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An unusual C_6–$C_{6''}$ linked flavonoid from *Miconia cabucu* (Melastomataceae)

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Abstract

Chromatographic fractionation of the methanolic extract from the leaves of *Miconia cabucu* Hoehne (Melastomataceae) afforded the first example of a C_6–$C_{6''}$ linked flavone dimer, 5-hydroxy-4′,7-dimethoxyflavone-(6-C-6″)-5″-hydroxy-3‴,4‴,7″-trimethoxyflavone as well as the known compounds, quercetin-3-*O*-α-L-rhamnopyranosyl-(2→1)-*O*-β-D-xylopyranoside, quercetin-3-*O*-α-L-rhamnopyranoside, myricetin-3-*O*-α-L-rhamnopyranoside, quercetin-3-*O*-β-D-glucopyranoside, kaempferol-3-*O*-β-D-(6″-coumaroyl)-glucopyranoside and gallic acid. Their chemical identities were established by application of NMR spectroscopic methods including 2D-NMR, as well as UV and ESI-MS analyses.
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Keywords: *Miconia cabucu*; Melastomataceae; Flavonoids; Flavone dimer

1. Introduction

Recent lists of the Central Brazil flora register around 6253 native vascular plant species included in 150–160 families (Mendonça et al., 1998). An ethnopharmacological survey carried out in the Cerrado of Central Brazil showed a high number of plants used to treat gastric pain and gastritis, including *Miconia* species (Silva et al., 2000).

Miconia is a genus of approximately 1000 species (Martins et al., 1996) occurring in tropical America (Renner, 1993; Judd and Skean, 1991). The genus belongs to the pantropic family Melastomataceae, with over 166 genera that include about 4300 species (Renner, 1993). Many of these plants are used as medicines by people living in the Cerrado area (Almeida et al., 1998). *Miconia* extracts and isolated compounds have demonstrated various biological activities such as antibiotic, antitumoral, analgesic and antimalarial properties (Hasrat et al., 1997; Cunha et al., 2003).

Previous phytochemical investigations of *Miconia* species resulted in isolation of triterpenes (Chan et al., 1992; Macari et al., 1990), flavanones (Li et al., 2001) and quinone compounds (Bernays et al., 1984). To our knowledge, no phytochemical work was reported on *Miconia cabucu*.

The present paper describes the isolation, purification and structure elucidation of a C_6–$C_{6''}$ linked flavone dimer, besides flavonol glycosides and gallic acid from the leaves of *M. cabucu*.

2. Results and discussion

Aerial parts of *M. cabucu* were sequentially extracted with $CHCl_3$, to remove lipids, and with MeOH. After concentration, the methanolic extract was partitioned between EtOAc and H_2O (1:1). The EtOAc fraction was subjected to column chromatography over Sephadex LH-20. Fractions eluted with methanol were purified by semi-preparative reversed-phase HPLC to afford compounds **1**–**7**.

Six known compounds were isolated and identified by comparison of their spectroscopic data (NMR, MS and UV) as gallic acid **1** (Almeida et al., 2005), quercetin-3

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-*O*-α-L-rhamnopyranosyl-(2→1)-*O*-β-D-xylopyranoside **2** (Bilia et al., 1996), quercetin-3-*O*-α-L-rhamnopyranoside **3**, myricetin-3-*O*-α-L-rhamnopyranoside **4** (Burghardt et al., 1997; Chung et al., 2004), quercetin-3-*O*-β-D-glucopyranoside **5** (Markhan et al., 1978; Harborne, 1994) and kaempferol-3-*O*-β-D-(6″-coumaroyl)-glucopyranoside **6** (Higuchi and Donnelly, 1978; Budzianowski and Skrzypczak, 1995).

Compound **7** was obtained as a pale yellow amorphous solid. The IR spectrum of **7** exhibited a broad vibrational band due to a hydroxyl group at 3448 cm^{-1} and a carbonyl band at 1658 cm^{-1}. The UV spectrum displayed bands at 276 and 336 nm, suggesting the presence of flavone moieties (Mabry et al., 1970). The presence of 35 signals in the ^{13}C NMR spectrum (Table 1) suggested a dimeric structure. The HR-ESI-QTOF displayed a pseudomolecular ion $[M+H]^+$ at *m*/*z* 625.3905, calc. 625.5900, which is in agreement with the molecular formula $C_{35}H_{28}0_{11}$ and confirmed the dimeric nature of compound **7** (Fig. 1).

