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Dichromenoxanthones from *Tovomita brasiliensis*

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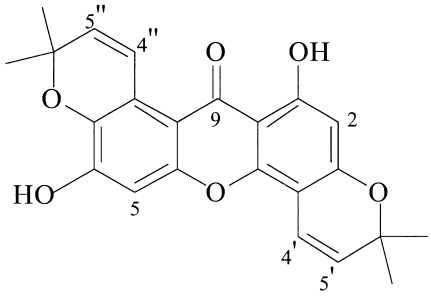
Abstract

Two dichromenoxanthones [1,6-dihydroxy-6′,6′-dimethylpyrano(2′,3′:3,4)-6″,6″-dimethylpyrano(2″,3″:7,8)xanthone (brasilixanthone A) and 1,6-dihydroxy-6′,6′-dimethylpyrano(2′,3′:2,3)-6″,6″-dimethylpyrano(2″,3″:7,8)xanthone (brasilixanthone B)], along with betulinic acid, friedelin, sitosterol and stigmasterol were isolated from the roots and stems of *Tovomita brasiliensis*. Their structures were characterized on the basis of ^{1}H and ^{13}C NMR spectral data, including 2D NMR experiments.

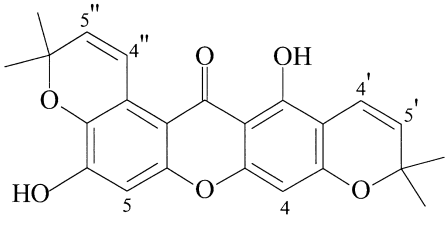
Keywords: *Tovomita brasiliensis*; Guttiferae; Dichromenoxanthones; Terpenes

1. Introduction

Natural xanthones occur widely among different higher plant families and ferns and have been reported to possess several biological activities (Bennett and Lee, 1989). Dichromenoxanthones have been found in only a few species of the Guttiferae (Waterman and Crichton, 1980; Waterman and Hussain, 1982; Delle Monache et al., 1984a; Bennett and Lee, 1989). The genus *Tovomita* is known to contain xanthones, benzophenones, triterpenes and steroids (Gottlieb and Gabriel, 1972; Oliveira et al., 1972, 1984; Mesquita et al., 1975; Braz-Filho et al., 1982; Delle Monache et al., 1984b,c; Bennett and Lee, 1989; Nagem et al., 1997; Seo et al., 1999), only one dichromenoxanthone, tovophyllin B, has been reported, from the wood of *T. macrophylla* (Oliveira et al., 1972) and *T. pyrifolium* (Mesquita et al., 1975). In a previous investigation from the trunk wood of *T. brasiliensis* (Mart.) Walp., popularly known as "manguarana or taxiubarana" (Le Cointe, 1934), revealed the presence of simple xanthones, terpenes and isocoumarins, the latter probably being due to infestation of the plant material by fungi (Braz-Filho et al., 1982). In the present study two isomeric dichromenoxanthones, **1**



1



2

(from roots) and **2** (from stems), are reported, together with the known betulinic acid, friedelin, lupeol, α- and β-amyrin, lanosta-7,24-dien-3β-ol, β-sitosterol and stigmasterol.

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2. Results and discussion

The dichloromethane extract of the roots and stems from *T. brasiliensis* were submitted to chromatographic separations to afforded betulinic acid, friedelin, two mixtures containing α- and β-amyrin, lupeol and lanosta-7,24-dien-3β-ol, and β-sitosterol and stigmasterol. The compounds were identified by comparing spectroscopic data with literature values (Akihisa et al., 1990; Kojima et al., 1990; Mahato and Kundu, 1994; Venkatraman et al., 1994). After a combination of gel filtration on Sephadex LH-20 and recrystallization the xanthones **1** (from roots) and **2** (from stems) were obtained.

