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STEROIDAL ALKALOID GLYCOSIDES FROM *SOLANUM ORBIGNIANUM*

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Key Word Index—*Solanum orbignianum*; Solanaceae; aerial parts; solanidane alkaloid glycosides; leptinidine 3-*O*-*β*-D-glucopyranoside; leptinine I; leptinine II.

Abstract—Aerial parts of *Solanum orbignianum* afforded a new steroidal alkaloid glycoside, leptinidine 3-*O*-*β*-D-glucopyranoside, together with the known alkaloids leptinidine, leptinine I and leptinine II. Their structures were established by spectroscopic methods. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Solanum species have already been studied chemically and found to contain mixtures of steroidal alkaloid glycosides. From the aerial parts of *Solanum orbignianum* Sendt., along with leptinidine (**1**), we have isolated three steroidal alkaloid glycosides, leptinidine 3-*O*-*β*-D-glucopyranoside (**2**), leptinine I (**3**) and leptinine II (**4**). Compound **2** is a new steroidal alkaloid glycoside. Leptinines I and II were previously isolated from *Solanum chacoense* [1], and their structures deduced on the basis of acid hydrolysis and biogenetic analysis [2]; their spectroscopic data are unknown. In this paper, we report the isolation of the compounds **1–4**, and their structures elucidation by spectroscopic methods, including two-dimensional NMR techniques.

RESULTS AND DISCUSSION

Leptinidine (**1**) was identified by its mass spectrum (EIMS) and mainly by comparison of its ^{13}C NMR spectrum (Table 2) with literature values [3].

Compound **2** was obtained as an amorphous powder and gave ion peaks at *m/z* 576 $[M+H]^+$ and *m/z* 412 $[M-C_6H_{11}O_5]^+$, in the FAB mass spectrum, suggesting a glycosidic structure. Peaks at *m/z* 413, *m/z* 166 and *m/z* 220 in the EIMS spectrum, were diagnostic fragments for a solanidane skeleton containing a hydroxyl in either rings E or F [4]. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) indicated that the aglycone was leptinidine [3]. The chemical shifts for the aglycone moiety of **2** agreed with those of **1**, except for C-3 (δ_H 3.95, δ_C 78.5). The ^{13}C NMR spectrum of **2** permitted the assignment of the sugar moiety linked at C-3 of the aglycone. The sugar moiety of **2** was determined as *β*-D-glucopyranose, based on the chemical shifts of the carbons, together with the coupling constant ($J_{1,2}=7.8$ Hz) for the anomeric proton. This was confirmed by acid hydrolysis of **2**, which yielded glucose as sugar component and leptinidine (**1**), as aglycone. The ^1H and ^{13}C NMR spectroscopic signals of **2** were assigned by ^1H-^1H 2D COSY, HETCOR and DEPT experiments. Thus, compound **2** was characterized as leptinidine 3-*O*-*β*-D-glucopyranoside.

The solanidane alkaloid glycosides **3** and **4** were identified by their FAB mass, ^1H and ^{13}C NMR (Tables 1 and 2) spectral data. Assignment of the proton and carbon resonances of **3** and **4** were achieved by a combination of ^1H-^1H 2D COSY, HETCOR and DEPT experiments. The FAB mass spectra of **3** gave peaks at *m/z* 868 $[M+H]^+$, *m/z* 722 [868–146(rhamnose)], *m/z* 576 [722–146(rhamnose)] and *m/z* 414 [576–162(hexose)], indicating a triglycoside structure. The solanidane skeleton was characterized by the presence of peaks at *m/z* 166 and *m/z* 220 in the EIMS spectra [4]. Acid hydrolysis of **3** gave rhamnose and glucose as sugar components, and leptinidine (**1**) as aglycone. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) for the aglycone moiety of **3** were similar to those of **1**, except for C-3 (δ_H 3.85, δ_C 77.8), indicating linkage of the sugar moiety at that position. The anomeric proton signals in the ^1H NMR spectra (Table 1), permitted assignment of the orientation of the rhamnopyranosyl units and glucopyranosyl unit to be *α* and *β*, respectively. The

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Table 1. ^{1}H NMR spectral data for compounds **1**–**4** (values in parentheses are J in Hz; δ in pyridine-d_5)