Table 1
NMR spectroscopic data for compound **7** (500 MHz, DMSO-d_6)[a]

Position	^1H NMR	^{13}C NMR	HMBC (δ^1H)	NOESY
2		163.4	H-3; H-2′; H-6′	
3	6.90 *s*	103.7		
4		181.9		
5		155.4		
6		107.0	H-8	
7		163.4	OCH_3-7; H-8	
8	6.89 *s*	90.4		
9		157.4	H-8	
10		104.0	H-8; H-3	
1′		122.8	H-3; H-6′/H-2′	
2′	8.07 *d* (8.0)	128.3		
3′	7.11 *d* (8.0)	114.6	H-5′	
4′		163.0		
5′	7.11 *d* (8.0)	114.6	H-3′	
6′	8.07 *d* (8.0)	128.3		
2″		163.4	H-3″/H2‴	
3″	7.05 *s*	104.6		
4″		181.9		
5″		155.4		
6″		107.0	H-8″	
7″		163.4	OCH_3-7″; H-8″	
8″	6.92 *s*	90.4		
9″		157.4	H-8″	
10″		104.0	H-8″; H-3″	
1‴		122.8	H-3″; H5‴	
2‴	7.60 *d* (2.0)	109.5	H-6‴	
3‴		149.0	H5‴	
4‴		152.2	H-2‴; H-6‴	
5‴	7.15 *d* (8.0)	111.7		
6‴	7.73 *dd* (2.0, 8.0)	120.1	H2‴	
OCH_3-7	3.93 *s*	55.9		H-8
OCH_3-7″	3.93 *s*	56.3		H-8″
OCH_3-4′	3.86 *s*	55.6		H-3′; H-4′
OCH_3-3‴	3.89 *s*	55.7		H-2‴
OCH_3-4‴	3.86 *s*	55.6		H-5‴
OH-5	13.01 *br s*			
OH-5″	13.01 *br s*			

[a] Chemical shifts (δ) are in ppm. Coupling constants (*J* in Hz) are given in parentheses.

The ^1H NMR (Table 1) and HR-ESI-QTOF mass spectra of **7** suggested the presence of two hydroxyl and five methoxyl groups as well as nine aromatic and two methine protons (Harborne, 1994). The ^1H NMR of **7** (in DMSO-d_6) showed a low-frequency broad singlet at δ 13.01 (2H) corresponding to two chelated hydroxyl groups at the 5 and 5″ positions. Resonances equivalent to five methoxyl groups were observed at δ 3.86 (6H), 3.89 (3H) and 3.93 (6H). Two singlets at δ 6.90 (1H) and 7.05 (1H) correspond to protons at H-3 and H-3″ and indicate that compound **7** is a biflavone derivative (Harborne, 1994). The presence of A_2B_2 doublets ($J = 8.0$ Hz) at δ 8.07 (2H) and 7.11 (2H) were attributed to the H-2′/H-6′ and H-3′/H-5′ of the B-ring of an apigenin unit, respectively. Three other proton signals at δ 7.73 (1H, *dd*, $J = 8.0$, 2.0 Hz), 7.60 (1H, *d*, $J = 2.0$ Hz) and 7.15 (1H, *d*, $J = 8.0$ Hz) corresponded to H-6‴, H-2‴ and H-5‴ of a luteolin unit, respectively. The absence of two sets of *meta*-coupled doublets and the presence of two singlets at δ 6.89 (1H) and 6.92 (1H) provided further evidence for a C→C connection between the two flavone moieties. The HMQC spectrum established a direct connection between protons and their respective carbons. Both signals at δ 6.89 and 6.92 are connected to carbons at δ 90.4. Therefore, the resonance at δ 90.4 could be ascribed either a C-6→C-6″, C-6→C-8″, C-8→C-6″ or C-8→C-8″ linkage.

In order to establish the connection between the two monomeric units, the ^{13}C NMR spectroscopic data of **7** (Table 1) was compared with several other known flavonoids. For example, agathisflavone [C-6→C-8″, δ 103.6 (C-6), 93.7 (C-8), 98.9 (C-6″) and at δ 99.4 (C-8″)] (Agrawal, 1989); agathisflavone 7,7″-dimethyl ether [C-6→C-8″, δ 103.5 (C-6), 90.8 (C-8), 95.5 (C-6″) and δ 99.8 (C-8″)] (Ofman et al., 1995); cupressuflavone [C-8→C-8″, δ 99.0 (C-6/C-6″) and at δ 98.7 (C-8/C-8″)] (Chari et al., 1977; Agrawal, 1989); cupressuflavone 7,7″-dimethyl ether [C-8→C-8″, δ 95.5 (C-6/C-6″) and at δ 98.8/99.0 (C-8/C-8″)] (Ofman et al., 1995). Then, the typical resonance of free C-6 and C-8 of biflavonoids is approximately δ 98.0 and δ 94.0, respectively. These data when compared to those of **7** at C-6/C-6″ (δ 107.0) and C-8/C-8″ (δ 90.4), indicate a C-6→C-6″ linkage between the two flavone moieties.

