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TWO NEW STEROIDAL GLYCOSIDES FROM *Solanum surattense*

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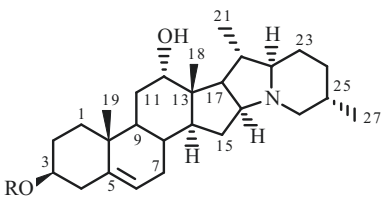
*Two new steroidal alkaloid glycosides, solanoside A (**1**) and solanoside B (**2**), were isolated from the whole plant of* Solanum surattense*. Their structures were elucidated on the basis of spectroscopic techniques (1D and 2D NMR, HR-EI-MS, HR-FAB-MS, IR), physical data, and chemical analysis.*

Keywords: steroidal alkaloid glycosides, solanoside A, solanoside B, *Solanum surattense*, Solanaceae, 1D and 2D NMR.

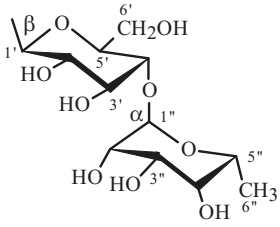
The genus *Solanum* comprises about 3000 species and is widely distributed in the tropical region of Asia, Australia, and the Polynesian Islands [1]. In Pakistan, it is widespread in waste places from plains to 1500 m [2]. *Solanum surattense* is a perennial prickly prostrate herb and that has been used in folk medicine for the treatment of bronchial asthma, nonspecific cough, vomiting, catarrhal fever, rheumatism, diarrhea, blood cancer, and to control stones in bladder. The plant is bitter, digestive, alterative, and astringent; this used as an expectorant, aperient, and carminative [3–5]. With regard to the saponin constituents of the genus *Solanum*, many steroidal saponins have been reported [6]. The pharmacological importance of *Solanum surattense* prompted us to investigate the steroidal alkaloids from the basified ethyl acetate fraction of the whole plant. Our detailed investigation has led to the discovery of two new saponins, solanoside A (**1**) as rubijervine 3-*O*-β-D-glucopyranosyl-(4→1)-α-L-rhamnopyranoside, and solanoside B (**2**) as rubijervine 3-*O*-[α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside. Both compounds were identified by IR, MS, 1D, and 2D NMR and comparison with co-TLC of authentic samples.

The methanolic extract of *S. surattense* was suspended in H_2O and partitioned successively with *n*-hexane, EtOAc, *n*-BuOH, and basified EtOAc. The basified EtOAc layer was dried and subjected to various chromatographic techniques to afford **1** and **2**.



1, 2

1: R =



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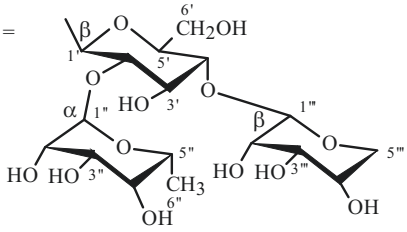

