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Flavonoids from *Brosimum acutifolium*☆

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Abstract

4′-Hydroxy-7,8-[2-(2-hydroxyisopropyl)dihydrofuran]flavan and 4′,7-dihydroxy-8-(3,3-dimethylallyl)flavan, together with 10 known plant constituents, were obtained from the trunk bark of *Brosimum acutifolium*. Their structures were elucidated by spectroscopic methods, including 2D NMR spectroscopic techniques. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Brosimum acutifolium*; Moraceae; Flavans; Chalcones; Brosimine A and B

1. Introduction

The trunk bark of *Brosimum acutifolium*, a plant which is distributed mainly in highland forests from east of Pará State (Brazil) to east of Suriname, is used in folk medicine in the Amazon region as an anti-inflammatory and anti-rheumatic agent (van den Berg, 1982). In a previous paper (Torres, Monteiro, Arruda, Muller & Arruda, 1997), we reported the isolation and structure elucidation of two flavans [4′-hydroxy-7,8-(2″,2″-dimethylpyran)flavan and 4′-hydroxy-7,8-(3″-hydroxy-2″,2″-dimethylpyran)flavan] from the trunk bark of *B. acutifolium* subsp. *acutifolium*. Further investigation led to the isolation of two new flavans, named brosimine A (**1**) and brosimine B (**2**), along with the known compounds 3′,7-dihydroxy-4′-methoxyflavan, isobavachin, liquiritigenin, 4-hydroxylonchocarpin, 4-hydroxyisocordoin, isoliquiritigenin, coniferaldehyde, syringaldehyde, sitosterol and stigmasterol. This paper describes the structure elucidation of the two new flavans.

2. Results and discussion

Compound **1** gave a violet spot on a TLC plate with ceric sulphate. The IR spectrum of compound **1** suggested the presence of a hydroxyl group (3436 cm^{-1}) and an aromatic ring (1617 and 1465 cm^{-1}). The HR mass spectrum gave a $[M]^+$ at m/z 326.1514 which corresponds to the molecular formula $C_{20}H_{22}O_4$ (calcd.: 326.1518). Its 300 MHz ^{1}H-NMR spectrum (Table 1) showed signals typical of a flavan [δ 5.01 (1H, *dd*, $J = 10.0$ and 2.0 Hz, H-2)], 2.01 and 2.16 (1H each, *m*, 2H-3), 2.72 (1H, *ddd*, $J = 11.8$; 6.4 and 3.2 Hz, H-4α) and 2.91 (1H, *ddd*, $J = 11.8$; 8.5 and 4.3 Hz, H-4β) (Torres et al., 1997; Saini & Ghosal, 1984). The substitution pattern of ring B was proposed with a hydroxyl group at the 4′-position from the ^{1}H-NMR [δ 7.29 (2H, *d*, $J = 8.5$Hz) and 6.84 (2H, *d*, $J = 8.5$Hz)] and EI mass spectra (m/z 120). The presence of a 2-(2-hydroxyisopropyl)-dihydrofuran ring was indicated by a set of peaks at δ 4.63 (1H, *dd*, $J = 8.9$ and 9.2 Hz), 3.05 (1H, *dd*, $J = 11.8$ and 8.9 Hz), 3.12 (1H, *dd*, $J =$ 11.8 and 9.2 Hz), 1.20 and 1.31 (3H each, *s*), attributed to an oxymethine H-2″, two benzylic hydrogens H-1″ and a *gem*-dimethyl group at C-3″, respectively. This was confirmed by a COLOC experiment (summarized in Fig. 1) which showed $^3J_{(CH)}$ and $^2J_{(CH)}$ correlations between the hydrogens Me-4″/Me-5″ and

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Table 1
^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data for compounds **1** and **2** ($CDCl_3$, δ)[a]

