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Pentaoxygenated xanthones and fatty acids from *Bredemeyera brevifolia*

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Abstract

The polar portion of an ethanol extract of *Bredemeyera brevifolia* yielded two pentaoxygenated xanthones whose structures were determined as 1-methoxy-2,3,7,8-dimethylenedioxyxanthone, and 1,7,8-trimethoxy-2,3-methylene-dioxyxanthone. The apolar fraction contained various known fatty acids, structure determinations were accomplished by spectral analysis, mainly NMR, chemical derivatization and comparison to literature values. © 2000 Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

The Polygalaceae family has been long known as a good source of polyoxygenated xanthones (Carpenter et al., 1969; Dreyer 1969). Although its phytochemical study has been restricted to *Polygala* (Andrade et al., 1977; Sultanbawa, 1980; Ghosal et al., 1981; Habib et al., 1987; Marston et al., 1993; Ykeya et al., 1991) and *Bredemeyera* genera (Silveira et al., 1995; Peres and Nagem, 1997), most of the xanthones isolated from this family shows a pentaoxygenated pattern. We have already reported on the phytochemical analysis of two species belonging to the genera *Bredemeyera* and *Polygala*, which presented some physiological properties (Andrade et al., 1977; Rao et al., 1990; Silveira et al., 1995). In the course of our investigation of the Polygalaceae native to northeastern Brazil, we report the phytochemical analysis of *Bredemeyera brevifolia*, a congener of *B. floribunda*, a liana type plant very common at "Chapada do Araripe" (Port. lit.: Araripes's Plateau), Crato County, Ceará State.

2. Results and discussion

Ground roots of *B. brevifolia* were soaked with hexane. After solvent evaporation a brownish viscous extract was obtained, designated BBR-H. The plant residue was then extracted with ethanol to yield a dark brown viscous extract designated BBR-E.

Coarse chromatography over silica gel of BBR-H yielded two fractions by elution with $CHCl_3$ and AcOEt. GC/MS analysis of the methylester mixture obtained after saponification of the $CHCl_3$ fraction, followed by methylation of the free fatty acids with BF_3/MeOH, allowed identification of palmitic (C_{16}:O, 9.2%), oleic (C_{18}:1, 51.1%), stearic (C_{20}:0, 2.1%), behenic (C_{22}:0, 0.8%) and lignoceric (C_{24}:0, 0.7%) acids. These findings are in agreement with the lipid constitution found for *B. floribunda* (Silveira et al., 1995). Mixture of an aliquot of BBR-E with silica gel followed by elution with hexane, $CHCl_3$, AcOEt and MeOH yielded fractions of complex mixture that were all discarded for future chromatography. A new attempt was accomplished by partitioning of the crude extract with hexane (BBRE-H) followed by $CHCl_3$ (BBRE-C). Part of BBRE-H was treated accordingly to the same procedure used for BBR-H allowing the identification of palmitic (4.8%), oleic (86.4%) and linoleic (8.8%) acids.

Coarse chromatography of BBRE-C over silica gel yielded a fraction that, after prep. TLC purification, allowed the isolation of a yellow material designated BBR-1. A more polar fraction yielded a yellowish ppt (BBR-2) after hot MeOH treatment, and another yellow material (BBR-3) from the mother liquors, with the same RF than BBR-2.

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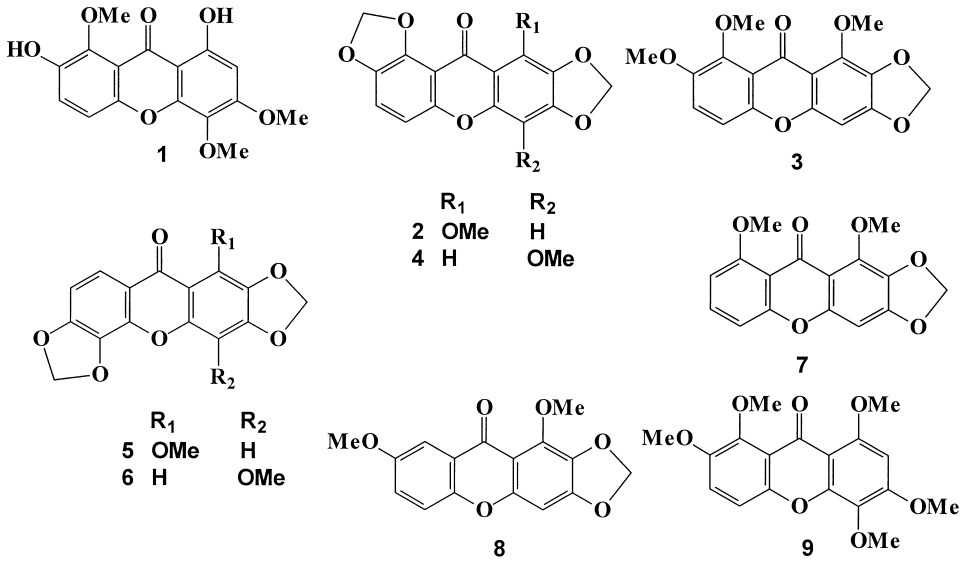