Brasilixanthones A (**1**) and B (**2**), were isolated as pale yellow amorphous powders, and identified as 1,3,6,7-tetraoxygenated dichromenoxanthones. The EIMS spectra showed a $[M]^+$ at *m/z* 392, corresponding to the molecular formula $C_{23}H_{20}O_6$, in accordance with the ^{13}C NMR spectral data (Table 1). Their IR spectra exhibited strong bands due to hydroxyl groups and a conjugated carbonyl group. The ^1H NMR spectra showed signals for a chelated hydroxyl group, two isolated aromatic hydrogens and two 2,2-dimethylpyran rings, which were supported by fragment ions at *m/z* 377 [M^+-15, **1** and **2** (100)] in the MS spectra. Another feature common to both compounds was the highly deshielded position of the olefinic hydrogens of the one 2,2-dimethylpyran unit [**1** δ 7.99 (H-4″) and 5.82 (H-5″); **2** δ 8.02 (H-4″) and 5.83 (H-5″)]. Such structural features suggests that these functionalities to be *peri* to the carbonyl group (Oliveira et al., 1972). Correlations among the hydrogens and the chemical shifts of the corresponding carbons were established in the ^1H-^1H COSY of both compounds and from the HMQC spectrum of **1** (Table 1).

Correlations were observed in the HMBC spectrum of **1** between: the signal of the chelated hydroxyl group at δ 13.40 with the carbons at δ 163.30 (C-1), δ 99.26 (C-2) and δ 104.03 (C-9a); the signal at δ 6.83 (H-5) with the quaternary carbons at δ 153.08 (C-10a), δ 151.15 (C-6), δ 137.12 (C-7); and, the signal at δ 7.99 (H-4″) with the carbons at δ 119.97 (C-8), δ 108.65 (C-8a), δ 137.12 (C-7) and δ 77.23 (C-6″). All assignments were compatible with location of the pyrano units in **1**.

Table 1
NMR data for compounds **1** and **2**[a]

	1[b]				**2**[b]		
$^1H/^{13}C$	δ_C	HMQC δ_H	^1H-^1H COSY	HMBC	δ_C	δ_H	^1H-^1H COSY
C							
1	163.30				159.56		
2	–				104.47		
3	160.17				158.61		
4	100.59				–		
4a	151.38				155.12		
6	151.15				150.94		
7	137.12				135.76		
8	119.97				121.50		
8a	108.65				110.14		
9	182.64				182.12		
9a	104.03				103.78		
10a	153.08				152.47		
6′	78.19				77.43		
6″	77.23				77.43		
CH							
2	99.26	6.20 *s*		C-1,C-3,C-4,C-9a	–		
4	–				94.28	6.26 *s*	
5	102.52	6.83 *s*		C-6,C-7,C-10a,C-8a	102.44	6.83 *s*	
4′	115.28	6.76 *d* (10.0)	H-5′	C-3, C-4a, C-6′	115.69	6.72 *d* (10.0)	H-5′
5′	126.98	5.57 *d* (10.0)	H-4′	C-4, C-6′, Me-6′	127.19	5.57 *d* (10.0)	H-4′
4″	121.08	7.99 *d* (10.2)	H-5″	C-7, C-8, C-8a, C-6″	120.99	8.02 *d* (10.3)	H-5″
5″	132.58	5.82 *d* (10.2)	H-4″	C-4″, C-6″, Me-6″	132.31	5.83 *d* (10.3)	H-4″
CH_3							
6′,6′	28.46	1.47 *s*		C-6′	28.38	1.47 *s*	
6″,6″	27.53	1.49 *s*		C-6″	27.39	1.49 *s*	
1-OH		13.40 *s*		C-1, C-2, C-9a		13.63 *s*	
6-OH		6.29 *br*		C-5, C-6, C-7			

[a] ^1H (**1**: 500 MHz; **2**: 300 MHz, $CDCl_3$); ^{13}C (**1**: 125 MHz; **2**: 75 MHz. $CDCl_3$).
[b] The hydrogenation patterns was deduced by DEPT; Chemical shifts (δ) expressed in ppm from internal TMS, coupling constants (*J*) in Hz.

The ^{1}H NMR spectral data of xanthone **2** was similar to both those for **1** (Table 1) as well as related compounds (Waterman and Crichton, 1980; Waterman and Hussain, 1982; Delle Monache et al., 1984). Therefore, comparison of the ^{13}C NMR spectral data with **1** revealed a significative difference mainly for the unsubstituted carbon at ring A (**1** δ 99.26 and **2** δ 94.28). The diamagnetic shift ($\Delta\delta = 4.98$) observed for **2** was in perfect agreement with a linear arrangement of the second pyrano unit. Additional evidence for the attachment of this unit at C-2 was deduced from its UV spectra, which did not show the same bathochromic shift with $AlCl_3$ as for **1**, since it complete only after 30 min. This behaviour is typical of chelated 1-OH compounds with a substituent at C-2 (Delle Monache et al., 1984a,c). Finally, comparison with model compounds described in the literature (Rocha et al., 1994; Rath et al., 1996; Nagem et al., 1997) confirmed these structures.

