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Isoflavone glycosides from *Centrosema pubescens*

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Abstract

Afromosin 7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside, was isolated from the seeds of *Centrosema pubescens*, together with the known afromosin 7-*O*-*β*-D-glucopyranoside and irisolidone 7-*O*-*β*-D-glucopiranoside. Their structures were established by spectroscopic and chemical methods.

Keywords: Centrosema pubescens; Leguminosae; Seeds; Isoflavone glycosides; Afromosin 7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside; Afromosin 7-*O*-*β*-D-glucopyranoside; Irisolidone 7-*O*-*β*-glucopyranoside

1. Introduction

We previously reported the isolation and structural elucidation of an isoflavone glycoside, called pubescidin, and sitosterol, stigmasterol and sitosterol 3-*O*-*β*-D-glucopyranoside from the seeds of *Centrosema pubescens* (Tostes, Silva, & Parente, 1997). We continued our investigation of the constituents of the seeds of this species and isolated a new isoflavone glycoside, afromosin 7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside (**1**), along with afromosin 7-*O*-*β*-D-glucopyranoside (**2**) (Shibata, Murata, & Fujita, 1963) and irisolidone 7-*O*-*β*-D-glucopyranoside (**3**) (Kubo, Fujita, Nishimura, Naruto, & Namba, 1973). This paper deals with the isolation and structural elucidation of the new and known compounds.

2. Results and discussion

Fractionation of a MeOH extract from the dried seeds of *C. pubescens* by a combination of adsorption chromatography on silica gel and repeated column fractionation on Sephadex LH-20, yielded the isoflavone glycoside (**1**).

The molecular formula of **1** calculated as $C_{28}H_{32}O_{14}$ by combination of its LSIMS (neg. ion mode) *m*/*z* 591[M–H]$^-$ and ^{13}C NMR spectral data (Table 1). The UV spectrum of **1** showed at 260 nm (4.15) and 320 nm (4.02). The chromatographic behavior of **1**, UV, IR 3400 cm^{-1} (OH) and 1622 cm^{-1} (>C═O), ^{1}H NMR *δ* 8.35 (1H, s, H-2) (Caballero, Smith, Fronczek, & Fischer, 1986; Tanaka, Ohsaki, & Takahashi, 1975), ^{13}C NMR *δ* 153.29 (CH, C-2) and 124.70 (C, C-3) (Jha, Zilliken, & Breitmaier, 1980; Murthy, Rao, & Ward, 1986) spectra established that **1** is an isoflavone glycoside. The ^{1}H spectrum displayed, in addition to a signal for two methoxyl groups, H-2 of an isoflavone nucleus, two doublets at *δ* 7.0 and 7.5 for H-3′, H-5′ and H-2′, H-6′, respectively. Two singlets at *δ* 7.56 and 7.38 integrating for single protons were assigned to H-5 and H-8, respectively. Two doublets at *δ* 5.13 (*J*=7.1 Hz) and 4.80 (*J*=3.1) integrating for single protons were attributed to H-1 of a glucose and H-1 of an apiose, respectively, indicating *β*-linkages Table 1 (Tostes et al., 1997).

The ^{13}C NMR spectrum showed two quartets which resonated at *δ* 55.07 and 55.93, and were assigned to the carbons of the two methoxyl-substituents at C-4′ and C-6, respectively. The signal at *δ* 174.70 was attributed to the carbonyl carbon. The resonance of the aromatic moiety was assigned by DEPT, ^{1}H–^{13}C COSY and ^{1}H–^{13}C COLOC and by comparison with data from the literature (Jha et al., 1980; Murthy et al., 1986; Tostes et al., 1997). The proposed structure **1** was fully supported by its ^{13}C NMR spectrum, which exhibited peaks for 28 carbon atoms Table 1.

On acid hydrolysis, compound **1** yielded afromosin (7-hydroxy-6,4′-dimethoxy isoflavone) (**4**) (McMurry & Theng, 1960), glucose and apiose. Mp and UV, IR, ^{1}H and ^{13}C NMR spectral data of **4** were in accordance with those reported in the literature (McMurry & Theng, 1960; Harborne, Gottlieb, & Magalhães, 1963; Shibata et al., 1963; Tanaka et al., 1975; Jha et al., 1980; Caballero et al., 1986; Murthy et al., 1986). Compound **4** revealed [M]$^+$ at *m*/*z* 298.2975, $C_{17}H_{14}O_5$. The molar carbohydrate