H	1	2	3	4
Aglycone moieties				
3	3.55 *m*	3.95*	3.85 *m*	3.96*
6	5.35 *d* (4.9)	5.33 *d* (4.9)	5.32 *d* (5.0)	5.32 *d* (5.2)
16	2.76 *m*	2.68 *m*	2.77*	2.76 *m*
18	0.83 *s*	0.92 *s*	0.94 *s*	0.94 *s*
19	1.03 *s*	0.84 *s*	0.98 *s*	0.98 *s*
21	0.98 *d* (6.6)	1.00 *d* (6.6)	1.00 *d* (6.2)	1.00 *d* (6.5)
23	3.95 *m*	3.95*	3.98 *m*	3.96*
26*eq*	2.89 *dd* (8.0, 3.0)	2.93 *dd* (8.0,3.2)	3.06 *d* (7.8)	3.10 *d* (9.1)
26*ax*	1.50 *m*	1.41*	1.50*	1.50 *m*
27	0.84 *d* (6.3)	0.81 *d* (6.6)	0.82 *d* (6.2)	0.81 *d* (6.5)
Sugar moieties				
Inner-Glucose				
1		5.04 *d* (7.8)	4.97 *d* (8.3)	
2		4.06 *dd* (7.8, 8.2)	4.23*	
3		4.29 *dd* (8.2, 8.9)	4.23*	
4		4.27 *t* (9.0)	4.39*	
5		3.95*	3.63	
6		4.41 *dd* (5.4,12.0)	4.08 *dd* (3.2, 12.5)	
		4.56 *dd* (2.1, 12.0)	4.20	
4-*O*-Rhamnose				
1			5.85 *brs*	
2			4.68 *dd* (3.2, 1.7)	
3			4.54 *dd* (3.2, 9.3)	
4			4.35 *dd* (9.3, 9.4)	
5			4.94 *dd* (9.4, 6.1)	
6			1.61 *d* (6.1)	
2-*O*-Rhamnose				
1			6.40 *brs*	6.29 *brs*
2			4.83 *dd* (3.2, 1.6)	4.90*
3			4.63 *dd* (3.2, 9.3)	4.61 *dd* (3.0, 9.1)
4			4.37*	4.35*
5			4.94 *dd* (9.4, 6.1)	4.90*
6			1.74 *d* (6.1)	1.70 *d* (6.1)
Inner-Galactose				
1				4.92 *d* (8.3)
2				4.70 t (8.8)
4				4.80 *d* (2.4)
3-*O*-Glucose				
1				5.18 *d* (7.6)
2				3.94 *d* (7.6)
6				4.48 *d* (10.1)

* Overlapping with other signals

sequence and connectivities of the sugar moiety were determined by comparing the ^{13}C NMR spectral data (Table 2) with those reported for solamargine [5]. Thus, the structure of **3** was determined as leptinidine 3-*O*-[α-L-rhamnopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside].

The mass spectral data of **4** suggested a triglycoside solanidane alkaloid, due the presence of peaks at m/z 884 $[M+H]^+$, and m/z 414 in the FAB mass spectrum, and m/z 220 and m/z 166 [4] in the EIMS spectrum. Acid hydrolysis of **4** gave glucose, galactose and rhamnose as sugar components, and leptinidine (**1**) as aglycone. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) for the aglycone moiety of **4** agreed with those of **1**, except for C–3 (δ_H 3.96, δ_C 78.2). This confirmed the aglycone to be lepitinidine (**1**), and indicated that the sugar moiety was linked at C–3. The ^{13}C NMR spectroscopic signals (Table 2), together with coupling constants for

Table 2. ^{13}C NMR spectral data for compounds **1–4** (δ values in pyridine-d_5)

