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A FLAVONE FROM LEAVES OF *ARRABIDAEA CHICA* f. *CUPREA*

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Abstract—A new flavone, 6,7,3′,4′-tetrahydroxy-5-methoxyflavone, named carajuflavone, was isolated from the leaves of the Brazilian plant *Arrabidaea chica* f. *cuprea*. The structure was established by spectroscopic analysis.

INTRODUCTION

The family Bignoniaceae comprises about 120 genera and 650 species that are distributed mainly in tropical America and Africa [1, 2]. The genus *Arrabidaea* occurs in tropical America, from south of Mexico to central Brazil. Although *A. chica* f. *cuprea* has a limited occurrence in the south of Brazil, Paraguay and northeast of Argentina, *A. chica* is very common in the Amazon region and has been widely used as an anti-inflammatory and astringent agent as well as a remedy for intestinal colic, sanguine diarrhoea, leucorrhoea, anaemia and leukaemia [3]. In the past, the chemical constituents of the leaves, which supply a precious red dye, were studied by Chapman *et al.* who identified the 3-desoxyanthocyanidin named carajurin [4]. Later, Harborne [5] and Scogin [1] proposed that the occurrence of this rare pigment in Bignoniaceae is probably restricted to the species *A. chica*. In a previous study of the leaves of *A. chica* (H&B) Verlot f. *cuprea* (Cham.) Sandw. locally called 'carajurú', the presence of anthocyanins, flavonoids, tannins and phytosterols in addition to 7,4′-dihydroxy-5-methoxyflavone (thevetiaflavone) [6] has been reported. In the present paper, the isolation and structural determination of a new flavone from the leaves is described.

RESULTS AND DISCUSSION

An ethyl acetate-soluble layer of 95% methanol extract of the leaves of *Arrabidaea chica* f. *cuprea* was subjected to column chromatography on silica gel, polyamide and polyclar, successively, to yield **1** and **2**. Compound **1**, obtained as a pale yellow amorphous powder, showed $[M]^+$ at *m/z* 316.0594 in the high resolution EI-mass spectrum, which corresponds to the molecular formula $C_{16}H_{12}O_7$ (calcd 316.0583). The UV absorption bands at 275 and 336 nm revealed that **1** was a flavone, and the bathochromic shifts observed by addition of shift reagents such as $AlCl_3$/HCl and NaOAc/H_3BO_3 indicated that **1** had free hydroxyl groups at C-7, and C-6 or C-8 on the A ring and an *ortho*-dihydroxyl group on the B ring in a flavone skeleton [7]. The ^{1}H NMR spectrum showed the presence of four hydroxyls [δ8.28, 8.37, 8.63 and 8.92 (1H each, *br s*)] and a methoxyl group [δ3.88 (3H, *s*)]. Among them, two hydroxyls and the methoxyl group were located on the A ring, and the remaining two hydroxyl groups were on the B ring, which was also supported by the EI-mass spectrum fragment ions caused by retro-Diels–Alder (RDA) cleavage (*m/z* 181 derived from the A ring and *m/z* 134 from the B ring). The ^{1}H NMR spectrum further exhibited three protons in a ABX system [δ6.98 (1H, *d*, J = 8 Hz), 7.41 (1H, *dd*, J, = 8, 2 Hz) and 7.46 (1H, *d*, J = 2 Hz)] and two one-proton singlets [δ6.45 and 6.88]. The above data suggested the partial structure of the B ring moiety is a 3′,4′-dihydroxyl substituent, which was confirmed by the HMBC experiment (Fig. 1). On the other hand, the methoxyl group appeared in a lower field at δ62.3 in the ^{13}C NMR spectrum, indicating that both *ortho*-positions of the methoxyl group were occupied by substituents. The aromatic carbons bearing *O*-function were observed at δ137.3, 145.9, 152.2 and 152.7 in the ^{13}C NMR spectrum, indicating the A ring moiety to be a 1,3,4,5-tetraoxygenated benzene. As no chelated hydroxyl group was observed in the ^{1}H NMR and the UV spectrum, the structure of **1** was either 6,7,3′,4′-tetrahydroxy-5-methoxy- or 7,8,3′,4′-tetrahydroxy-5-methoxyflavone. In the HMBC spectrum, the methoxyl group (δ3.88) was correlated to the aromatic carbon at δ145.9 assigned to C-5. The aromatic proton (δ6.88) assignable to H-8 was correlated to three aromatic carbons with *O*-function (δ137.3, 152.2 and 152.7) in addition to a quaternary

