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PUBESCIDIN, AN ISOFLAVONE GLYCOSIDE FROM *CENTROSEMA PUBESCENS*

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Key Word Index—*Centrosema pubescens*; Leguminosae; seeds; isoflavone glycoside; pubescidin.

Abstract—A new isoflavone glycoside, irisolidone 7-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside was isolated from the seeds of *Centrosema pubescens* along with sitosterol, stigmasterol and sitosterol 3-*O*-β-D-glucopyranoside. The structures of new and known compounds were established by spectroscopic and chemical methods. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Centrosema, a genus of climbing plants (Leguminosae) has 26 reported species in Brazil [1], some of which are used in folk medicine to treat dropsy [2, 3]. *Centrosema pubescens* Benth., known in Brazil as 'patinho', is widely grown as a fodder crop. The chemical study of this plant revealed amino acids [4–6], flavonoids [7], isoflavonoids [8, 9], and cyclohexitols and galactosyl-pinitol [10]. In this paper we report the isolation and structural elucidation of a new isoflavone glycoside, pubescidin (**1**) along with sitosterol, stigmasterol and sitosterol 3-*O*-β-D-glucopyranoside.

RESULTS AND DISCUSSION

Fractionation of a $CHCl_3$–MeOH (80:20) extract from the dried seeds of *Centrosema pubescens* by a combination of adsorption chromatography on non-ionic resin Amberlite XAD-2 and silica gel afforded pubescidin (**1**).

The molecular formula of **1** calculated as $C_{28}H_{32}O_{15}$ by combination of its LSIMS (neg. ion mode) *m/z* 607 $[M-H]^-$ and ^{13}C NMR spectral data (Table 1). The UV spectrum of **1** showed single maxima at 264 nm (4.65) and 329 nm (4.02). The chromatographic behaviour of **1**, UV, IR 3400 cm^{-1} (OH) and 1660 cm^{-1} (>C=O), ^1H NMR δ 8.40 (1H, *s*, H-2) [11], ^{13}C NMR δ 155 (CH, C-2) and 122.80 (C, C-3) [12] spectra established that **1** is an isoflavone glycoside. UV bathochromic shifts with $AlCl_3$ and $AlCl_3$–HCl and a signal at δ 12.88 [13] in the ^1H NMR spectrum indicated the presence of a 5-hydroxyl group. The ^1H NMR spectrum displayed, in addition to signals for two methoxyl groups, H-2 of an isoflavone nucleus and 5-OH, two doublets at δ 7.0 and 7.5 for H-3′, H-5′ and H-2′ and H-6′, respectively. A singlet at δ

Table 1. ^1H and ^{13}C NMR spectral data for pubescidin (**1**) in DMSO-d_6

Attribution	δ ^{13}C	δ ^1H (*J* = Hz)	DEPT
2	155.00	8.40 *s*	CH
3	122.80		C
4	180.80		C
4a	106.60		C
5	152.60		C
6	132.60		C
7	156.60		C
8	94.40	6.90 *s*	CH
8a	152.90		C
1′	121.80		C
2′ and 6′	130.20	7.50 *d*(9.0)	CH
3′ and 5′	113.80	7.00 *d*(9.0)	CH
4′	159.30		C
5-OH		12.88 *s*	
6-OCH_3	60.12	3.78 *s*	CH_3
4′-OCH_3	55.25	3.76 *s*	CH_3
Glc-1	100.30	5.10 *d*(7.2)	CH
2	77.15		CH
3	76.01		CH
4	70.02		CH
5	76.77		CH
6	61.27		CH_2
Api-1	109.50	4.80 *d*(3.1)	CH
2	76.60		CH
3	78.77		C
4	73.39		CH_2
5	64.92		CH_2

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Table 2. Summary of the 2D-NMR correlations of **1**

H	COSY (^{1}H)	HETCOR (^{13}C)	COSY (^{1}H) LR
2		2	
8		8	
2′	3′	2′	
3′	2′	3′	4′-OCH_3
5′	6′	5′	4′-OCH_3
6′	5′	6	
6-OCH_3		6-OCH_3	
4′-OCH_3		4′-OCH_3	3′, 5′
Glc-1	Glc-2	Glc-1	
2			
3			
4		4	
5			
6		6	
Api-1	Api-2	Api-1	
2			
3			
4			
5		5	

