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# PUBESCIDIN, AN ISOFLAVONE GLYCOSIDE FROM CENTROSEMA PUBESCENS

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**Abstract**—A new isoflavone glycoside, irisolidone 7-O- $\beta$ -D-apiofuranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucopyranoside was isolated from the seeds of *Centrosema pubescens* along with sitosterol, stigmasterol and sitosterol 3-O- $\beta$ -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- $\beta$ -D-glucopyranoside.

#### RESULTS AND DISCUSSION

Fractionation of a CHCl<sub>3</sub>-MeOH (80:20) extract from the dried seeds of *Centrosema pubescens* by a combination of adsorption chromatography on nonionic 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 <sup>13</sup>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<sup>-1</sup> (OH) and 1660 cm<sup>-1</sup> (>C=O), <sup>1</sup>H NMR  $\delta$  8.40 (1H, s, H-2) [11], <sup>13</sup>C NMR  $\delta$  155 (CH, C-2) and 122.80 (C, C-3) [12] spectra established that 1 is an isoflavone glycoside. UV bathochromic shifts with AlCl<sub>3</sub> and AlCl<sub>3</sub>-HCl and a signal at  $\delta$  12.88 [13] in the <sup>1</sup>H NMR spectrum indi-

Table 1. <sup>1</sup>H and <sup>13</sup>C NMR spectral data for pubescidin (1) in DMSO- $d_6$ 

Attribution	$\delta$ <sup>13</sup> C	$\delta^{-1}$ H ( $J$ = Hz)	DEPT
2	155.00	8.40 s	СН
3	122.80		С
4	180.80		С
4a	106.60		С
5	152.60		С
6	132.60		С
7	156.60		С
8	94.40	6.90 s	CH
8a	152.90		С
17	121.80		С
2' and 6'	130.20	7.50 d(9.0)	СН
3' and 5'	113.80	7.00 d(9.0)	СН
4′	159.30		С
5-OH		12.88 s	
6-OCH <sub>3</sub>	60.12	3.78 s	$CH_3$
4′-OCH <sub>3</sub>	55.25	3.76 s	$CH_3$
Glc-1	100.30	5.10 d(7.2)	CH
2	77.15		CH
3	76.01		СН
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		СН
3	78.77		С
4	73.39		CH <sub>2</sub>
5	64.92		CH <sub>2</sub>

cated the presence of a 5-hydroxyl group. The <sup>1</sup>H NMR spectrum displayed, in addition to signals for two methoxyl groups, H-2 of an isoflavone nucleus and 5-OH, two doublets at  $\delta$  7.0 and 7.5 for H-3', H-5' and H-2' and H-6', respectively. A singlet at  $\delta$ 

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Table 2. Summary of the 2D-NMR correlations of	∙f	1	l	
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Н	COSY ('H)	HETCOR ( <sup>13</sup> C)	COSY ( <sup>1</sup> H) LR
2		2	
8		8	
2′	3′	2'	
3'	2′	3'	4'-OCH <sub>3</sub>
5'	6′	5'	4'-OCH
6′	5'	6	v
6-OCH3		6-OCH <sub>3</sub>	
4'-OCH <sub>3</sub>		4'-OCH <sub>3</sub>	3', 5'
Gie-1	Glc-2	Glc-1	
2			
3			
4		4	
5			
6		6	
Api-1	Api-2	Api-t	
2	·	•	
3			
4			
5		5	

Table 3. <sup>1</sup>H and <sup>13</sup>C NMR spectral data for irisolidone (2) in CDCl<sub>3</sub>

Attribution	$\delta$ <sup>13</sup> C	$\delta$ <sup>1</sup> <b>H</b> ( $J$ = <b>H</b> z)	DEPT
2	152.70	7.90 s	СН
3	123.00		С
4	181.20		С
4a	106.30		С
5	152.50		С
6	130.30		С
7	155.10		С
8	93.07	6.50 s	СН
8a	153.30		С
1′	122.80		С
2' and 6'	130.00	7.45 d(10)	CH
3' and 5'	114.00	7.00 d(10)	СН
4′	159.70		С
5-OH		13.10 s	
7-OH		6.60 s	
4′-OCH3	55.25	3.80	CH <sub>3</sub>
6-OCH3	60.76	4.10 s	CH <sub>3</sub>

6.90 integrating for one proton represented H-8. Two doublets at  $\delta$  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  $\beta$ -linkages.

The <sup>13</sup>C NMR spectrum showed two quartets resonated at  $\delta$  55.25 and 60.12, assigned to the carbons of the 2 methoxy-substituents at C-4' and C-6, respectively. The signal at  $\delta$  180.80 was attributed to the carbonyl carbon. The resonance of the aromatic moiety was assigned by DEPT (Table 1), HETCOR (<sup>13</sup>C) (Table 2) and by comparison with data from the literature [12, 14–16]. The proposed structure 1 was fully supported by its <sup>13</sup>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 <sup>1</sup>H and <sup>13</sup>C NMR spectral data of pubescidin (1) and DEPT experiments. Compound 2 revealed [M]<sup>+</sup> at m/z 314.2969, C<sub>17</sub>H<sub>14</sub>O<sub>6</sub>. 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. Dglucose 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 <sup>13</sup>C NMR spectrum. C-2 of glucosyl unit was observed at  $\delta$  77.15, showing that the apiosyl unit is linked to it.