BBR-1, mp. 213.2–214.3°C, was identified as the known 1,7-dihydroxy-3,4,8-trimethoxyxanthone, **1**, after spectral data (MS, UV, IR, ^{1}H and ^{13}C NMR spectroscopy) comparison, confirmed by co-chromatography with an authentic sample of **1** (lit. 213–214°C) obtained from *B. floribunda* (Silveira et al., 1995).

BBR-2, mp. 265–270°C, showed a characteristic xanthone absorption for $\nu_{O\text{-}H}$ (3080–3000 cm^{-1}), ν_{Csp^3}-H (2950–2850 cm^{-1}), $\nu_{C=O}$ (1695 cm^{-1}), $\nu_{\phi\text{-}O}$ (1300–1200 cm^{-1}),$\nu_{C=C}$ (1590–1454 cm^{-1}) and $\delta_{\phi\text{-}H}$ (1170–700 cm^{-1}). Its HREIMS showed $[M]^+$ at *m/z* 314.0428 (calc. for $C_{16}H_{10}O_7$, 314.0426). The UV spectra in both neutral and alkali conditions were superimposable, revealing the absence of hydroxyl groups. The ^{1}H NMR spectrum revealed an AB aromatic system at δ7.05 (*d*, 1H, J=8.2 Hz, H-6) and δ6.80 (*d*, 1H, J=8.2 Hz, H-5), a singlet at δ6.56 (1H, H-4), two singlets at δ6.17 and δ6.02, integrating 2H each (2×O-CH2-O), and finally an aromatic methoxyl at δ4.08 (3H, s, OCH_3). The ^{13}C NMR spectrum showed a characteristic non-chelated xanthone carbonyl at δ174.6, nine non-hydrogenated sp^2 carbons at δ154.8, 153.8, 149.8, 146.0, 143.8, 142.0, 134.5, 110.5 and 109.8, three monohydrogenated sp^2 carbons at δ113.0, 108.2 and 93.0, and finally a methoxyl at δ61.2. From these data one can infer the penta-oxygenated character of BBR-2, with tri- and dioxygenated rings, respectively. Moreover the methoxyl group is sterically crowded. Two methylenedioxide moieties and the crowded methoxyl on a xanthone framework show an AB coupling system that allows just four structural possibilities: one methylenedioxy at C-2 & C-3 and the other one at C-7 & C-8, **2** and **4**, or at C-5 & C-6, **5** and **6**, with the methoxy either at C-1 or C-4.

The absence of any hydrogen absorption below δ7.00 (hydrogens *peri* to the carbonyl) in its ^{1}H NMR spectrum, strongly suggested position C-1 as bearing the methoxyl. A search in the literature for ^{13}C NMR spectral data comparison revealed xanthones **7** (Marston et al., 1993) and **8** (Fujita et al., 1992). Indeed, the $\Delta\delta_C$ observed for ring A of **7** and **8**, in comparison with the trioxygenated ring of BBR-2, **2** (Table 1), showed a good match for the MeO- at C-1. The final structure determination, with the MeO- located at C-1, was accomplished by a heteronuclear long-range correlation observed through a hydrogen channel detected two-dimensional heteronuclear experiment (HMBC), particularly for the methoxy hydrogens (δ4.08) with the non-hydrogenated C-1 (δ142.0). All other observed long-range correlations are depicted in Fig. 1. This experiment also allows the unambiguous assignment of all ^{13}C absorptions (Table 1). Thus, BBR-2 is 1-methoxy-2,3,7,8-dimethylenedioxyxanthone, **2**.