Table 3. ^{1}H and ^{13}C NMR spectral data for irisolidone (**2**) in $CDCl_3$

Attribution	δ ^{13}C	δ ^{1}H (J = Hz)	DEPT
2	152.70	7.90 *s*	CH
3	123.00		C
4	181.20		C
4a	106.30		C
5	152.50		C
6	130.30		C
7	155.10		C
8	93.07	6.50 *s*	CH
8a	153.30		C
1′	122.80		C
2′ and 6′	130.00	7.45 *d*(10)	CH
3′ and 5′	114.00	7.00 *d*(10)	CH
4′	159.70		C
5-OH		13.10 *s*	
7-OH		6.60 *s*	
4′-OCH_3	55.25	3.80	CH_3
6-OCH_3	60.76	4.10 *s*	CH_3

6.90 integrating for one proton represented H-8. Two doublets at δ 5.10 (J = 7.2 Hz) and 4.80 (J = 3.1) integrating for single protons were assigned to H-1 of a glucose and H-1 of an apiose, respectively, indicating β-linkages.

The ^{13}C NMR spectrum showed two quartets resonated at δ 55.25 and 60.12, assigned to the carbons of the 2 methoxy-substituents at C-4′ and C-6, respectively. The signal at δ 180.80 was attributed to the carbonyl carbon. The resonance of the aromatic moiety was assigned by DEPT (Table 1), HETCOR (^{13}C) (Table 2) and by comparison with data from the literature [12, 14–16]. 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, pubescidin (**1**) yielded irisolidone (5,7-dihydroxy-6,4′-dimethoxy isoflavone) (**2**) [17], glucose and apiose. Mp and UV spectral data of **2** were in accordance with those reported in the literature [17, 18]. Its IR spectrum was consistent with the structure of irisolidone (**2**). The ^{1}H and ^{13}C NMR spectral data of **2** (Table 3) were identified from direct comparison with the ^{1}H and ^{13}C NMR spectral data of pubescidin (**1**) and DEPT experiments. Compound **2** revealed $[M]^+$ at m/z 314.2969, $C_{17}H_{14}O_6$. The molar carbohydrate 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 was detected. D-Apiose was identified by GC-EI mass spectrometry of the pertrimethylsilylated methylglycoside form (characteristic ions in the EI mass spectrum at m/z 275 and 103 and CI mass spectrum at m/z 398 $[M+18]^+$, 381 $[M+H]^+$, 366 $[M-32+18]^+$ and 349 $[M-32+H]^+$).

The methylation analysis of **1** showed a 2-linked glucopyranose and a terminal apiofuranose. On periodate oxidation of **1**, TLC and PC examinations of the hydrolysate did not show any sugar. The conclusions of these chemical reactions were corroborated by the chemical shifts of glycosidated carbon atoms in the ^{13}C NMR spectrum. C-2 of glucosyl unit was observed at δ 77.15, showing that the apiosyl unit is linked to it.

Hence **1** was established as irisolidone 7-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside. The identification of sitosterol 3-*O*-β-D-glucopyranoside was made on the basis of acid hydrolysis, and comparison with authentic sample by mp, IR, ^{1}H and ^{13}C NMR, and LSI-mass spectrometry. Sitosterol and stigmasterol were also found in the plant.

EXPERIMENTAL

General. Mps are uncorr. OR measured at 20°. IR spectra: KBr discs. ^{1}H NMR: 200 MHz, in $CDCl_3$, DMSO-d_6 or pyridine-d_5. TMS as int. standard. ^{13}C NMR edited DEPT spectra: 50 MHz from $CDCl_3$, DMSO-d_6 or pyridine-d_5 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 and positive LSIMS carried out using an HMPA–glycerol mixt. and glycerol as matrices, respectively, 35 kV anodic voltage, 8 kV accelerating voltage using Cs ions. Silica gel columns (230–400 mesh ASTM, Merck) and Amberlite XAD-2 nonionic polymeric adsorbent (20–60 mesh, Aldrich) used for CC. TLC was performed on silica gel coated plates (Merck) using the following solvent systems: (A) $CHCl_3$–MeOH (4:1) for isoflavone glycoside and (B) $CHCl_3$–MeOH (19:1) for isoflavone aglycone, and (C) *n*-BuOH–pyridine–H_2O (6:4:3) for sugars. Compounds **1** and **2** 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) [19].

Plant material. Seeds of *C. pubescens* Benth. collected at Mangaratiba, Rio de Janeiro, in September 1975, and identified by Vania P. Barbosa. A voucher specimen (no. 172177) is deposited at the Botanical Garden, Rio de Janeiro, Brazil.

