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TWO METHOXYLATED FLAVONE GLYCOSIDES FROM *BIDENS PILOSA*

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Abstract—Two methoxylated flavone glycosides were identified as the novel quercetin 3,3′-dimethyl ether 7-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside and the known quercetin 3,3′-dimethyl ether 7-*O*-β-D-glucopyranoside from the roots of *Bidens pilosa*. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Bidens pilosa is a plant widely found in tropical and subtropical regions of the world and is used in traditional medicine as an antiinflamatory, a diuretic, an antirheumatic, an antibiotic and against diabetes [1–3]. In the Amazon, the roots of *B. pilosa* are used in the treatment of liver disorders caused by malaria [4,5]. Several phytochemical studies have been done on the aerial parts of this plant but few with its roots [6–13]. We describe now the isolation and structural elucidation of two methoxylated flavone glycosides from its roots.

RESULTS AND DISCUSSION

The concentrated ethanolic extract from the roots of *Bidens pilosa*, yielded a precipitate separated by filtration. Repeated MPLC of this fraction allowed the isolation of **1** and **2**. Compound **1** was isolated as a yellow amorphous powder. TLC and HPLC analysis indicated low polarity of **1** in comparison to an authentic sample of rutin. Total acid hydrolysis and TLC with samples of sugars reveled the presence of glucose and rhamnose in the molecule. The presence of a glucorhamnosyl moiety on the structure was confirmed by FABMS spectrum, which showed a molecular ion at *m/z* 639 $[M+1]^+$ (15%), follow by ions at *m/z* $[M+1-\text{rhamnose}]^+$ 493 (10%) and at *m/z* $[M+1-\text{rhamnose–glucose}]^+$ 331 (100%). The molecular weight of 331 suggested a molecular formula for the aglycon, of $C_{17}H_{14}O_7$, compatible with a flavone bearing three hydroxyl and two methoxyl groups.

The chemical shift assignment of the ^1H NMR spectrum of **1** (400 MHz, pyridine d_5) exhibited signals attributed to a quercetin moiety. The presence of a doublet at δ_H 5.72 (1H, $J = 7.5$ Hz) and a singlet at 5.55 (1H) were assigned to H-1 of a β-D-glucopyranoside and a α-L-rhamnopyranose, respectively. A doublet at δ_H 1.61 (3H, $J = 9.0$ Hz) was assigned for the rhamnosyl Me and the two singlets at δ_H 3.82 and 3.90 (3H each) were assigned for two—OMe groups. Two doublets attributed to one proton each at δ_H 6.82 and 6.98 ($J = 2.5$ Hz) were assigned to H-6 and H-8 of ring A of aglycone. Another three signals at $_H$ 7.23 (1H, *d*, $J = 9$ Hz), 7.85 (1H, *dd*, $J = 9$ and 2.4 Hz) and 8.14 (1H, *d*, $J = 2.5$ Hz) were assigned to protons at C-5′, C-6′, and C-2′ of ring B. Normal and DEPT ^{13}C NMR exhibited signals for the anomeric carbons of the sugars at δ_C 102.3 and 102.0 ppm. The interglycosidic linkage was determined by DEPT spectrum, which showed a single signal for methylene carbon at δ_C 67.3, assigned to C-6 of the glucopyranoside. The presence of intense cross peak between the H-1 of rhamnopyranosyl and the H_2-6 of glucopyranoside in the NOESY spectrum supported this assumption.