H/C	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2	5.01 (1H, *dd*, $J = 9.9, 1.9$)	77.5	4.98 (1H, *dd*, $J = 10.1, 2.4$)	77.6
3	2.01 (1H, *m*); 2.16 (1H, *m*)	29.7	2.15 (1H, *m*); 1.98 (1H, *m*)	30.0
4β	2.91 (1H, *ddd*, $J = 11.8, 8.5, 4.3$)	24.6	2.92 (1H, *ddd*, $J = 16.3, 11.0, 5.6$)	24.9
4α	2.72 (1H, *ddd*, $J = 11.8, 6.4, 3.2$)	24.6	2.72 (1H, *ddd*, $J = 16.3, 5.2, 3.4$)	24.9
5	6.84 (1H, *d*, $J = 8.1$)	128.7	6.82 (1H, *d*, $J = 8.2$)	127.3
6	6.37 (1H, *d*, $J = 8.1$)	101.5	6.40 (1H, *d*, $J = 8.2$)	108.1
7		159.2[b]		153.0[c]
8		113.4		114.6
9		151.7[b]		153.6[c]
10		114.1		113.9
1′		134.0		137.5
2′,6′	7.29 (2H, *d*, $J = 8.5$)	127.4	7.29 (*d*, $J = 8.7$)	127.3
3′,5′	6.84 (2H, *d*, $J = 8.5$)	115.3	6.84 (*d*, $J = 8.7$)	115.2
4′		155.3		155.0
1″	3.15 (1H, *dd*, $J = 11.8, 8.9$); 3.12 (1H, *dd*, $J = 11.8, 9.2$)	28.3	3.41 (*d*, $J = 7.2$)	22.4
2″	4.63 (1H, *dd*, $J = 9.2, 8.9$)	89.9	5.27 (*t*, $J = 7.2$)	122.2
3″		72.1		134.0
4″	1.20 (3H, *s*)	23.6[d]	1.74 (3H, *s*)	25.8
5″	1.31 (3H, *s*)	26.1[d]	1.72 (3H, *s*)	17.8
7-OH			5.20 (1H, *s*)	

[a] Coupling constants (J in Hz) are given in parentheses.
[b,c] Interchangeable signals.
[d] Solution in $CDCl_3$ referenced to $CHCl_3$ at δ 7.26 ppm (^{1}H) and δ 77.23 ppm (^{13}C).

carbons C-2″ (δ 89.9) and C-3″ (δ 72.1). Based on the presence of two *ortho* coupled doublets in ^{1}H-NMR [δ 6.84 (1H, *d*, $J = 8.1$ Hz, H-5) and 6.37 (1H, *d*, $J = 8.1$Hz, H-6)] and comparison with ^{1}H-NMR spectroscopic data of the two flavans isolated previously (Torres et al., 1997), the dihydrofuran ring was placed at the C-7/C-8 positions, which was confirmed by a NOE experiment. Irradiation of H-4α (δ 2.72) resulted in the enhancement of the H-5 signal (δ 6.84). A possible isomeric structure is 4′-hydroxy-7,8-(2″,2″-dimethylpyran)flavan (**1a**), previously obtained by us from the same species (Torres et al., 1997); however, its ^{1}H- and ^{13}C-NMR data are not totally superimposable on those of **1**, and the signals at δ_H 4.63 (H-2″) and δ_C 89.9 (C-2″) are characteristics of a dihydrofuran ring bonded to an aromatic ring (Macias, Massanet, Rodrigues-Luis & Salvá, 1989). Therefore, structure **1a** for compound **1** was eliminated. The foregoing is consistent with structure **1**, for which the name brosimine A is proposed. The ^{13}C-NMR signals (Table 1) were assigned using HETCOR and COLOC experiments and by comparison of the observed values with those reported for model compounds (Torres et al., 1997; Macias et al., 1989).