Isolation of the constituents. Dried and powdered seeds of *C. pubescens* (990 g) extracted successively with cold $CHCl_3$ and $CHCl_3$–MeOH (80:20). Evapn of the $CHCl_3$ gave a residue (13 g). A part of the residue (5 g) was submitted to CC (30 × 1 cm) on silica gel which was eluted with hexane–CH_2Cl_2 mixts of increasing polarity (up to 10% CH_2Cl_2). Identity of the compounds (sitosterol and stigmasterol) was established by comparison with authentic samples through mp, IR, ^1H and ^{13}C NMR, and EIMS. Another part (8 g) was chromatographed on silica gel (GATEAU) with hexane, EtOAc, Me_2CO and MeOH successively. The EtOAc fr. was evapd to provide an amorphous powder, which was crystallized from MeOH to give sitosterol 3-*O*-*β*-D-glucopyranoside. Hydrolysis with vapour from conc. HCl, 90°, 1 hr, yielded glucose (co-chromatography on MeOH–$CHCl_3$–MeOH, 8:5:1) and sitosterol (co-chromatography on $CHCl_3$–MeOH, 9:1).

Sitosterol 3-O-*β*-D-*glucopyranoside.* Mp 295–297° (dec.). LSIMS: *m/z* 599 $[M+Na]^+$.

To obtain **1**, the $CHCl_3$–MeOH, 4:1 extract was concd *in vacuo* to give a brown powder (23 g) which was chromatographed on Amberlite XAD-2 (500 g). Frs eluted with MeOH yielded a mixt. of 2 compounds. Evapn of the MeOH gave a residue (2.3 g) which was chromatographed on silica gel (100 g). Frs eluted with $CHCl_3$–MeOH–H_2O (13:7:2, lower phase) on repeated chromatographic purification yielded one TLC homogeneous compound (320 mg), R_f 0.48 which gave a dark green colour with alcoholic $FeCl_3$.

Pubescidin (**1**). Yellowish amorphous powder from MeOH, mp 154–156°, $[\alpha]_d^{27}$ −93° (DMSO, *c* 0.7). UV λ^{MeOH} nm (log ε): 264 (4.65), 329 (4.02), ($AlCl_3$): 274, 381; ($AlCl_3$+HCl): 277, 383; (NaOMe): 267, 360; (NaOAc): 265, 329; (NaOAc+H_3BO_3): 265, 330. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1660 (>C=O), 1615, 1580, 1300, 1255, 1180, 1020, 830. ^1H and ^{13}C NMR spectra data shown in Tables 1 and 2. Negative LSIMS, *m/z* (rel. int.): 607 $[M-H]^-$ (39), 313 $[M-295]$ (100).

Acid hydrolysis of pubescidin (**1**). Compound **1** (100 mg) refluxed in 2 M HCl (10 ml) for 2 hr. Aglycone was extracted with EtOAc and evapd to dryness *in vacuo*. The residue was dissolved in MeOH and the soln on concn yielded a yellow compound which on further crystallization gave irisolidone (**2**, 42 mg mp) 193–195°, (lit. [17] mp 191–192°). Irisolidone gave a blue colour with $FeCl_3$. UV λ^{MeOH} nm (log ε): 267 (4.60), 330 (4.10); ($AlCl_3$): 275, 378; ($AlCl_3$+HCl): 277, 381; (NaOMe): 249, 275, 340; (NaOAc): 273, 340; (NaOAc+H_3BO_3): 265, 330. IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1660 (>C=O), 1615, 1580, 1300, 1255, 1180, 1020. ^1H and ^{13}C NMR: Table 3. EIMS (probe) 70 eV, *m/z* (rel. int.): 314$[M]^+$ (100), 299 (59), 271 (62), 132 (12), 117 (7). Aq. layer was adjusted to pH 6 by addition of $NaHCO_3$. After lyophilization, sugars were extracted with pyridine and analysed by silica gel-TLC in the above described system. After spraying, apiose gave a weak yellow spot at R_f 0.78, and glucose gave a blue spot at R_f 0.70. For co-chromatography the hydrolysate of bredemeyeroside **B** [20], and glucose was used.

Molar carbohydrate composition and D,L *configurations.* Monosaccharides were analysed as their TMSi methylglycosides obtained after methanolysis (0.5 M HCl in MeOH, 24 hr, 80°) and trimethylsilylation according to Kamerling *et al.* [21]. The configurations of the glycosides were established by capillary GC of their TMSi (−)-2-butylglycosides [22].

Methylation analysis. Pubescidin (**1**) was methylated with DMSO–lithium methylsulphinyl carbanion–CH_3I [23]. The methyl ethers were obtained either (a) after hydrolysis (4H, TFA, 100°) and analysed as partially polyol-acetates by GC-MS [24] or (b) after methanolysis (0.5 M HCl in MeOH, 24 hr 80°) and analysed as partially methylated methylglycosides by GC-MS [25].

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