A set of 1D-gNOESY experiments permitted assignment of all methoxy groups as indicated in Table 1. Irradiation of the proton signal at δ 6.89 showed a spatial interaction with the methoxyl group at δ 3.93 and located this methoxyl at C-7/C-7″. The HMBC experiment also showed a long range correlation between the C-7-methoxyl (δ 3.93) with the carbon signals at δ 163.4 (C-7/C-7″), thus establishing the chemical shift of C-7. Key-correlations were the additions observed between the proton resonances at δ 6.89/6.92 (H-8/H-8″) and the carbon signals at δ 163.4 (C-7/C-7″), 157.4 (C-9/C-9″) and 104.0 (C-10/C10″), which corroborated the (C-6→C-6″) interflavonoid linkage. The structure of **7** was thus established as 5-hydroxy-4′, 7-dimethoxyflavone-(6→6″)-5″-hydroxy-3‴,4‴,7″-trimeth-

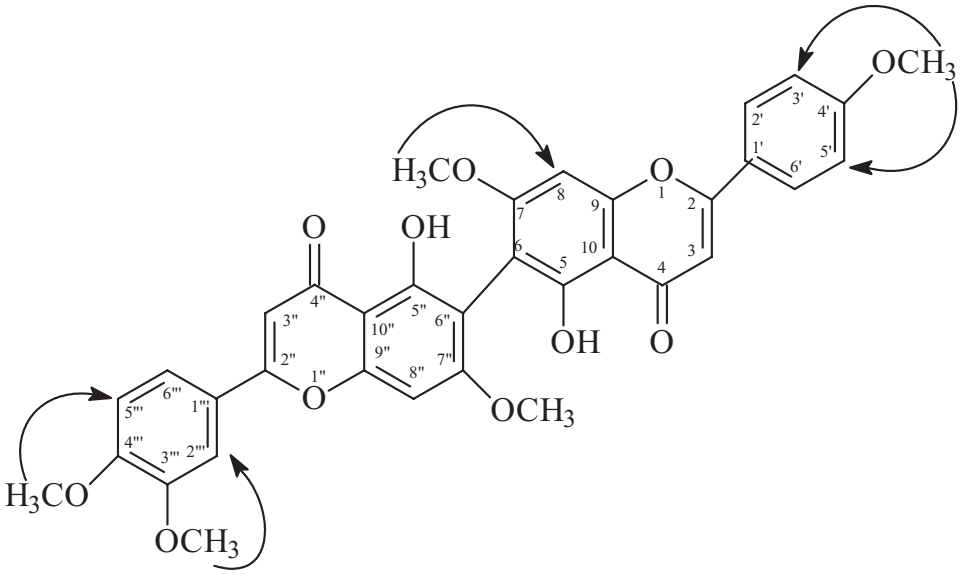

Fig. 1. Flavone dimer **7** isolated from *M. cabucu* with selected observed NOE interactions.

oxyflavone, the first flavone dimer with apigenin and luteolin derived units coupled through carbons C-6→C-6″.

The majority of the naturally occurring biflavonoids contain carbon–carbon linked monomers, with ring A usually being involved in the interflavonoid linkage (Locksley, 1973; Geiger and Quinn, 1975). The combinations so far found in nature are (C-6→C-8), (C-3′→C-6″), (C-8→C-8″), (C-3′→C-8″) and (C-3→C-8″). The biflavonoids linked only at C6→C6″ has been obtained by total synthesis. For example, the C-6→C-6″ biapigenin hexamethylether was generated in four steps from benzyl 4-iodo-3,5-dimethoxyphenylether (Lin et al., 2001).

However, to our knowledge, there is no report of any biflavonoids linked through the C-6→C-6″ position, isolated from a natural product origin. This report thus demonstrates the unusual structure of the compound isolated in this work.