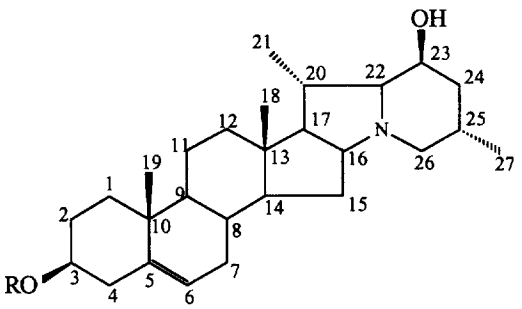
C	1	2	3	4
Aglycone moieties				
1	37.2	37.3	36.9	37.3
2	31.5	30.1	29.9	29.9
3	71.6	78.5	77.8	78.2
4	42.2	39.2	38.9	38.5
5	140.9	140.9	140.9	140.9
6	121.5	121.8	121.8	121.7
7	31.9	32.2	31.0	30.8
8	31.5	31.6	31.5	31.5
9	50.2	50.2	50.2	50.1
10	36.5	36.8	37.3	36.9
11	20.7	20.9	20.8	20.8
12	40.3	40.8	40.7	40.6
13	40.9	41.0	40.9	40.6
14	57.6	57.6	57.5	57.5
15	32.0	31.5	32.2	32.2
16	69.3	69.5	69.3	69.2
17	62.1	62.5	62.2	62.1
18	16.5	16.7	16.6	16.5
19	19.3	19.2	19.1	19.1
20	30.5	30.7	30.8	30.8
21	18.4	18.3	18.0	17.8
22	78.2	78.4	78.4	78.3
23	65.1	64.8	64.6	64.5
24	39.5	39.7	39.7	39.7
25	25.3	25.5	25.3	25.2
26	60.2	60.5	60.2	60.2
27	18.9	19.2	19.1	19.1
Sugar moieties				
Inner-Glucose				
1		102.5	100.2	
2		75.3	78.0[a]	
3		78.5	77.6[a]	
4		71.6	78.4	
5		78.0	76.8	
6		62.2	61.1	
4-*O*-Rhamnose				
1			102.9	
2			72.6	
3			72.7	
4			73.7[b]	
5			70.2[c]	
6			18.2	
2-*O*-Rhamnose				
1			101.9	102.1
2			72.3	72.3
3			72.3	72.6
4			73.9[b]	73.9
5			69.3[c]	69.6
6			18.4	18.4
Inner-Galactose				
1				100.3
2				76.3
3				84.7
4				70.1
5				74.7
6				62.5

continued

Table 2—*continued.*

C	1	2	3	4
3-*O*-Glucose				
1				105.7
2				74.7
3				77.4[a]
4				71.4
5				78.3[a]
6				62.5

[a-c] May be exchanged.



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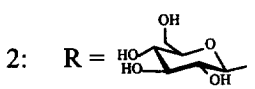

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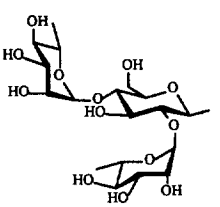

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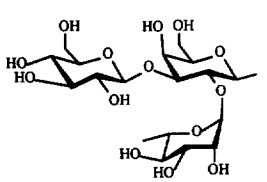

4: R =

the anomeric protons (Table 1), indicated that the sugar moiety was consisted of a β-D-glucopyranosyl unit, a β-D-galactopyranosyl unit and a α-L-rhamnopyranosyl unit. Finally, connections of the sugar components were determined by comparison of the ^{13}C NMR signals with those of solasonine [6]. Therefore, compound **4** was determined to be leptinidine 3-*O*-[β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-galactopyranoside].

EXPERIMENTAL

General

Mps: uncorr. ^1H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) were recorded on a Varian Gemini 2000 spectrometer, in pyridine-d_5 or $CDCl_3$ with TMS as int. standard. IR spectra were recorded as KBr pellets on a Jasco model IR 700 spectrometer. FABMS were taken in the positive mode on a Fison model Quattro GC-MS spectrometer, using glycerol as matrix. TLC: Silica gel GF_{254} (0.25 mm thick). CC: Silica gel 60 (0.0063–0.200 mesh, Merck); alumina 90 (70–230 mesh ASTM, Merck).

Plant material

Aerial parts of *S. orbignianum* were collected in August 1994 in Porto Rico, State of Paraná, Brazil. A voucher specimen (HUM2483) is deposited at the herbarium of the Universidade Estadual de Maringá, Paraná.

Extraction and isolation

The air-dried and powdered aerial parts (1.5 Kg) were extracted with MeOH (5 l) and the solvent was evapd under red. pres. The methanolic extract (79.9 g) was dissolved in 0.5 M HCl (400 ml), the soln extracted with hexane–Et_2O 1:1 (3 × 150 ml) and the aq. layer basified with K_2CO_3 powder to pH 10. The basic soln was stirred for 5 h and the resulting ppt. was filtered and dried to give 5.53 g of alkaloid material. This material was sepd into 5 frs over alumina CC with *n*-hexane–$CHCl_3$ 2:1 (200 ml), hexane–$CHCl_3$ 1:1 (300 ml), $CHCl_3$ (150 ml), $CHCl_3$–MeOH 1:1 (250 ml) and MeOH (200 ml). Recrystallization of the residue from the *n*-hexane–$CHCl_3$ 1:1 and $CHCl_3$ eluates with Me_2CO, afforded 750 mg of **1**. The residue of the $CHCl_3$–MeOH 1:1 eluate (1.19 g) was subjected to silica gel CC using mixts of $CHCl_3$–MeOH 9.5:0.5, 9:1, 8:2, 7:3, 6:4 and 1:1 as eluent; 25 frs were collected.