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TABLE 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) of Solanoside A (**1**) and Solanoside B (**2**) (pyridine-d_5, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1eq	1.90 (ddd, J = 12.9, 4.0, 3.5)	37.4	1.91 (ddd, J = 12.7, 4.1, 3.6)	37.4
1ax	1.10 (ddd, J = 12.9, 6.9, 3.0)		1.10 (ddd, J = 12.7, 6.9, 3.6)	
2eq	1.64 m	30.2	1.65 m	30.3
2ax	1.56 m		1.56 m	
3	3.49 (dt, J = 8.7, 5.7)	76.8	3.49 (dt, J = 8.7, 5.8)	76.8
4eq	2.50 (dd, J = 12.1, 5.7)	39.4	2.50 (dd, J = 12.1, 5.8)	39.6
4ax	2.29 (dd, J = 12.1, 8.7)		2.29 (dd, J = 12.1, 8.7)	
5	–	140.4	–	140.6
6	5.38 (br.d, J = 5.5)	121.6	5.38 (br.d, J = 5.5)	121.6
7eq	1.94 (dt, J = 12.9, 7.5)	31.8	1.94 (dt, J = 12.8, 7.5)	31.8
7ax	1.59 m		1.59 m	
8	1.83 m	31.6	1.84 m	31.6
9	1.81 m	49.4	1.81 m	49.5
10	–	35.6	–	35.6
11	1.28 m	30.5	1.28 m	30.5
12	3.92 (dd, J = 6.2, 2.1)	74.6	3.92 (dd, J = 6.4, 2.3)	74.6
13	–	44.4	–	43.5
14	1.52 m	43.6	1.53 m	48.4
15	1.30 m	29.2	1.32 m	29.3
16	3.07 (dt, J = 9.9, 7.2)	69.5	3.07 (dt, J = 9.8, 7.4)	69.5
17	1.88 (dd, J = 9.9, 8.4)	61.1	1.89 (dd, J = 9.8, 8.3)	61.2
18	1.01 s	17.0	1.02 s	17.3
19	1.12 s	18.9	1.12 s	18.9
20	1.69 m	36.9	1.69 m	36.9
21	1.09 (d, J = 6.2)	17.0	1.10 (d, J = 6.5)	17.1
22	1.62 m	75.0	1.62, m	75.2
23	1.71 m	28.9	1.71, m	28.9
24	1.77 m	31.5	1.77 m	31.5
25	1.80 m	30.9	1.81 m	30.9
26	3.22 (dd, J = 9.9, 4.5)	61.0	3.23 (dd, J = 10.1, 4.6)	61.1
	2.81 (dd, J = 10.1, 3.9)		2.82 (dd, J = 10.1, 4.0)	
27	0.99 (d, J = 7.2)	19.4	0.99 (d, J = 7.3)	19.4
1′	4.93 (d, J = 7.6)	102.6	4.91 (d, J = 7.2)	102.2
2′	4.10 m	76.4	4.11 m	76.8
3′	4.03 m	88.0	4.03 m	88.5
4′	3.78 m	71.0	3.79 m	70.5
5′	3.76 m	77.7	3.76 m	77.9
6′a	4.44 (dd, J = 11.2, 7.9)	62.9	4.44 (dd, J = 11.4, 7.9)	63.4
6′b	4.14 (dd, J = 11.2, 7.8)		4.13 (dd, J = 11.3, 7.9)	
1″	6.38 (d, J = 4.4)	102.4	6.39 (d, J = 4.1)	101.9
2″	4.51 m	72.6	4.75 m	72.8
3″	4.75 m	72.8	4.52 m	72.6
4″	4.30 m	73.9	4.26 m	74.4
5″	5.00 m	70.3	4.78 m	69.5
6″	1.70 (d, J = 5.6)	18.5	1.62 (d, J = 6.5)	19.5
1‴	–	–	4.92 (d, J = 7.3)	105.1
2‴	–	–	3.96 m	74.9
3‴	–	–	4.08 m	78.6
4‴	–	–	4.10 m	70.8
5‴a	–	–	4.28 m	67.3
5‴b			3.70 m	

Solanoside A (**1**) was isolated as a gummy solid, positive to Lieberman Burchard, Molisch, and Dragendorff tests. The molecular formula $C_{39}H_{63}NO_{11}$ was deduced from ^{13}C NMR data (Table 1) and positive-ion FAB-MS showing the $[M + H]^+$ ion at *m/z* 722 (calcd for $C_{39}H_{64}NO_{11}$). Strong IR absorptions were observed at 3330 (OH), 2895 (CH), 1635 (C=C),

and 1070 cm^{-1}, indicating the glycoside and olefinic nature of molecule. The ^1H NMR spectrum of **1** in pyridine-d_5 showed three methyl signals as doublet at δ 1.70 (J = 5.6 Hz), 1.09 (J = 6.2 Hz), and 0.99 (J = 7.2 Hz) and two methyl signals as singlet at δ 1.12 and 1.01, while an olefinic signal appeared at δ 5.38 (br.d, J = 5.5 Hz). Characteristic signals for the anomeric protons were observed as doublets at δ 4.93 (J = 7.6 Hz) and δ 6.38 (J = 4.4 Hz), and a signal at δ 3.49 (dt, J = 8.7, 5.7 Hz) accounted for the hydroxymethine of aglycone due to the attached glycosidic moiety. The hydroxymethine signals of disaccharides appeared at δ 3.76–5.00 (8H, m). The presence of a hydroxymethyl was evident from the downfield signals at δ 4.44 (1H, dd, J = 11.2, 7.9 Hz) and 4.14 (1H, dd, J = 11.2, 7.8 Hz). The ^{13}C NMR spectra (BB and DEPT) revealed 39 carbon signals due to 3 quaternary carbons, 21 methines, 10 methylenes, and 5 methyls, of which 27 were assigned to the aglycone portion and the remaining 12 were ascribed to the sugar moiety.