Compound **2** also gave a violet color with ceric sulfate. It was assigned the molecular formula $C_{20}H_{22}O_3$ by HR-MS (*m*/*z* 310.1568, $[M]^+$, calcd. 310.1569). The ^{1}H-NMR spectrum (Table 1) of **2** was similar to that of **1**, but with signals assigned to a 3,3-dimethylallyl moiety [δ 5.27 (1H, *t*, $J = 7.2$ Hz, H-2″), 3.41 (2H, *d*, $J = 7.2$ Hz, H-1″), 1.72 (3H, *s*, H-4″) and 1.74 (3H, *s*, H-5″)] replacing those of the 2-(2-hydro-

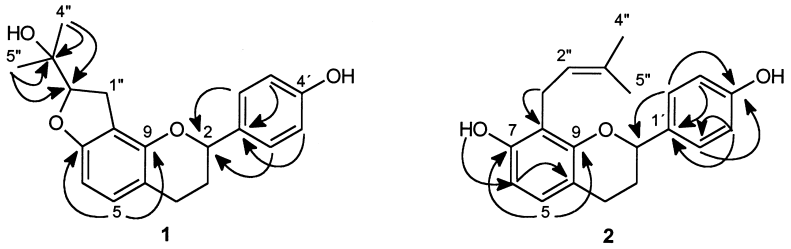
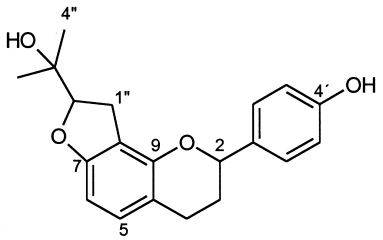
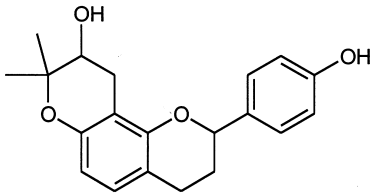


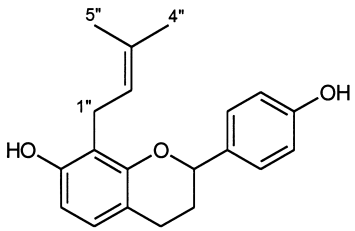
Fig. 1. CH long-range correlations in COLOC spectra of **1** and **2**.



1



1a



2

xyisopropyl)-dihydrofuran ring of **1**. The EI mass spectrum showed characteristic retro Diels–Alder fragments at *m*/*z* 120 and 190 indicating the presence of a 4′-OH group in the B-ring and of 3,3-dimethylallyl and hydroxyl groups in the A-ring. The prenyl and OH groups were located at the 8 and 7-positions in ring A, respectively, based on the presence of two *ortho*-coupled aromatic hydrogens [δ 6.82 and 6.40 (*d*, $J = 8.2$ Hz)] and by NOE and COLOC (Fig. 1) experiments. A NOE was observed for H-5 (δ 6.82) when H-4α (δ 2.72) was irradiated, and the OH-7 hydrogen (δ 5.20) exhibited a cross-peak to the aromatic carbon C-6 (δ 108.1). Thus **2**, for which the trivial name brosimine B is proposed, is 4′,7-dihydroxy-8-(3,3-dimethylallyl)flavan. The ^{13}C-NMR frequencies (Table 1) of **2** were assigned on the basis of HETCOR and COLOC (Fig. 1) NMR experiments.

The known compounds 3′,7-dihydroxy-4′-methoxyflavan (Masaoud, Ripperger, Porzel & Adam, 1995) isobavachin (Komatsu, Yokoe & Shirataki, 1978), liquiritigenin (Achenbach, Stocker & Constenla, 1988; Fukai, Wang, Inami & Nomura, 1990), hydroxylonchocarpin (Braz Filho, Gottlieb, Mourão, Rocha & Oliveira, 1975), hydroxyisocordoin (Pisteli, Speka, Flamini, Mele & Morelli, 1996) isoliquiritigenin (Achenbach et al., 1988; Fukai et al., 1990), coniferaldehyde (Borges-Del-Castilho, Bradley-Delso, Ferrero, Bueno & Rodrigues-Luis, 1983), syringaldehyde (Barakat, Nawwar, Buddrus & Michael, 1987), sitosterol and stigmasterol, were identified by comparison of their spectral and physical data with those reported in the literature.