Hence 1 was established as irisolidone 7-O- $\beta$ -D-apiofuranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucopyranoside. The identification of sitosterol 3-O- $\beta$ -D-glucopyranoside was made on the basis of acid hydrolysis, and comparison with authentic sample by mp, IR, <sup>1</sup>H and <sup>13</sup>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. <sup>1</sup>H NMR: 200 MHz, in CDCl<sub>3</sub>, DMSO- $d_6$  or pyridine- $d_5$ . TMS as int. standard. <sup>13</sup>C NMR edited DEPT spectra: 50 MHz from CDCl<sub>3</sub>, DMSO- $d_6$  or pyridine- $d_5$  solns. GC carried out with FID, using a capillary column (0.3 mm  $\times$  25 m) OV 101. EIMS and GC-MS: recorded at 70 eV. Negative and positive LSIMS carried out using an HMPAglycerol 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<sub>3</sub>-MeOH (4:1) for isoflavone glycoside and (B) CHCl<sub>3</sub>-MeOH (19:1) for isoflavone aglycone, and (C) n-BuOH pyridine-H<sub>2</sub>O (6:4:3) for sugars. Compounds 1 and 2 detected under UV 254 and 366 nm and by spraying with orcinol-H<sub>2</sub>SO<sub>4</sub>, 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<sub>3</sub> and CHCl<sub>3</sub>-MeOH (80:20). Evapn of the CHCl<sub>3</sub> gave a residue (13 g). A part of the residue (5 g) was submitted to CC ( $30 \times 1$  cm) on silica gel which was eluted with hexane-CH<sub>2</sub>Cl<sub>2</sub> mixts of increasing polarity (up to 10% CH<sub>2</sub>Cl<sub>2</sub>). Identity of the compounds (sitosterol and stigmasterol) was established by comparison with authentic samples through mp, IR, <sup>1</sup>H and <sup>13</sup>C NMR, and EIMS. Another part (8 g) was chromatographed on silica gel (GATEAU) with hexane, EtOAc, Me<sub>2</sub>CO and MeOH successively. The EtOAc fr. was evapd to provide an amorphous powder, which was crystallized from MeOH to give situaterol  $3-O-\beta$ -D-glucopyranoside. Hydrolysis with vapour from conc. HCl, 90°, 1 hr, yielded glucose (co-chromatography on MeOH-CHCl<sub>3</sub>-MeOH, 8:5:1) and situaterol (co-chromatography on CHCl<sub>3</sub>-MeOH, 9:1).

Sitosterol 3-O- $\beta$ -D-glucopyranoside. Mp 295–297° (dec.). LSIMS: m/z 599 [M + Na]<sup>+</sup>.

To obtain 1, the CHCl<sub>3</sub>-MeOH, 4:1 extract was coned *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<sub>3</sub>-MeOH-H<sub>2</sub>O (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<sub>3</sub>.

*Pubescidin* (1). Yellowish amorphous powder from MeOH, mp 154–156°,  $[a]_{4}^{27}$  –93° (DMSO, *c* 0.7). UV  $\lambda^{MeOH}$  nm (log  $\varepsilon$ ): 264 (4.65), 329 (4.02), (AlCl<sub>3</sub>): 274, 381; (AlCl<sub>3</sub>+HCl): 277, 383; (NaOMc): 267, 360; (NaOAc): 265, 329; (NaOAc+H<sub>3</sub>BO<sub>3</sub>): 265, 330. IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3400 (OH), 1660 (>C=O), 1615, 1580, 1300, 1255, 1180, 1020, 830. <sup>1</sup>H and <sup>13</sup>C NMR spectra data shown in Tables 1 and 2. Negative LSIMS, *m/z* (rel. int.): 607 [M-H]<sup>-</sup> (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<sub>3</sub>. UV  $\lambda^{MeOH}$  nm (log  $\varepsilon$ ): 267 (4.60), 330 (4.10); (AlCl<sub>3</sub>): 275, 378; (AlCl<sub>3</sub>+HCl): 277, 381; (NaOMe): 249, 275, 340; (NaOAc): 273, 340; (NaOAc + H<sub>3</sub>BO<sub>3</sub>): 265, 330. IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3460 (OH), 1660 (>C=O), 1615, 1580, 1300, 1255, 1180, 1020.

<sup>1</sup>H and <sup>13</sup>C NMR: Table 3. EIMS (probe) 70 eV, m/z(rel. int.): 314[M]<sup>+</sup> (100), 299 (59), 271 (62), 132 (12), 117 (7). Aq. layer was adjusted to pH 6 by addition of NaHCO<sub>3</sub>. 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,  $80^\circ$ ) 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<sub>3</sub>I [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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