Acid hydrolysis of **1** yields L-rhamnose and D-glucose as carbohydrate components and a white crystalline powder of aglycone (mp 240.5°C) by comparison of its spectral and physical data with that reported in the literature [7–9]. The above data were clearly indicative of **1** being a rubijervine diglycoside. It was also clearly evident, from the loss of 162 amu from the molecular-ion peak and the loss of 178 amu from the resulting fragment in the positive-ion FAB mass spectrum, that the L-rhamnose was attached to the D-glucose, which itself was attached to the aglycone. The HMBC and ^1H–^1H COSY spectra determine the sugar linkage sequence. In the HMBC spectrum, correlations of δ_H 4.93 (Glc H-1′) with δ_C 76.8 (C-3 of Agl), δ_H 6.38 (Rha H-1″) with δ_C 71.0 (Glc C-4′), and δ_H 1.70 (Rha H-6″) with δ_C 73.9 (Rha C-4″) were observed. The absolute configurations of glucose and rhamnose were assumed to be D and L, respectively, because only these forms are found in terpenoid glycosides [10]. A literature search of the ^{13}C NMR chemical shifts of sugars revealed that the values observed for **1** are close to those reported for α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside [11]. These results confirmed the structure of compound **1** as rubijervine 3-*O*-β-D-glucopyranosyl-(4→1)-α-L-rhamnopyranoside.

Solanoside B (**2**) was isolated as a gummy solid and gave positive Lieberman Burchard, Molisch, and Dragendorff tests. The molecular formula ($C_{44}H_{71}NO_{15}$) was established by ^{13}C NMR data (Table 1) and positive mode FAB-MS showing the $[M + H]^+$ ion at *m/z* 854.2, consistent with $C_{44}H_{72}NO_{15}$. The IR, mass, ^1H NMR (1D and 2D), and ^{13}C NMR spectral features of the aglycone nucleus were similar to **1**, except for the signals due to the glycoside portion. The ^{13}C NMR (BB and DEPT) displayed 44 signals which consist of 5 methyls, 11 methylenes, 25 methines, and 3 quaternary carbons. Acid hydrolysis of **2** yielded rubijervine [7–9] as the aglycone, and glucose, rhamnose, and xylose as the carbohydrate components [11]. The carbohydrate components were confirmed by co-TLC with authentic samples and by GLC analysis of the thiazolidine derivatives [12]. The above methods proved the absolute configuration of D-glucose, L-rhamnose, and D-xylose [12]. The ^1H NMR spectrum of the anomeric protons and their correlated carbons of D-glucose, L-rhamnose, and D-xylose were observed at δ 4.91 (d, J = 7.2 Hz), 6.39 (d, J = 4.1 Hz), 4.92 (d, J = 7.3 Hz) and 102.2, 101.9, and 105.1, respectively. The coupling constant values indicated their β- and α-orientation, respectively [13, 14]. The attachment of one xylose and one rhamnose to glucose and then to the aglycone was confirmed by inspection of the chemical shifts of C-2′ and C-4′ and the upfield shift of C-1′ and C-5′ [15]. The HMBC spectrum showed the correlation of H-1′ (δ_H 4.91) of glucose with C-2′ (δ_C 76.8) and C-3′ (δ_C 88.5), and H-1″ (δ_H 6.39) of rhamnose with C-2′ (δ_C 76.8) and C-2″ (δ_C 72.8). Similarly H-1‴ (δ_H 4.92) of xylose showed HMBC correlations with C-4′ (δ_C 70.5) and C-5‴ (δ_C 67.3). Thus, the rhamnopyranosyl-(1→2)-[xylopyranosyl]-(1→4)-[glucopyranosyl] structure was revealed. On the basis of these cumulative data, compound **2** was determined as rubijervine 3-*O*-[α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside.

EXPERIMENTAL

Melting points were obtained on a Buchi melting point apparatus and uncorrected. Optical rotations were taken on a JASCO DIP 360 polarimeter. The IR spectra were recorded on an FTIR-8900 Shimadzu spectrometer. The 1D and 2D NMR spectra were recorded in pyridine-d_5 at 500 MHz on a Bruker AV 500 spectrometer. The chemical shift values are reported in ppm (δ) units, and coupling constants (J) are shown in Hz. EI-MS, HR-EI-MS, and HR-FAB-MS were recorded on a JMS-HX-110 spectrometer with a data system and on a JMS-DA 500 mass spectrometer. Aluminum sheets precoated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick, E-Merck) were used for TLC, and silica gel (230–400 mesh) was used for column chromatography. Precoated RP-18 gel (E-Merck) glass plates were used for TLC. GC-18A equipped with FID was used for GLC. Standard carbohydrates (D-glucose, L-glucose, D-xylose, L-rhamnose) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA).