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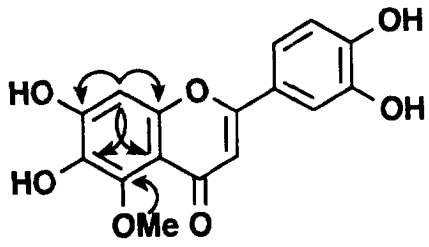

Fig. 1. HMBC spectrum of 1 (correlations observed in the B and C rings were omitted).

aromatic carbon (δ112.6, C-10) through ^2J and ^3J. Therefore the methoxyl group was located at the *para*-position (C-5) to the aromatic proton (δ6.88). The structure of carajuflavone was, then, concluded to be 6,7,3',4'-tetrahydroxy-5-methoxyflavone (**1**).

In addition to **1**, luteolin (**2**) was isolated and the structure was elucidated by means of spectral analysis. Biological assays, using the isolated compounds, revealed that **1** had a weak activity on the inhibition of enzyme release and superoxide production by rabbit neutrophils stimulated by formyl-methionyl-leucyl-phenylalanine (FMLP), and **2** showed strong inhibitory effects on these responses of neutrophils (Takemura *et al.*, unpublished data).

EXPERIMENTAL

Plant material. Leaves of *Arrabidaea chica* f. *cuprea* were collected at Alto da Glória district, Curitiba City, Paraná, Brazil in April 1989. The specimen was identified by Dr Gerdt Hatschbach, Botanical Garden of Curitiba City. A voucher specimen is deposited in the Herbarium of Department of Botany, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba City, under registration number n° 20.549.

Extraction and isolation. Dried leaves (2.0 kg) were exhaustively extracted with 95% MeOH. The concd extract was partitioned with petrol, toluene, $CHCl_3$ and EtOAc, successively. Upon concentration, the EtOAc layer (17.8 g) was subjected to CC on silica gel using $CHCl_3$, EtOAc and MeOH as eluent. The EtOAc elution (7.29 g) was rechromatographed on silica gel using $CHCl_3$–MeOH–HCO_2H (40:10:1). The initial frs I and II were further transferred to another chromatograph on polyamide 200 eluted with CH_2Cl_2–MeOH–H_2O (40:10:1). Fr. I was finally submitted to chromatography on polyclar eluted with $CHCl_3$–EtOH–MeCOEt–Me_2CO (40:20:5:1) to give **2** (18 mg). The fr. II was also subjected to chromatography over polyclar eluted with $CHCl_3$–MeOH–MeCOEt–Me_2CO (20:10:5:1) with gradual increase in MeOH in $CHCl_3$ to give **1** (26 mg).

Compound **1**, *carajuflavone*, 6,7,3'4'-*tetrahydroxy*-5-*methoxyflavone*. A pale yellow amorphous powder [MeOH]. HR-EIMS *m/z* 316.0594 (calcd 316.0583 for $C_{16}H_{12}O_7$). EIMS *m/z* (rel. int.): 316 (M^+, 91), 301 (26), 298 (100), 273 (23), 270 (10), 242 (10), 181 (5), 167 (6), 153 (14), 139 (7), 137 (13), 135 (10), 134 (26), 69 (27), 44 (21). UV λ_{max} (nm, MeOH): 275, 336; + NaOH: 265sh, 296, 403; + $AlCl_3$: 260sh, 315, 370sh, 473; + $AlCl_3$/HCl: 275, 338, 413; + NaOAc: 270, 318sh, 364; + NaOAc/H_3BO_3: 252sh, 283, 356. ^{1}H NMR (400 MHz, acetone-d_6) δ:3.88 (3H, *s*, OMe), 6.45 (1H, *s*, H-3), 6.88 (1H, *s*, H-8), 6.98 (1H, *d*, J = 8 Hz, H-5'), 7.41 (1H, *dd*, J = 8, 2 Hz, H-6'), 7.46 (1H, *d*, J = 2 Hz, H-2'), 8.28, 8.37, 8.63 and 8.92 (1H each, *br s*, OH × 4). ^{13}C NMR (100 MHz, acetone-d_6): δ62.3 (MeO), 100.3 (C-8), 106.6 (C-3), 112.6 (C-10), 113.9 (C-2'), 116.6 (C-5'), 119.5 (C-6'), 124.5 (C-1'), 137.3 (C-6), 145.9 (C-5), 146.4 (C-3'), 149.4 (C-4'), 152.2 (C-7*), 152.7 (C-9*), 162.1 (C-2), 176.9 (C-4), (*: interchangeable). All carbons were assigned with the aid of HMBC spectrum.

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