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STEROID ALKALOID GLYCOSIDES FROM *SOLANUM ROBUSTUM**

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Key Word Index—*Solanum robustum*; Solanaceae; steroid alkaloid glycosides; solamargine; *N*-hydroxysolamargine; solasonine; *N*-hydroxyrobustine.

Abstract—Solamargine, solasonine, *N*-hydroxyrobustine and a new glycoalkaloid, *N*-hydroxysolamargine, has been isolated from the leaves of *Solanum robustum*, the structure of which has been elucidated as (25*R*)-3β-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranosyloxy}-22α*N*-spirosol-5-en-*N*-ol.

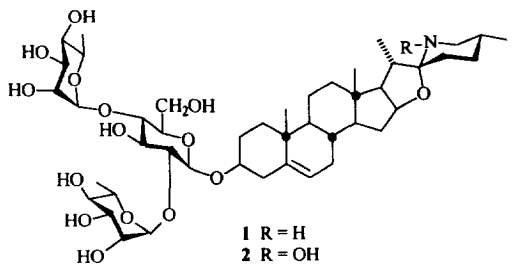
INTRODUCTION

β_1-Solamargine, robustine, *N*-hydroxyrobustine and 25-acetoxyrobustine had been isolated from the roots of *Solanum robustum* [2]. In addition to solamargine (**1**), solasonine and *N*-hydroxyrobustine, a new alkaloid (*N*-hydroxysolamargine) has been isolated from the leaves of this species. The structure of which has been elucidated as (25*R*)-3β-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranosyloxy}-22α*N*-spirosol-5-en-*N*-ol (**2**).

RESULTS AND DISCUSSION

Solamargine (**1**) was identified by its liquid secondary ion mass spectrum (LSI-MS) and mainly by comparison of its ^{13}C NMR spectrum with literature values [3–7, cf. 2].

In the LSI-mass spectrum of **2**, $[M - 2H]^+$ and $[M - O]^+$ ions were detected. The aglycone ^{13}C NMR signals (see Experimental) agreed with those of *N*-hydroxyrobustine [2] indicating the aglycone to be *N*-hydroxysolasodine. The assignments of the ^{13}C signals of all compounds investigated in this publication were supported by APT measurements. The ^{13}C signals of the sugar part (Table 1) corresponded to the signals of solamargine [3–7]. These observations led to structure **2** for the alkaloid. There were differences in the assignments of the sugar signals of solamargine (**1**) in the literature. Therefore, the ^{1}H NMR signals of **1** were assigned from the ^{1}H–^{1}H COSY 2D spectrum (Table 1) and, afterwards, the ^{13}C signals by HMQC measurements (Table 1). This assignment did not completely agree with any of the numerous literature assignments [3–7].



Solasonine and *N*-hydroxyrobustine were identified by their LSI-mass spectra and mainly by ^{13}C NMR spectra, which were compared with literature values [2, 4, 5].

Whereas normal phase chromatography separates steroid alkaloid glycosides mainly according to the sugar moieties, the relative retention times (RR_t) of these compounds in the reverse-phase HPLC system used in this study strongly depend on their aglycones; solanidane glycosides had RR_t 1.0, spirosolane glycosides 1.46–1.82, *N*-hydroxysolasodine glycosides 3.27–3.58 and the 25-acetoxysolasodine glycoside, 25-acetoxyrobustine, 6.70.

This and other recent papers have shown that *Solanum* species often contain complex mixtures of steroid alkaloid glycosides, which still present separation difficulties. Therefore, many of the older publications using less sophisticated technique are worth repeating.

EXPERIMENTAL

Seeds of *Solanum robustum* Wendl. were obtained from the collection of the Institute of Plant Genetics and Crop Plant Research, Gatersleben. Plants were grown in a field in Halle (Saale) and harvested in August. A voucher specimen is retained in the Institute of Plant Biochemistry, Halle.

Isolation of alkaloids. Leaves were dried at 60°, ground and extracted with MeOH at room temp. Evapn of MeOH *in vacuo* gave a residue which was partitioned

*Part 134 in the series '*Solanum* alkaloids'. For part 133 see ref. [1].