Plant Material. The dried whole plant (6 kg) of *Solanum surattense* Burm. f. was collected from Cholistan Desert near Bahawalpur and identified by Dr. Muhammad Arshad, Plant Taxonomist, Cholistan Institute of Desert Studies (CIDS). A voucher specimen (CIDS/IUB/10) has been deposited at the Islamic University of Bahawalpur, Bahawalpur, Pakistan.

Extraction and Isolation. The shade-dried crushed plant (6 kg) was chopped and soaked in methanol (3 × 25 L) thrice, and the combined methanolic extracts were concentrated under reduced pressure to give a crude extract (700 g). The crude extract was partitioned between *n*-hexane (125 g), EtOAc (85 g), and *n*-BuOH (255 g). The aqueous portion was basified with NH_4OH and extracted with EtOAc at pH 8.0–9.0 to obtain a gummy EtOAc-soluble extract (40 g), it was then applied to a silica gel column and eluted with $CHCl_3$–methanol in increasing order of polarity. The fractions obtained from $CHCl_3$–methanol (40:60) were combined and again chromatographed over RP-18 PTLC using the solvent system $CHCl_3$–methanol (1:1 + 1 mL of H_2O, total volume 10 mL) to obtain **1** (R_f 0.7) and **2** (R_f 0.4) in a 32 and 28 mg amounts, respectively.

Solanoside A (1). Gummy solid, $[\alpha]_D^{25}$ –51.8° (*c* 1.01, MeOH). IR (KBr, ν_{max}, cm^{-1}): 3330 (OH), 2895 (CH), 1635 (C=C), 1070. FAB-MS (*m/z*, $I_{rel.}$, %): $[M + H]^+$ 722 (85), 560 (45), 414 (100), 386 (80), 382 (68). EI-MS (*m/z*, $I_{rel.}$, %): 396 $[M - Glc - Rha - H_2O]^+$ (25), 378 (18), 282 (11), 215 (26), 138 (93), 114 (100). For ^1H NMR (500 MHz, Py-d_5) and ^{13}C NMR (125 MHz, Py-d_5), see Table 1.

Solanoside B (2). Gummy solid, $[\alpha]_D^{25}$ –39.5° (*c* 1.00, MeOH). IR (KBr, ν_{max}, cm^{-1}): 3394 (OH), 2880, (CH), 1639 (C=C), 1075. FAB-MS (*m/z*, $I_{rel.}$, %): $[M + H]^+$ 854 (70), 704 (55), 692 (67), 542 (52), 414 (100), 364 (90). EI-MS (*m/z*, $I_{rel.}$, %): 396 $[M - Glc - Rha - Xyl - H_2O]^+$ (10), 378 (5), 282 (15), 215 (5), 138 (78), 114 (100). For ^1H NMR (500 MHz, Py-d_5) and ^{13}C NMR (125 MHz, Py-d_5), see Table 1.

Acid Hydrolysis and Sugar Analysis of 1 and 2. Compounds **1** and **2** (5 mg each) were refluxed with 10% aq. HCl for 3 h at 100°C. On cooling, the aglycone was recrystallized from $CHCl_3$ and identified as rubijervine by comparison of its spectral data with the literature [11]. The aqueous hydrolysate was recrystallized with silver carbonate and concentrated. The sugars were found to be D-glucose and L-rhamnose in the case of compound **1**, while for compound **2**, D-glucose, L-rhamnose, and D-xylose were identified using different solvent systems.

The concentrated residue was further treated with L-cysteine methyl ester hydrochloride (1 mg) in pyridine (0.125 mL) at 60°C for 1 h [16]. The supernatant was applied to GLC. GLC conditions: column Supelco SPBTM -1, 30 m × 0.25 mm, column temperature 230°C, N_2 flow rate 0.8 mL min^{-1}; t_R of derivatives: D-glucose, 10.49 min (L-glucose, 10.96 min), L-rhamnose, 9.40 min (D-rhamnose, 9.72 min), and D-xylose, 7.79 min (L-xylose, 8.29 min).

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