2**). The most polar fractions from BFRCM-C showed a spot on TLC corresponding to a more polar compound than **1**. The yellow solid was washed with warm $CHCl_3$ to yield a residue (33.0 mg), not soluble in $CHCl_3$, homogeneous by TLC, mp 225–226°, designated **2**; HR-EI-MS *m/z* 304.0588 ($C_{15}H_{12}O_7$ requires 304.0583); EI-MS *m/z* (rel. int.): 304 $[M]^+$ (80), 289, (100), 286 (25), 271 (40), 261 (50), 246 (73), 218 (27), 162 (13), 123 (28), 93 (18), 69 (65); ^{1}H NMR (200 MHz, Me_2CO-d_6): Table 1; ^{13}C NMR (50 MHz, Me_2CO-d_6): Table 2; UV λ^{MeOH} nm (log ε): 239 (4.38), 267 (4.48), 320 (4.01), 335sh (3.98); $\lambda^{MeOH+AlCl_3}$: 237, 283, 335; $\lambda^{MeOH+NaOH}$: 247, 282, 355; $\lambda^{MeOH+NaOAc}$: 241, 277, 350.

Methylation of **2** (20 mg) with excess CH_2N_2 yielded a mixture of methyl ethers with very similar R_fs. The less polar of them was sepd (5.3 mg), mp 191–193°, and was shown to be **4** by TLC co-chromatography and ^{1}H NMR comparison.

1,3,6-*Trihydroxy*-2,7-*dimethoxyxanthone* (**3**). The vitreous resin, BFR-E, (119 g) was placed in a Soxhlet thimble and extracted in a glass Soxhlet apparatus with hexane, followed by $CHCl_3$, EtOAc and finally MeOH, to yield 0.3 g (BFRE-H), 13.9 g (BFRE-C), 12.7 g (BFRE-A) and 80.1 g (BFRE-M) after evapn of the respective solvents. Successive CC over silica gel of BFRE-C yielded a fraction which on rotational TLC (Chromatotron) gave 15 mg of **1** and 37 mg of an amorphous pale yellow solid designated **3**: mp 229–230° (lit. 231–233°) [2]; HR-EI-MS 304.0581 ($C_{15}H_{12}O_7$ requires 304.0583); EI-MS *m/z* (rel. int.): 304 $[M]^+$ (100), 289 (93), 286 (21), 261 (90), 246 (28), 218 (10), 152 (8), 83 (25), 69 (28); ^{1}H NMR (400 MHz pyridine-d_5): Table 1; ^{13}C NMR (100 MHz, DMSO-d_6): Table 2; UV λ^{MeOH} nm (log ε): 242 (4.45), 258 (4.41), 323 (4.28), 363 (4.12).

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