Table 1. NMR spectral data (δ values, J Hz) of compounds **1**–**2** (oligosaccharide portions, pyridine-d_5, 125 MHz for ^{13}C, 500 MHz for ^1H)

		1			**2**
Sugar	Position	δ_C	δ_H	J (H, H)	δ_C
2-*O*-rhamnose	1	102.1	6.44	0.0 (1, 2)	102.0
	2	72.6	4.86	2.7 (2, 3)	72.5
	3	72.9	4.66	9.4 (3, 4)	72.85
	4	74.2[a]	4.39	9.5 (4, 5)	74.2[a]
	5	69.6[b]	4.99	6.1 (5, 6)	69.5[b]
	6	18.8	1.79*	—	18.7
4-*O*-rhamnose	1	102.9	5.90	0.0 (1, 2)	102.9
	2	72.6	4.71	2.4 (2, 3)	72.5
	3	72.8	4.57	9.4 (3, 4)	72.75
	4	74.0[a]	4.36	9.5 (4, 5)	73.9[a]
	5	70.5[b]	4.99	6.1 (5, 6)	70.4[b]
	6	18.6	1.66*	—	18.5
glucose	1	100.3	4.97	8.2 (1, 2)	100.3
	2	78.0[c]	4.26	9.2 (2, 3)	78.0[c]
	3	77.8[c]	4.25	7.6 (3, 4)	77.8[c]
	4	78.6	4.44	8.0 (4, 5)	78.8
	5	77.0	3.66	2.9 (5, 6)	76.9
	6	61.3	4.11	1.8 (5, 6′)	61.4[d]
			4.24	13.1 (6, 6′)	

[a–c] May be exchanged.

*Assigned by comparison with the corresponding signal of β_1-solamargine [9].

[d] May be exchanged with the signal of C-26, cf. Experimental.

between H_2O and C_6H_6–Et_2O (1:1). After addition of $KHCO_3$ to the aq. layer, the latter was extracted with $CHCl_3$–EtOH (2:1). Evapn of solvents *in vacuo* gave a mixt. of alkaloids. This was chromatographed over Merck silica gel with $CHCl_3$–MeOH–conc NH_3 (12:5:4) and over Merck LiChroprep RP-8 with MeOH–0.4% HOAc (1:1, 3:2 or 2:1). Alkaloids were purified by HPLC, performed on a Eurosil Bioselect 100–10 C8 column, 250 × 32 mm, 6.6 MPa, 20 ml min^{-1}, detection at 210 nm, elution with MeOH-buffer soln. [3:2 or 1:1, buffer soln: 0.1 M $(NH_4)H_2PO_4$, H_3PO_4 added to pH 3]. Frs containing alkaloids were basified with conc NH_3, MeOH evapd *in vacuo*, the aq. soln extracted with $CHCl_3$–EtOH (2:1) and the solvents evapd *in vacuo*. TLC: Merck TLC aluminium sheets silica gel 60 WF_{254s}, $CHCl_3$–MeOH–conc NH_3 (3:3:1), detection by Dragendorff's reagent [R_f(1)] or Merck TLC plates RP-8 F_{254s}, MeOH-buffer soln (2:1; buffer soln: 50 g of NH_4OAc dissolved in 50 ml of H_2O, 560 ml of 1 M HCl and 720 ml of H_2O added, detection by I_2 vapour) [R_f(2)]. Analytical HPLC: Eurosil Bioselect 100–10 C8, 250 × 4 mm, 8.2 MPa, 1 ml min^{-1}, detection at 210 nm, MeOH-buffer soln [1:1; buffer soln: 0.1 M $(NH_4)H_2PO_4$, H_3PO_4 added to pH 3.] (RR_t related to solanine).

Solamargine (**1**). From MeOH–H_2O; yield 0.58%. Mp. 279–280°, ref. [8]: 301°. $[\alpha]_D^{22}$ –101.5° (pyridine; *c* 0.65), ref. [8] : – 114° (pyridine). R_f(1) 0.49, R_f(2) 0.46. RR_t 1.71. ^1H NMR (derived from HMQC, 500 MHz, pyridine-d_5, TMS) : δ 1.00 (H-1), 1.74 (H-1), 1.85 (H-2), 2.08 (H-2), 3.87 (H-3), 2.72 (H-4), 2.79 (H-4), 5.31 (H-6), 1.49 (H-7), 2.05 (H-7), 1.57 (H-8), 0.90 (H-9), 1.41 (H-11), 1.45 (H-11), 1.13 (H-12), 1.71 (H-12), 1.08 (H-14), 1.47 (H-15), 1.87 (H-15), 4.43 (H-16), 1.77 (H-17), 0.87 (H-18), 1.05 (H-19), 1.96 (H-20), 1.09 (H-21), 1.72 (H-23), 1.63 (H-24), 1.57 (H-25), 2.77 (H-26), 0.81 (H-27), oligosaccharide signals derived from the ^1H–^1H COSY 2D spectrum cf. Table 1. ^{13}C NMR (125 MHz, pyridine-d_5, TMS) : δ 15.8 (C-21), 16.7 (C-18), 19.5 (C-19), 19.9 (C-27), 21.3 (C-11), 30.3 (C-2), 31.2 (C-24), 31.7 (C-25), 31.8 (C-8), 32.5 (C-15), 32.7 (C-7), 34.8 (C-23), 37.2 (C-10), 37.6 (C-1), 39.0 (C-4), 40.2 (C-12), 40.7 (C-13), 41.7 (C-20), 48.2 (C-26), 50.4 (C-9), 56.8 (C-14), 63.6 (C-17), 78.1 (C-3), 78.9 (C-16), 98.5 (C-22), 121.9 (C-6), 140.8 (C-5), oligosaccharide signals cf. Table 1. LSI-MS (matrix glycerol, positive ions, 9 kV) *m/z* (rel. int.) : 868 $[M + H]^+$ (100), 722 $[M + H - C_6H_{10}O_4]^+$ (16), 704 $[M + H - C_6H_{12}O_5$ (rhamnose)$]^+$ (13), 414 $[C_{27}H_{43}NO_2$ (solasodine) $+ H]^+$ (28), 396 $[414 - H_2O]^+$ (39).