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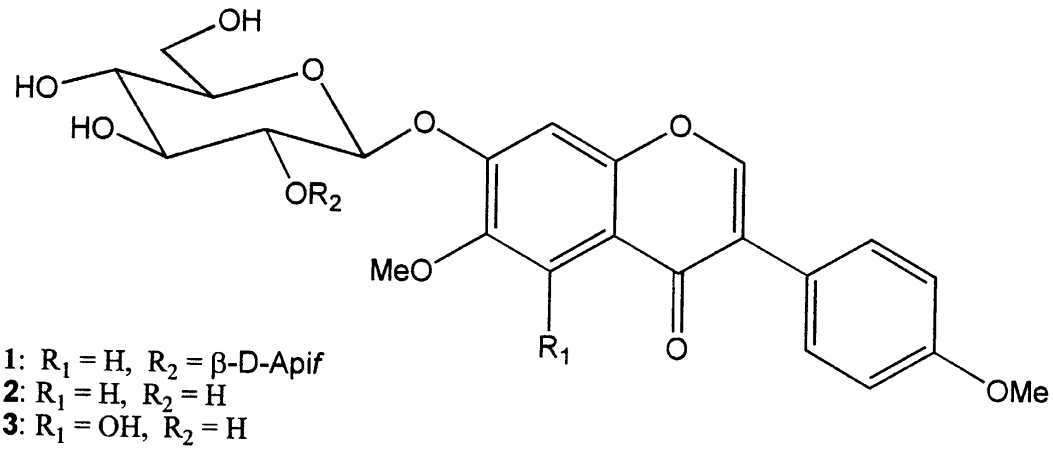

Structure 1.

composition of **1** indicated the presence of two neutral monosaccharides glucose:apiose (1.0:0.9). Their absolute configurations were determined by GC of their TMSi (−)-2-butylglycosides. D-Glucose and D-apiose were identified by GC-EIMS of the pertrimethylsilylated methylglycosides.

The methylation analysis of **1** showed a 2-linked glucopyranose and a terminal apiofuranose. The conclusions of these chemical reactions were corroborated by the chemical shifts of glycosidated carbon atoms in the ^{13}C NMR spectrum. The C-2 of a glucosyl unit was observed at δ 77.13, showing that the apiosyl unit was linked to it.

Table 1
^{1}H and ^{13}C NMR spectral data for compounds **1**–**3** in DMSO-d$^{a,b,c}_{6}$

	1		**2**		**3**	
Attribution	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$
2	153.29 d	8.35 s	153.29 d	8.35 s	155.00 d	8.40 s
3	124.70 s		124.70 s		122.80 s	
4	174.70 s		174.70 s		180.80 s	
4a	117.84 s		117.83 s		106.60 s	
5	104.80 d	7.56 s	104.80 d	7.54 s	152.60 s	
6	147.00 s		147.13 s		132.00 s	
7	154.50 s		154.50 s		156.60 s	
8	103.80 d	7.38 s	103.80 d	7.33 s	94.40 d	6.90 s
8a	152.60 s		152.60 s		152.90 s	
1′	123.60 s		123.60 s		121.80 s	
2′ and 6′	130.10 d	7.50 d(9.0)	130.10 d	7.50 d(9.0)	130.20 d	7.50 d(9.0)
3′ and 5′	113.70 d	7.00 d(9.0)	113.70 d	7.00 d(9.0)	113.80 d	7.00 d(9.0)
4′	159.15 s		159.15 s		159.12 s	
5-OH						12.90 s
6-OCH_3	55.93 q	3.79 s	55.93 q	3.79 s	55.91 q	3.78 s
4′-OCH_3	55.07 q	3.77 s	55.07 q	3.77 s	55.25 q	3.76 s
Glc-1	100.13 d	5.13 d(7.1)	99.93 d	5.13 d(7.1)	100.28 d	5.10 d(7.2)
2	77.13 d		73.22 d		73.24 d	
3	76.00 d		77.37 d		77.38 d	
4	70.01 d		69.81 d		69.83 d	
5	76.75 d		76.80 d		76.83 d	
6	61.30 t		60.79 t		60.80 t	
Api-1	109.53 d	4.80 d(3.1)				
2	76.80 d					
3	78.74 q					
4	73.38 t					
5	64.93 t					

[a] Coupling constants (J in Hz) in parentheses.[b]Individual protons assigned by 2-D-COSY, ^{1}H ^{13}C COSY and ^{1}H and ^{13}C COLOC experiments.[c]Multiplicities were determined by DEPT experiments.

Hence **1** was established as afromosin 7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside. The identification of afromosin 7-*O*-*β*-D-glucopyranoside and irisolidone 7-*O*-*β*-D-glucopyranoside was made by comparison of mp, $[\alpha]_D$, UV, IR and ^{1}H NMR data Table 1 with the literature (Shibata et al., 1963; Kubo et al., 1973; Tanaka et al., 1975; Ali, El-Elmary, El-Moghazi, Darwish, & Frahm, 1983), and ^{13}C NMR and LSI-mass spectrometry.

3. Experimental

3.1. General

Mps are uncorr. OR measured at 20°C. IR spectra: KBr discs. ^{1}H NMR: 200 MHz, in $CDCl_3$ or DMSO-d_6. TMS as int. standard. ^{13}C NMR edited DEPT spectra: 50 MHz from $CDCl_3$ and DMSO-d_6 solns. GC carried out with FID, using a capillary column (0.3 mm × 25 m) OV-101 EIMS and GC–MS: recorded at 70 eV. Negative LSIMS carried out using an HMPA–glycerol mixt. as matrix, 35 kV anodic, 8 kV anodic voltage, 8 kV accelerating voltage using Cs ions. Silica gel columns (230–400 mesh ASTM Merck) and Sephadex LH-20 used for CC. TLC was performed on silica gel coated plates (Merck) using the following solvent systems: (a) $CHCl_3$–MeOH (4:1) for compound **1**, (b) $CHCl_3$–MeOH (5:1) for compounds **2** and **3**, (c) $CHCl_3$–MeOH (19:1) for isoflavone aglycone and (d) *n*-BuOH–pyridine–H_2O (6:4:3) for sugars. Compounds **1**, **2**, **3** and afromosin detected under UV 254 and 366 nm and by spraying with orcinol–H_2SO_4, sugars by spraying with aniline–diphenylamine–85% orthophosphoric acid–MeOH (1:1:5:43).