Frs 12–14 (11.2 mg) were recrystrallized from Me_2CO to yield 10.0 mg of **2**. The residue of frs 17–22 (350.1 mg) was recrystallized from Me_2CO–MeOH to give 207.8 mg of **3**. The residue (870.1 mg) of the MeOH eluate from alumina CC was subjected to silica gel CC with mixts of $CHCl_3$–MeOH, 9:1, 8:2, 6:4 and 1:1; 15 frs were collected. Frs 7–10 were recrystallized from Me_2CO to yield 310.0 mg of **3**. Recrystallization of the residue from frs 13–15 with MeOH yielded **4** (270.0 mg).

Acid hydrolysis of the glycoalkaloids

The glycoalkaloid (5 mg) was refluxed with 5 ml of 5% HCl–MeOH soln for 2 hr. The soln was diluted with H_2O and extracted with $CHCl_3$ and the aglycone was sepd. The aq. filtrate was neutralized with $BaCO_3$ and evapd. The resulting residue was chromatographed on TLC (silica gel; $CHCl_3$–MeOH–Me_2CO–H_2O, 3:3:3:1) against ref. sugars. Compounds **2**, **3** and **4** yielded leptinidine (**1**) as aglycone, which was identified by comparison of the EIMS and ^1H NMR spectroscopic data with those of the authentic sample. The sugar components were identified as glucose for compound **2**; glucose and rhamnose for compound **3**; glucose, galactose and rhamnose for compound **4**.

Leptinidine (**1**)

Crystals from Me_2CO, mp 248–249°, ref [7]: 245–247°. $[\alpha]_D^{22} - 29°$ ($CHCl_3$, c 0.30), ref [7]: −24° ($CHCl_3$). EIMS (probe) 70 eV *m/z* (rel. int.): 413 $[M]^+$ (9), 369 (4), 342 (6), 220 (34), 166 (100). ^1H NMR (300 MHz, $CDCl_3$): Table 1. ^{13}C NMR (75.5 MHz, $CDCl_3$): Table 2.

Leptinidine 3-O-β-D-glucopyranoside (**2**)

Amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1665 (C=C), 1055 (C–O). EIMS (probe) 70 eV *m/z* (rel. int.): 413 (4), 220 (25), 166 (100). FABMS *m/z* (rel. int.): 576 $[C_{33}H_{53}NO_7+H]^+$ (100), 414 $[C_{27}H_{43}NO_2+H]^+$ (25), 412 (30). ^1H NMR (300 MHz, pyridine-d_5): Table 1. ^{13}C NMR (75.5 MHz, pyridine-d_5): Table 2.

Leptinidine 3-O-[α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside], leptinine I (**3**)

Amorphous powder. $[\alpha]_D^{22} - 85°$ (pyridine, c 0.30). IR ν_{max}^{KBr} cm^{-1}: 3395 (OH), 1665 (C=C), 1050 (C–O). FABMS *m/z* (rel. int.): 868 $[C_{45}H_{73}NO_{15}+H]^+$ (100), 722 (30), 576 (5), 414 $[C_{27}H_{43}NO_2+H]^+$ (28). EIMS (probe) 70 eV *m/z* (rel. int.): 413 (3), 220(27), 166 (100). ^1H NMR (300 MHz, pyridine-d_5): Table 1. ^{13}C NMR (75.5 MHz, pyridine-d_5): Table 2.

Leptinidine 3-O-[β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside], leptinine II (**4**).

Amorphous powder. $[\alpha]_D^{22} - 60°$ (pyridine, c 0.30). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1667 (C=C), 1055 (C–O). FABMS *m/z* (rel. int.): 884 $[C_{45}H_{73}NO_{16}+H]^+$, 722 (20), 414 $[C_{27}H_{43}NO_2+H]^+$. EIMS (probe) 70 eV *m/z* (rel. int.): 413 (6), 220 (27), 166 (100). ^1H NMR (400 MHz, pyridine-d_5): Table 1. ^{13}C NMR (75.5 MHz, pyridine-d_5): Table 2.

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