The location of the substituents on the aglycone was evidenced by the use of diagnostic reagents in UV spectra and 2D-NMR spectra. Acid hydrolysis of **1** gave an aglycone whose UV spectrum in MeOH/NaOAc showed a consistent bathochromic shift (255–275 nm), absent in spectrum of **1**, indicating a free position at C-7. This finding, together with the NOESY cross peaks between the H-1 of glucopyranoside and H-6 and H-8 of the aglycon, confirmed the presence of the sugar chain at C-7. The

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marked bathochromic effect observed with the addition of NaOH, and the absence of a free 3′,4′-dihydroxysystem after adition of $AlCl_3$/HCl and $NaOAc/H_3BO_3$ in UV spectra suggested the presence of one methoxyl group at C-3′. The presence of signals for methoxyl groups at δ_C 55.6 and 59.9 in the ^{13}C NMR spectrum confirms the hypothesis that one —OMe group should be located on C-3′ and another on C-3 of the aglycone [14]. Thus **1** has the novel structure quercetin 3,3′-dimethyl ether 7-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside.

Compound **2** was also isolated as a yellow amorphous powder and its UV spectrum showed the same characteristic bands for a flavone glycoside. Acid hydrolysis of **2** yielded only glucose and its FABMS spectrum showing a molecular ion at *m/z* $[M+1]^+$ 493 (22), followed by an ion at *m/z* $[M+1-\text{glucose}]^+$ 331 (100), has confirmed the presence of one glucose. In addition, the ^1H and ^{13}C NMR spectrum were superimponsable with **1**, except for the absence of the signals attributed to a rhamnosyl group. On the basis of these data, **2** was identified as quercetin 3,3′-dimethyl ether 7-*O*-β-D-glucopyranoside [15].

EXPERIMENTAL

General

Mps uncorr. UV: VIS/UV Shimadzu Model UV 2400 in MeOH, with subsequent addition of the usual reagents: NaOAc, NaOH, $AlCl_3$, HCl, H_3BO_3. ^1H and ^{13}C NMR: 400 and 100 or 360.13 and 90 MHz. δ [ppm] relative to int. TMS (0 ppm) and *J* in Hz. FABMS: ZAp-HP, glycerol matrix (positive ion mode). MPLC: Buechi Column (26 × 400 mm), with silica-gel (230–400 mesh) or RP-18 and Sephadex LH-20 (Pharmacia). TLC: solvent systems (a) AcOEt:HCOOH:CH_3COOH:H_2O (100:11:11:26) (b) $CHCl_3$:MeOH:H_2O (8:5:1); spray reagents (c) $AlCl_3$/MeOH (d), NP/PEG and (e) diphenylamine phosphoric acid, followed by heating.

Plant material

The roots were collected around Pampulha Lake, Belo Horizonte, Brazil and identified by T.S.M. Grandi. A voucher specimen (No. GR/101) was deposited at the Pharmacognosy Laboratory, Federal University of Minas Gerais (UFMG), Belo Horizonte.

Extraction and isolation

300 g of *B. pilosa* roots were percolated exhaustively with 90% ethanol and the soln evaporated at a maximum temperature of 50°C. The concd ethanolic extract, stored in the refrigerator, yielded a ppt (8.4 g) that was separated by filtration. It was submitted to MPLC with a mixt. of $CHCl_3$:MeOH:H_2O (8:5:1). **1** (16.8 mg) and **2** (3.5 mg) were obtained and purified by MPLC on reverse-phase material, using MeOH:H_2O (9:1) mixtures and Sephadex LH-20 with MeOH.