N-*Hydroxysolamargine* (**2**). Yield 0.046%, amorphous. $[\alpha]_D^{24}$ – 82.8° (pyridine; *c* 0.50). R_f(1) 0.49, R_f(2) 0.22. RR_t 3.58. ^{13}C NMR (125 MHz, pyridine-d_5, TMS): δ 16.2[a] (C-21), 16.9[a] (C-18), 19.4 (C-19), 19.5[a] (C-27), 21.3 (C-11), 29.9[b] (C-2), 30.0[b] (C-15), 31.6 (C-8), 31.9 (C-25), 32.5 (C-7), 34.1[c] (C-24), 36.7[c] (C-23), 37.2 (C-10), 37.6 (C-1), 39.0 (C-4), 40.4 (C-12), 40.8 (C-13), 43.4 (C-20), 50.6 (C-9), 56.2 (C-14), 61.3[d] (C-26), 65.1 (C-17), 78.2 (C-3), 85.1 (C-16), 103.4 (C-22), 121.9 (C-6), 140.8 (C-5), [a–c] may be exchanged, [d] may be exchanged with the signal of C-6

of glucose, cf. Table 1, oligosaccharide signals cf. Table 1. LSI–MS (matrix glycerol, positive ions, 9 kV) : *m/z* (rel. int.): 881 $[M-2H]^+$ (100), 867 $[M-O]^+$ (21), 735 $[881-C_6H_{10}O_4]^+$ (18), 717 $[881-C_6H_{12}O_5$(rhamnose)$]^+$ (8).

Solasonine. From MeOH; yield 0.051%. Mp. 280–282°, lit. [8] : 301 – 303°. $[\alpha]_D^{22}$ −76.1° (pyridine; *c* 0.68), ref. [8] : −88° (pyridine). $R_f(1)$ 0.27, $R_f(2)$ 0.48. RR_t 1.50. ^{13}C NMR (125 MHz, pyridine-d_5, TMS) : δ 15.7 (C-21), 16.5 (C-18), 19.4 (C-19), 19.6 (C-27), 21.1 (C-11), 30.1 (C-2), 30.6 (C-24), 31.7 (C-8, C-25), 32.3 (C-15), 32.6 (C-7), 34.3 (C-23), 37.2 (C-10), 37.5 (C-1), 38.8 (C-4), 39.9 (C-12), 40.7 (C-13), 41.8 (C-20), 47.6 (C-26), 50.2 (C-9), 56.6 (C-14), 63.3 (C-17), 77.4 (C-3), 78.5 (C-16), 98.3 (C-22), 121.7 (C-6), 140.8 (C-5), 100.3, 76.5, 84.8, 70.4, 75.1[a], 62.6[b] (C-1–C-6 of galactose), 102.2, 72.5, 72.8, 74.2, 69.4, 18.7 (C-1–C-6 of rhamnose), 105.8, 74.9[a], 78.5, 71.6, 78.3, 62.5[b] (C-1–C-6 of glucose), [a, b] may be exchanged. LSI-MS (matrix glycerol, positive ions, 9 kV) : 884 $[M+H]^+$ (48), 720 $[M+H-C_6H_{12}O_5$ (rhamnose)$]^+$ (6), 704 $[M+H-C_6H_{12}O_6$ (glucose)$]^+$ (7), 414 $[C_{27}H_{43}NO_2$ (solasodine) + H$]^+$ (30), 396 $[414-H_2O]^+$ (82), 138 (93), 114 (100).

N-*Hydroxyrobustine*. Yield 0.080%, amorphous. $[\alpha]_D^{24}$ −65.0° (pyridine; *c* 0.61), ref. [2] : – 63.4° (pyridine). $R_f(1)$ 0.27, $R_f(2)$ 0.22. RR_t 3.27. NMR and LSI-MS as described in ref. [2].

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