BBR-3, mp. 157.5°C (with decomposition), like BBR-2, did not show any bathochromic shift in its UV spectra under neutral and alkali conditions, which indicated no hydroxyl groups. The HREIMS of BBR-3 gave a $[M]^+$ at *m/z* 330.0739 (calc. for $C_{17}H_{14}O_7$, 330.0739). Its ^{1}HNMR spectrum showed a very similar hydrogenation pattern to BBR-2, particularly for the aromatic rings, except for the presence of only one methylenedioxy group and two more methoxyl functionality (see Table 2). Comparison of the ^{13}C NMR spectral data of BBR-3 with **7** and **8**, and also BBR-2, (Table 1) revealed that the methylenedioxy group (expected at δ103.1) for ring B was absent. Comparison of the chemical shifts of the B ring of BBR-3 with those of **9**, the permethylated derivative of **1** (Silveira et al., 1995), indicated a close structural relationship (Table 2). Thus, BBR-3 is the 1,7,8-trimethoxy-2,3-methylenedioxyxanthone, **3**. Unfortunately, the HBMC experiment for **3** did not provide a large number of correlations but the few long-range correlations observed for BBR-3 (Fig. 1), are in agreement with the proposed structure.

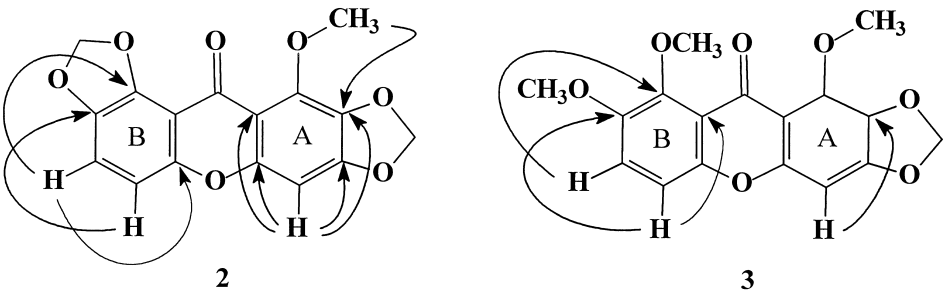

Fig. 1. ^{1}H, ^{13}C-long-range correlation observed through HMBC for xanthones **(2)** and BBR-3 **(3)**.

Table 1
^{13}C NMR ($CDCl_3$) spectroscopic comparison of the trioxygenated ring of xanthone, **2**, and model compounds **7** (Marston et al., 1993) and **8** (Fujita et al., 1992) from literature

	2	7	8
C-1	142.0	142.1	142.1
C-2	134.5	134.2	135.0
C-3	153.8	153.7	153.0[a]
C-4	93.0	93.1	92.7
C-4a	154.8	154.9	153.3[a]
C-4b	149.8	149.8	160.4
C-5	108.2	118.5	105.6
C-6	113.0	123.7	133.6
C-7	143.8	156.1	109.2
C-8	146.0	106.1	157.1
C-8a	109.8	122.7	112.0
C-8b	110.5	110.3	113.0
C-9	174.6	175.5	177.1
O-CH_2-O	102.1	102.1	102.0
O-CH_2-O	103.1	–	–
OCH_3	61.2	61.1	61.1

[a] Values with the same superscript can be interchanged.

Table 2
^{13}C NMR ($CDCl_3$) spectroscopic comparison of the trioxygenated ring of xanthon, **3**, and model compounds **9** (Fujita et al., 1992) and, **2** (this work)

	9	3	2
C-1	157.3	142.2	142.0
C-2	91.0	133.8	134.5
C-3	156.8	153.3	153.8
C-4	129.5	92.3	93.0
C-4a	151.1	153.9	154.8
C-4b	148.5	150.2	149.8
C-5	112.3	112.1	108.2
C-6	118.5	119.0	113.0
C-7	149.9	149.0	143.8
C-8	149.3	148.5	146.0
C-8a	118.0	117.8	109.8
C-8b	107.8	111.0	110.5
C-9	175.5	175.4	174.6
O-CH_2-O	–	101.9	102.1
O-CH_2-O	–	–	103.1
OCH_3	61.6	57.1	61.2
OCH_3	61.5	60.9	–
OCH_3	156.8	61.7	–
OCH_3	56.3	–	–
OCH_3	56.1	–	–

All xanthones were tested, according to The Research Institute of Pharmaceutical Sciences protocols, against *Giardia intestinalis* (ATCC 3088), *Critidia fasciculata* (ATC 11745), HSV-1 in Vero cell host, human chronic myelogenous leukemia K562 (CCL 243), human oral epidermoid carcinoma KB (CCL 17), human ovary carcinoma SK-OV-3 (HTB 77) and African green monkey kidney Vero (CCL81) as normal cell line, but showed no cytotoxicity, antiprotozoal or antiviral activities. 1,7,8-trimethoxy-2,3-methylenedioxyxanthone, **3**, displayed marginal antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*.