Quercetin 3,3′-dimethyl ether 7-O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside **1**. Yellow powder; mp: browns at 133° and decomposes at 224°; $[\alpha]_D$ −28; TLC R_f 0.36 in (a) (rutin 0.30); UV λmax (nm/MeOH): 210, 255, 268, 355; +$AlCl_3$: 210, 268, 280, 355, 405; +$AlCl_3$–HCl: 210, 268, 280, 355, 405; +NaOH: 220, 270, 385. FAB-MS *m/z*: $[MH]^+$ 639 (15), $[MH-\text{rha}]^+$ 493 (10), $[MH-\text{rha}-\text{glu}]^+$ 331 (100). ^1H NMR(400 MHz, pyridine d_5): δ 6.71 (H-6, *d*, *J* = 2.5 Hz), 6.84 (H-8, *d*, *J* = 2.5), 8.00 (H-2′, *d*, *J* = 2.1), 7.16 (H-5′, *d*, *J* = 8.1), 7,78 (H-6′, *dd*, *J* = 2.1 and 8.1), 3.80 (OMe, 3H, *s*), 3.83 (OMe, 3H, *s*), 5.55 (glu-1=H-1″, *d*, 7.5 Hz), 4.6 (H-3″, *dd*, *J* = 1.5 and 3.5), 4.62 (H-6″, *dd*, *J* = 1.5 and 11.5), 5.31 (H-1‴, *s*), 1.53 (Me—rha, *d*, *J* = 9.0 Hz). ^{13}C NMR (pyridine d_5): δ 157.8 (C-2), 139.0 (C-3), 179.0 (C-4), 157.1 (C-5), 100.4 (C-6), 164.8 (C-7), 94.8 (C-87), 151.2 (C-9), 99.8 (C-10), 123.3 (C-1′), 112.2 (C-2′), 148.0 (C-3′), 148.2 (C-4′), 116.4 (C-5′), 121.2 (C-6′), 55.8 (OMe-C3′), 59.9 (OMe-C3), 102.3 (glu-1=C-1″), 74.6 (C-2″), 78.2 (C-3″), 73.9 (C-4″), 77.5 (C-5″), 67.6 (C-6″), 102.0 (rha-1=C-1‴), 71.3 (C-2‴), 72.7 (C-3‴), 72.7 (C-4‴), 69.5 (C-5‴), 18.5 (C-6‴). NOESY (pyridine d_5) cross-peaks: H-1″ (δ 5.55)/H-6 (7.78) and H-8 (δ 6.84), H-1‴ (δ 5.31)/H-6″ (δ 4.62), H-6′ (δ 7.78)/H-5′ (7.16), H-6 (δ 6.71)/H-8 (6.84).

Quercetin 3,3′-dimethyl ether 7-O-β-D-glucopyranoside **2**. [15]: Yellow pulver; mp decomposition at 202°C; $[\alpha]_D$ −22; TLC R_f 0.48 in (c); UV λ_{max} (nm/MeOH): 210, 255, 355; +$AlCl_3$: 210, 268, 280, 355, 405; +NaOH: 222, 270, 385; NaOH–HCl, 210, 255, 355. FAB-MS (*m/z*): 493 $[MH]^-$, 331 $[MH-\text{glu}]^+$. ^1H NMR (MeOD): 6.51 (H-6, *d*, *J* = 2.5 Hz), 6.79 (H-8, *d*, *J* = 2.5 Hz), 7.63 (H-2′, *d*, *J* = 2.5 Hz), 7.10 (H-5′, *d*, *J* = 8.5 Hz), 7.69 (H-6′, *dd*, *J* = 2.5 and 8.5 Hz), 3.80 (OMe, 3H, *s*), 3.95 (OMe, 3H, *s*), 5.10 (H-1‴, *d*, 7.5 Hz).

Total acid hydrolysis

Compound **1** (2.0 mg) was dissolved in 1 N HCl (3 ml) and heated at 110°C in a sealed tube for 4 h, then diluted with water. The aglycone was extracted with CH_2Cl_2 and submitted to UV spectra and TLC analysis. UV λ_{max} (nm/MeOH): 208, 255, 270, 355; +$AlCl_3$: 208, 265, 355; +NaOAc: 220, 278, 380: +NaOH: 215, 275, 292. The remaining aq. layer give glucose and rhamnose (system b).

Micro hydrolysis on the TLC plate

0.5 mg of **1** and **2** were applied to a TLC plate and submitted to hydrolysis in a chamber with HCl (30 min at 105°C). After elimination of HCl, the plate was submitted to system b with samples of sugars. Rhamnose and glucose were detected for **1** but only glucose for **2**.

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