3. Experimental

3.1. General

Mp uncorr. UV: MeOH. IR: KBr. NMR: 200, 300 and 500 MHz (^{1}H) and 50.3, 75 and 125 MHz (^{13}C) in $CDCl_3$ relative to TMS. ^{13}C multiplicities were determined using DEPT pulse sequences. MS: direct inlet, 70 eV. CC: silica gel (Merck, 0.063–0.200 mm). GPC: Sephadex LH-20 (MeOH).

3.2. Plant material

Roots and stems of *Tovomita brasiliensis* (Mart.) Walp. were collected at Acará, Pará State, Brazil, and identified by a specialist from the Museu Paraense Emílio Goeldi (MPEG/PA), where a voucher specimen (MG-0151737) was deposited.

3.3. Extraction and isolation

The air-dried and ground roots (1270 g) and stems (2400 g) were extracted successively with *n*-C_6H_{14}, CH_2Cl_2 and MeOH, respectively, using a Soxhlet apparatus. After solvent removal in vacuo, the CH_2Cl_2 extracts (roots: 12 g; stems: 18 g) were subjected to silica gel column, chromatography with *n*-C_6H_{14} as eluant containing increasing amounts of EtOAc to afford, after recrystallization from MeOH, betulinic acid (300 mg), friedelin (320 mg), and mixtures of α- and β-amyrin, lupeol and lanosta-7,24-dien-3β-ol (776.3 mg), and β-sitosterol and stigmasterol (18.6 mg). Further separation by gel filtration on Sephadex LH-20 (MeOH), and recrystallization from MeOH, the compound **1** (80 mg) from roots and 2 (5.7 mg) (from stems) were obtained.

3.4. 1,6-Dihydroxy-6′,6′-dimethylpyrano(2′,3′:3,4)-6″,6″-dimethylpyrano(2″,3″:7,8) xanthone [brasilixanthone A] (1)

Yellow amorphous powder, mp. 220.4–221.1°C (MeOH). UV (MeOH) λ_{max} nm (log ε): 264 (4.53), 285 (4.38), 330 (4.27), 343 (4.18), 397 (3.94); (NaOAc): 250 (4.71), 302 (4.24), 355 (4.32), 396 (4.23); ($AlCl_3$): 260 (4.48), 281 (4.61), 305 (4.41), 355 (4.41), 452 (3.89). IR ν_{max} (KBr) cm^{-1}: 3491, 3354, 2928, 2852, 1628, 1568, 1527, 1494, 1452, 1395, 1366, 1242, 1214, 1160, 1119, 1021, 949, 892, 862, 836, 758. EIMS (probe) 70 eV, *m*/*z* (rel. int.): 392 $[M]^+$ (31), 377 (100), 279 (2), 259 (18), 203 (6), 181 (9), 91 (4), 77 (6). ^{1}H (500 MHz) and ^{13}C NMR (125 MHz, $CDCl_3$, δ): Table 1.

3.5. 1,6-Dihydroxy-6′,6′-dimethylpyrano(2′,3′:2,3)-6″,6″-dimethylpyrano(2″,3″:7,8) xanthone [brasilixanthone B] (2)

Yellow amorphous powder, mp. 207°C (decomp.). UV (MeOH) λ_{max} nm (log ε): 287 (3.95), 290 (3.94), 310 (3.81), 385 sh (3.33); (NaOAc): 278 (3.98), 300 (3.84), 329 (3.70), 387 (3.62); ($AlCl_3$, after 30 min): 301 (3.92), 312 (3.96), 363 (3.88), 431 sh (3.26). IR ν_{max} (KBr) cm^{-1}: 3491, 3355, 2928, 2852, 1621, 1562, 1525, 1461,1396, 1365, 1291, 1214, 1177, 1126, 1078, 892, 862, 761. EIMS (probe) 70 eV, *m*/*z* (rel. int.): 392 $[M]^+$ (38), 377 (100), 359 (8). ^{1}H (300 MHz) and ^{13}C NMR (75 MHz, $CDCl_3$, δ): Table 1.

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