3. Experimental

3.1. General

Mps uncorrected. IR were recorded in NaCl film. ^{1}H- and ^{13}C-NMR spectra were recorded at 300 and 75 MHz, respectively, in $CDCl_3$ on a Varian GEMINI 300 instrument. EIMS were obtained by direct probe insertion at 70 eV.

3.2. Plant material

Brosimum acutifolium was collected in Portel county, State of Pará, Brazil and identified by Dr. Maria Elisabeth van den Berg of the Botanic Department of Museum Paraense Emílio Goeldi, Belém, Pará.

3.3. Extration and isolation

The powdered bark (2.3 kg) was extracted with hexane, CH_2Cl_2, EtOAc and MeOH, successively, at room temperature. After evaporation of the solvent the CH_2Cl_2 extract (4 g) was subjected to CC on silica gel (70–230 mesh) using hexane–CH_2Cl_2–MeOH at different ratios of increasing polarity furnishing 40 frs. The frs. were analyzed by silica gel TLC with the solvent system hexane–CH_2Cl_2–MeOH (10:10:1) or hexane–Me_2CO (10:3), and were detected by UV light and by spraying with 10% alcoholic H_2SO_4 or ceric sulfate.

Fr. 2 afforded a mixture of coniferaldehyde and syringaldehyde (35 mg). Frs. 15 and 29 afforded stigmasterol (23 mg) and sitosterol (31 mg), respectively. Frs. 24–28 were rechromatographed on silica gel (70–230 mesh) using hexane–CH_2Cl_2–MeOH in order of increasing polarity, furnishing **2** (8 mg) and hydroxylonchocarpin (20 mg). Frs. 33–34 were rechromatographed on silica gel (70–230 mesh) using hexane–Me_2CO in order of increasing polarity to yield **1** (16 mg), 3-7-dihydroxy-4′-methoxyflavan (16 mg) and hydroxyisocordoin (17 mg).

The EtOAc extract (10.77 g) was subjected to CC on

silica gel (70–230 mesh) eluted with a hexane–Me_2CO gradient to give 22 frs. Frs. 10–12 were further purified by CC on silica gel (230–400 mesh) and eluted with CH_2Cl_2–EtOAc (95:5) to furnish isobavachin (12 mg). Fr. 17 after purification on silica gel using hexane–EtOAc–MeOH gradient yielded liquirtigenin (7 mg) and isoliquiritgenin (4 mg).

3.4. Brosimne A (*1*)

$[\alpha]_D^{25}$ −7.06° ($CHCl_3$, *c* 0.35); IR ν_{max}^{film} (cm^{-1}): 3436, 1617, 1465, 1268, 1232; ^1H- and ^{13}C-NMR spectral data: see Table 1, HR-MS: *m/z* 326.1514 for $C_{20}H_{22}O_4$ (calcd: 326.1518); EIMS *m/z* (rel. int. %): 326 $[M]^+$ (3), 308 $[M - H_2O]^+$ (1), 293 $[308 - Me]^+$ (1), 173 $[C_{11}H_9O_2]^+$ (10), 120 $[C_8H_8O]^+$ (35), 107 $[C_7H_7O]^+$ (20), 69 $[C_5H_5]^+$ (53), 55 $[C_4H_7]^+$ (100).

3.5. Brosimine B (*2*)

$[\alpha]_D^{25}$ −4.88° ($CHCl_3$, *c* 0.5); IR ν_{max}^{film} (cm^{-1}): 3387, 1608, 1514, 1450, 1062, ^1H- and ^{13}C-NMR spectral data: see Table 1, HR-MS: *m/z* 310.1568 for $C_{20}H_{22}O_3$ (calcd: 310.1569); EIMS *m/z* (rel. int. %): 310 $[M]^+$ (62), 256 $[C_{16}H_{15}O_3]^+$ (53), 190 $[C_{12}H_{14}O_2]^+$ (36), 135 $[C_8H_7O_2]^+$ (98), 120 $[C_8H_8O]^+$ (100), 107 $[C_7H_7O]^+$ (90), 91 $[C_7H_7]^+$ (55), 55 $[C_4H_7]^+$ (19).

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