3. Experimental

3.1. General

Mps.: uncorr.; IR: KBr pellets; ^{1}HNMR: 200 and 400 MHz; ^{13}C NMR: 50 and 100 MHz, Bruker AC-200 or Varian Unity Plus, respectively; GC/MS Low resolution EIMS: (70 eV) Hewlett–Packard HP-5995-A; HR/MS: VG Analytical 7070 E-HF.

3.2. Plant material

Bredemeyera brevifolia roots were collected in February 1994. Plant identification was done by Dr. Afrânio G. Fernandes and Edson de Paula Nunes (Botanists, Universidade Federal do Ceará) and a voucher specimen (♯20605) representing the collection has been stored at Herbário Prisco Bezerra, Departamento de Biologia, Universidade Federal do Ceará, Brazil.

3.3. Extraction and isolation of constituents

Ground, oven-dried (50°C), roots of B. brevifolia (5.0 kg) were exhaustively extracted with cold hexane to yield a turbid orange, viscous extract (84.0 g), designated BBR-H. The plant residue, was then extracted

with cold EtOH to yield a brown viscous extract (620.0 g), designated BBR-E. Coarse chromatography over silica gel of an aliquot of BBR-H, yielded four fractions after elution with *n*-hexane followed by $CHCl_3$, EtOAc and finally MeOH. ^{1}HNMR of the hexane, $CHCl_3$ and EtOAc showed its lipid character. KOH hydrolysis of a portion of the $CHCl_3$ fraction, followed by methylation with BF_3/MeOH of the fatty acids, and GC/MS analysis of the methylesters mixture allowed the identification of palmitic (C_{16}:O, 9.2%), oleic (C_{18}:1, 51.1%), stearic (C_{20}: 2.1%), behenic (C_{22}: 0.8%) and lignoceric (C_{24}: 0.7%) acids.

Coarse chromatography over silica gel of an aliquot of BBR-E (53 g), gave very low yield fractions after elution with hexane, $CHCl_3$, EtOAc and MeOH. More over they represented a very complex mixture of compounds which was discarded. The remainder of BBR-E (485 g) was transferred to a 2 l Erlenmeyer flask and partitioned with cold hexane (BBRE-H, 34.8 g) followed by $CHCl_3$ (BBRE-C, 19.5 g). A portion of BBRE-H (3.5 g) was treated in the same way as for BBR-H to give palmitic (4.8%), oleic (86.4%) and linoleic (8.8%) acids.

BBRE-C (19.0 g) was adsorbed onto a silica gel column (100 g) packed over a layer of of silica gel (40.0 g) in a cylindrical funnel (40 mm ϕ). This was eluted with $CHCl_3$ to give fractions [BBRE-C(1) to (9)], then with $CHCl_3$–EtOAc 1:1 to give fractions [BBRE-C(10) and (11)], with EtOAc to give fractions [BBRE-C(12) to (22)], and finally with MeOH to give fractions [BBRE-C(23)].

3.4. 1,7-Dihydroxy-3,4,8-trimethoxyxanthone **1**

Successive silica gel CC of BBRE-C(3) (521 mg), yielded pure 1 (31 mg) after silica gel prep. TLC purification (benzene/ethyl acetate 15%), mp. 213.3–214.2°C. EIMS, UV, ^{1}H and ^{13}C NMR identical to the literature (Silveira et al., 1995). Co-chromatography with authentic **1** confirmed its identification.

3.5. 1-Methoxy-2,3,7,8-dimethylenedioxyxanthone, **2**

The more polar fractions obtained from the chromatography analysis of BBRE-C (3) were compared by TLC and similar fractions were pooled to yield a yellow solid material. Addition of hot MeOH dissolved part of the material leaving a yellow insoluble portion. Filtration of this material gave (20 mg). HREIMS 314.0428 (calc. for $C_{16}H_{10}O_7$, 314.0426). EI-MS *m*/*z* (rel. int.): 314 $[M]^+$ (90.0), 313 (9.6), 286 (41.4, M^+-CO), 285 (41.2, 286-H), 284 (13/0, M^+-H_2CO), 283 (24.4, M^+-OMe), 271 (46.8, 286- Me), 257 (23.6, 285-CO), 256 (13.4, 284-CO), 255 (25.2, 285-H_2CO). FT-IR ν_{max}^{KBr} cm^{-1}: 3080–3000, 2960–2850, 1695, 1590, 1480, 1454, 1280, 1200, 1170, 1111, 900-700. UV λ_{max}^{MeOH} nm (log ϵ): 259 (4.50), 278 (4.40), 296 (4.15), 389 (3.91). + NaOH: 258, 278, 294, 388. ^{1}HNMR (CDCl3) δ: 7.05 (*d*, 1H, *J* = 8.2 Hz, H-7), 6.80 (*d*, *J* = 8.2 Hz, H-6), 6.56 (*s*, H-4), 6.17 (*s*, 2H, O-CH_2-O), 6.03 (*s*, 2H, O-CH_2-O). ^{13}C NMR: Table 1.