3.2. Plant material

Seeds of *C. pubeseens* Benth. were collected at Mangaratiba, Rio de Janeiro in September 1975 and identified by V. P. Barbosa. A voucher specimen (No. 172177) is deposited at the Botanical Garden, Rio de Janeiro.

3.3. Extraction and isolation

Dried and powdered seeds of *C. pubescens* (2 kg) extracted with cold MeOH (5 l). Evapn of the MeOH gave a residue (32 g). The residue was submitted to CC (120 × 3 cm) on silica gel which was eluted with $CHCl_3$–MeOH mixts of increasing polarity (up to 35% MeOH) to afford 2 frs: fr. 1 (232 mg, $CHCl_3$–MeOH 67:23) and fr. 2 (712 mg, $CHCl_3$–MeOH, 75:25). Compounds **2** (72 mg, R*f* 0.45) and **3** (84 mg, R*f* 0.41) were isolated pure from the fr. 1 through column fractionation on silica gel (50 × 1 cm) using $CHCl_3$–MeOH (80:15) as solvent. Fr.2 was chromatographed on silica gel (100 × 1 cm) to yield 2 frs: fr. 2a (248 mg) and fr. 2b (412 mg). Pure **1** (183 mg, R*f* 0.52) was isolated from the fr. 2a through repeated column fractionation on Sephadex LH-20, using MeOH as solvent.

3.4. Afromosin 7-O-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside (**1**)

Colorless amorphous powder from MeOH, mp 146–148°, $[\alpha]_D^{20}$ −97° (DMSO, *c* 0.001). UV λ_{max}^{MEOH} nm (log *ε*): 260 (4.15), 320 (3.60). IR ν_{max}^{Kbr} cm^{-1}: 3400 (OH), 1622 (>C=O), 1607, 1580, 1298, 1255, 1178, 1020, 827. ^{1}H and ^{13}C NMR spectra data shown in Table 1. Negative LSIMS, *m*/*z* (rel. int.): 591 $[M–H]^-$ (10), 297 [M-295] (100). Compound **1** (100 mg) was hydrolyzed as previously reported for pubescidin (Tostes et al., 1997) to afford afromosin (**4**, 38 mg). Mp and UV, IR, ^{1}H and ^{13}C NMR spectral data of **4** were in accordance with those reported in the literature (McMurry & Theng, 1960; Harborne et al., 1963; Shibata et al., 1963; Tanaka et al., 1975; Jha et al., 1980; Caballero et al., 1986; Murthy et al., 1986). EIMS (probe) 70 eV, *m*/*z* (rel. int.): 298 $[M]^+$ (100), 283 (15), 267 (3), 166 (48), 132 (9), 117 (6) (Caballero et al., 1986); HRMS found: $[M]^+$ 298.2975, $C_{17}H_{14}O_5$ requires 298.2977. Molar carbohydrate composition and *D*, *L* configurations of the sugars and their methylation analysis were done according to our previous work (Tostes et al., 1997).

3.5. Afromosin 7-O-*β*-D-glucopyranoside (**2**)

Colorless needles from MeOH mp 210°C (Shibata et al., 1963), $[\alpha]_D^{20}$ −66° (DMSO, *c* 0.001). UV λ_{max}^{EtOH} nm (log *ε*): 262 (4.33), 320 (3.80) (Shibata et al., 1963; Harborne et al., 1963). IR ν_{max}^{Kbr} cm^{-1}: 3400 (OH), 1622(>C=O), 1606, 1580 (Harborne et al., 1963). ^{1}H and ^{13}C NMR spectral data shown in Table 1. Negative LSIMS, *m*/*z* (rel. int): 459 $[M–H]^-$ (62), 297 [M-163] (100).

3.6. Irisolidone 7-O-*β*-D-glucopyranoside (**3**)

Colorless needles from MeOH, mp 239°C (Kubo et al., 1973), $[\alpha]_D^{20}$ −79° (DMSO, *c* 0.001). UV λ_{max}^{EtOH} nm (log *ε*): 270 (4.59), 336 (3.94) (Kubo et al., 1973; Ali et al., 1983). IR ν_{max}^{Kbr} cm^{-1}: 3400 (OH), 1659 (>C=O), 1614, 1580, 1300, 1255, 1179, 1020, 830. ^{1}H and ^{13}C NMR spectral data are shown in Table 1. Negative LSIMS, *m*/*z* (rel. int): 475 $[M–H]^-$ (48), 313 [M-163] (100).

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