3. Experimental

3.1. General experimental procedure

Melting points were measured on a digital MQ APF-301 (Microquímica®, Brazil) apparatus. Elemental analysis of carbon, nitrogen and oxygen were performed on a CE Instruments EA 1110 – CHNS-O analyzer. UV spectra were recorded on a HACH UV–Vis DR/4000 spectrophotometer, whereas IR spectra were obtained using Shimadzu FT-IR 8300 spectrophotometer. 1D and 2D NMR spectra were obtained in DMSO-d_6 using a Varian® INOVA 500 operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C using TMS as internal standard. Electrospray mass spectrometry (ESI-MS) was performed using a Fisons VG Platform instrument in the positive mode (70 eV) for compounds **1**–**6**. Samples were dissolved in methanol and injected directly into the mass spectrometer through a Rheodyne injector. CH_3CN was used as solvent and nitrogen gas was used as both the drying gas and nebulization gas. HREIMS of **7** was performed using an ultrOTOF$_Q$ – ESI-TOF Mass Spectrometer Bruker Daltonics (Billerica, MA – USA) instrument. Fractions were purified by HPLC equipped with R401 refractive index detector, a Phenomenex® Luna reversed-phase C-18 column (25 cm × 1 cm i.d., 10 mm) and a Rheodyne injector with a 100 μl sample loop. TLC were performed on silica gel 200 μm (Sorbent Technologies®) and visualized using UV light (254 and 365 nm).

3.2. Plant material

Aerial parts of *M. cabucu* were collected in April of 2005 at Pariquera-Açu, São Paulo State, Brazil, and authenticated by Prof. Dr. Jorge Yoshio Tamashiro from the Instituto de Biologia, Unicamp, São Paulo. A *voucher* specimen (no. 1430) has been deposited in the Herbarium of the Universidade Estadual de Campinas, Brazil.

3.3. Extraction and isolation

The dried leaves of *M. cabucu* (600 g) were separated, powdered and extracted exhaustively at room temperature with $CHCl_3$ (2 l) and MeOH (3 l), one week for each solvent. Solvents were evaporated at 35 °C under reduced pressure to afford the $CHCl_3$ (14.0 g, 2.33%) and MeOH (20.0 g, 3.33%) extracts, respectively.

The MeOH extract was partitioned three times with EtOAc/H_2O (1:1), with the EtOAc layer evaporated at 35 °C under reduced pressure, to afforded a residue (1.4 g) which was applied to a Sephadex LH-20 (Pharmacia) column (100 cm × 5 cm i.d.) eluted with MeOH. Fractions (8.0 ml) were collected and analyzed by TLC [$CHCl_3$/MeOH/*n*-PrOH–H_2O (5:6:1:4)] and sprayed with anisalde-

hyde/H_2SO_4 solution (Wagner et al., 1984) for visualization. Fractions were further purified by reversed-phase C-18 HPLC eluted with MeOH/H_2O (3:2). Fractions 102–107 (100 mg) afforded gallic acid (**1**, 38 mg) and quercetin-3-*O*-α-L-rhamnopyranosyl-(2→1)-*O*-β-D-xylopyranoside (**2**, 38 mg), fractions 120–129 (91 mg) afforded quercetin-3-*O*-α-L-rhamnopyranoside (**3**, 33 mg), where fraction 130–149 (160 mg) afforded myricetin-3-*O*-α-L-rhamnopyranoside (**4**, 5 mg), quercetin-3-*O*-β-D-glucopyranoside (**5**, 5 mg) and kaempferol-3-*O*-β-D-(6″-coumaroyl)-glucopyranoside (**6**, 10 mg). Additionally fractions 108–119 (119 mg) gave, after further purification as above, 5-hydroxy-4′,7-dimethoxyflavone-(6-C-6″)-5″-hydroxy-3‴, 4‴,7″-trimethoxyflavone (**7**, 11 mg).

3.4. 5-Hydroxy-4′,7-dimethoxyflavone-(6-C-6″)-5″-hydroxy-3‴,4‴,7″-trimethoxyflavone (7)

Yellow solid (mp 151–153 °C); UV λ_{max}^{MeOH} nm (log ε): 217 (3.61), 241 (3.42), 276 (3.37), 336 (3.48); IR ν_{max}^{MeOH} (KBr) cm^{-1}: 3448, 1658; TOFMS ES$^+$ *m/z* 625.3905 $(M+H)^+$, 463.3212 $(M-C_{10}H_{10}O_2+H)^+$, 419.2933 $(M-C_{11}H_{10}O_4+H)^+$, 313.1228 $(M-C_{17}H_{12}O_6+H)^+$. Anal. calc.: $C_{35}H_{28}0_{11}$ requires: C, 67.32; H, 4.50. Found: C, 67.30; H, 4.52. For ^1H and ^{13}C NMR spectroscopic analyses, see Table 1.

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