3.6. 1,7,8-Trimethoxy-2,3-methylenedioxyxanthone, **3**

The mother liquor from 2 above was concentrated and subject to silica gel chromatography to yield pure **3**, as a yellow solid mp 265–270°C. HREIMS *m*/*z* 330.0739 (calc. for $C_{17}H_{14}O_7$, 330.0739); EIMS *m*/*z* (rel. int.): 330 $[M]^+$ (52.4), 315 (100.0, M^+-15), 300 (5.2, M^+-H2CO), 287 (12.0, 315-CO), 272 (52.8, 300-CO), 269 (M^+-OCH_3), 241 (4.8, 269-CO). FT-IR ν_{max}^{KBr} cm^{-1}: 3080–3000, 2955–2856, 1665, 1479, 1436, 1300-1200, 1168, 1109, 871-705. UV λ_{max}^{MeOH} nm (log ϵ): 227 (4.39), 258 (4.60), 293 (4.20), 373 (3.94). + NaOH: 257, 291, 372. ^{1}HNMR ($CDCl_3$) δ: 7.23 (*d*, 1H, *J* = 9.5 Hz, H-6), 7.80 (*d*, 1H, *J* = 9.5 Hz, H-5), 6.53 (1H, *s*, H-4), 6.00 (s, 2H, O-CH_2-O), 4.11 (*s*, 3H, OCH_3), 4.00 (*s*, 3H, OCH_3) and 3.88 (*s*, 3H, OCH_3). ^{13}C NMR: Table 2.

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References

Andrade, C.H.S., Braz-Filho, R., Gottlieb, O.R., Silveira, E.R., 1977. The chemistry of Brasilian Polygalaceae. I. Xanthones from *Polygala spectabilis*. Journal of Natural Products 40 (4), 344–346.

Carpenter, I., Locksley, H.U., Scheinmann, F., 1969. Xanthones in higher plants: Biogenetic proposals and a chemotaxonomic survey. Phytochemistry 8, 2013–2026.

Dreyer, D.L., 1969. Extractives of *Polygala macradenia* Gray (Polygalaceae). Tetrahedron 25, 4415–4420.

Fujita, T., Liu, D.-Y., Ueda, S., Takeda, Y., 1992. Xanthones from *Polygala tenuifolia*. Phytochemistry 31 (11), 3997–4000.

Ghosal, S., Basumatari, P.C., Banerjee, S., 1981. 1,2,3-Trioxygenated glucosyloxyxanthones from *Polygala triphylla*. Phytochemistry 20, 489–492.

Habib, A.M., Reddy, K.S., McCloud, T.G., Chang, C.J., Cassady, J.M., 1987. New xanthones from *Psorospermum febrifugum*. Journal of Natural Products 50 (2), 141–145.

Marston, A., Hamburger, M., Sordat-Diserens, I., Msonthi, J.D., Hostettmann, K., 1993. Xanthones from *Polygala syikensis*. Phytochemistry 33 (4), 809–812.

Peres, V., Nagem, T.J., 1997. Naturally occurring pentaoxygenated, hexaoxygenated and dimeric xanthones: a literature survey. Química Nova 20 (4), 388–397.

Rao, V.S.N., Menezes, A.M.S., Viana, G.S.B., Gadelha, M.G.T., Silveira, E.R., 1990. Experimental evaluation of *Bredemeyera floribunda* in acute gastric lesions induced by ethanol, acetylsalicylic acid and histamine. Fitoterapia LXI (I), 9–12.

Silveira, E.R., Falcão, M.J.C., Menezes, A. Jr, Kingston, D.G.I., Glass, T.E., 1995. Pentaoxygenated xanthones from *Bredemeyera floribunda*. Phytochemistry 39 (6), 1433–1436.

Sultanbawa, M.U.S., 1980. Xanthanoids of tropical plants. Tetrahedron 36, 1465–1506.

Ykeya, Y., Sugana, K., Okada, M., Mitsuhaski, H., 1991. Two xanthones from *Polygala tenuifolia*. Phytochemistry 30 (6), 2061–2065.