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STEROIDAL ALKALOIDS FROM *SOLANUM KHASIANUM*: APPLICATION OF ^{13}C NMR SPECTROSCOPY TO THEIR STRUCTURAL ELUCIDATION

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Key Word Index—*Solanum khasianum*; Solanaceae; glycoalkaloids; khasianine; solamargine; solasonine; ^{13}C NMR.

Abstract—The solasodine glycosides, solasonine, solamargine and khasianine have been isolated from berries of *Solanum khasianum* and characterized by ^{13}C NMR spectroscopy. By application of this method, the structure of khasianine has been elucidated as *O*-α-L-rhamnosyl (1→4_{glu})-*O* (3)-β-D-glucopyranosyl-solasodine (β_2-solamargine).

INTRODUCTION

Solanum khasianum C. B. Clarke, which grows wild in the Jorhat region, Assam, is also cultivated in other parts of India due to its importance as a potential source for solasodine. Varying yields of solasodine from the berries of the plant have been reported by different workers [1–5]. Though preliminary reports on isolation of glycosides from this plant have appeared [6–8] no systematic chemical work appears to have been done on the characterization of these compounds. Moreover, although ^{13}C NMR data of tomatine and solasonine (in DMSO-d_6) are available [9] no other glycoalkaloid appears to have been studied by ^{13}C NMR spectroscopy. This paper deals with the application of ^{13}C NMR spectroscopy to structural elucidation of steroidal alkaloids isolated from *Solanum khasianum*.

RESULTS AND DISCUSSION

The ethanol extract of the dried, finely ground berries of *Solanum khasianum* was extracted with 5% HOAc and the acid extract was neutralized with ammonia. The basic residue on chromatography over Si gel yielded the silicate salts of khasianine (**2**), solamargine (**3**) and solasonine (**4**) which were deionized and then subjected to ^{13}C NMR spectroscopy, which promises to be a powerful tool in elucidating structures of natural glycosides without chemical degradation provided that ^{13}C data on their aglycones and sugar moieties are available [10, 11].

^{13}C NMR spectra of several steroidal alkaloids have been determined by Ripperger and co-workers [12] with full signal assignments. We determined the ^{13}C chemical shifts of solasodine (**1**), solamargine (**3**) and solasonine (**4**) and applied these results to elucidate the structure of khasianine (**2**), a genuine glycoside isolated and characterized for the first time from a natural source. It is noteworthy that partial hydrolysis of solamargine leads to two different *O*-α-L-rhamnopyranosyl-*O*(3)-β-D-glucopyranosyl-solasodines, β_1 and β-solamargine. The solasodine–rhamnoglucoside (mp 245–247°, $[\alpha]_D - 100°$) obtained by Kuhn *et al.* [13] by partial hydrolysis of solamargine seems to be a mixture. On the other hand, the uncharacterized rhamnosyl–glucosyl-solasodine (mp 261°, $[\alpha]_D - 89°$) isolated by Briggs *et al.* [14] from *Solanum marginatum* may be identical with β_1-solamargine, the *O*-α-L-rhamnopyranosyl-(1 → 2_{glu})-*O* (3)-β-D-glucopyranosyl-solasodine. According to the ^{13}C NMR results, khasianine (mp 226–228°, $[\alpha]_D - 95°$) has the structure *O*-α-L-rhamnopyranosyl-(1 → 4_{glu})-*O* (3)-β-D-glucopyranosyl-solasodine and is therefore identical with β_2-solamargine.

Pyridine-d_5 was the solvent used as glycosides are generally soluble in this. ^{13}C NMR spectra of methyl-β-D-glucopyranoside (**5**), methyl-β-D-galactopyranoside (**6**) and methyl-α-L-rhamnopyranoside (**7**) in pyridine-d_5 were examined and ^{13}C NMR signals of compounds **5**–**7** were assigned by comparison with literature data in D_2O [15–17] (Table 2). Similarly, ^{13}C NMR signals of solasodine (**1**) were assigned by comparison with data in $CDCl_3$ [12]. The signal assignments in compounds **2**–**4** were fairly straightforward, by comparison with those of solasodine and sugar moieties. It may be mentioned that assignments of the carbon signals of the sugar moiety of solasonine reported by Weston *et al.* [9] need to be revised as follows: galactose C-2: δ 73.7 and C-3: 83.7 in DMSO-d_6, because it has been found that glycosylation shift by α-L-rhamnopyranosylation is somewhat smaller than that of β-D-glucopyranosylation [18]. ^{13}C NMR chemical shifts of the sugar moiety of solamargine were assigned by comparison with those of dioscin [19] whose sugar moiety is identical with that of solamargine. Glycosylation shifts [18, 20–22], i.e. chemical shift changes from aglycone and methyl glycoside to glycoalkaloid, may be calculated from the ^{13}C NMR chemical shifts shown in Table 1. Characteristic signal shifts are observed at the α-, β- and γ-positions of the OH group in which the glycosylation takes place. Particularly, carbinyl carbon (α-carbon) signals of aglycone alcohols are shifted downfield by + 7 ppm and the signals of the two β-methylene carbons are shifted upfield by -2.4 ± 0.1 ppm (in the case of the pro-*R* carbon) and -4.5 ± 0.5 ppm (in the case of pro-*S*

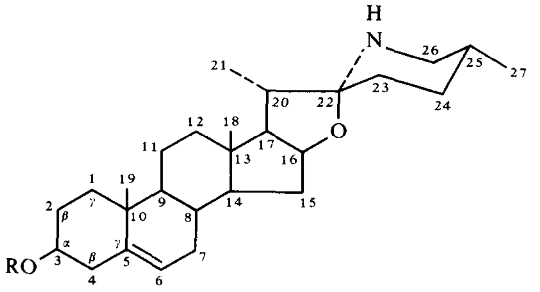
Table 1. ^{13}C NMR chemical shifts δ_C ($\pm$0.1) of solasodine (**1**), khasianine (**2**), solamargine (**3**) and solasonine (**4**)

Carbon No.	**1**		**2**		**3**		**4**
1	37.8		37.4		37.5		37.4
2	32.5		30.2		30.1		30.1
3	71.3		78.3		78.3^d		78.3
4	43.3		39.3		38.8		38.8
5	140.0		140.7		140.8		140.7
6	121.0		121.7		121.7		121.6
7	32.5^a		32.3^a		32.4		32.5
8	32.4^a		32.5^a		32.4		32.5
9	50.6		50.3		50.3		50.3
10	37.0		37.0		37.1		37.1
11	21.3		21.2		21.1		21.1
12	40.2		40.1		40.1		40.1
13	40.7		40.6		40.6		40.6
14	56.8		56.7		56.7		56.7
15	31.8		31.6		31.5^a		31.7
16	78.9		78.7		78.7^d		78.7
17	63.6		63.5		63.4		63.5
18	16.5		16.5		16.5		16.5
19	19.6		19.4		19.3		19.3
20	41.7		41.6		41.6		41.5
21	15.6		15.6		15.6		15.6
22	98.3		98.3		98.2		98.2
23	34.6		34.6		34.6		34.6
24	31.0		31.1		31.0		31.1
25	31.6		31.6		31.7^a		31.7
26	48.1		48.0		48.0		47.9
27	19.6		19.7		19.7		19.7
1′		Glc	102.7^b		100.2	Gal	100.3
2′			75.5		78.2^d		76.3
3′			77.0		72.5		84.8
4′			78.3		77.7^d		70.2
5′			76.6		76.7		74.9^a
6′			61.6		61.3		62.4^c
1″		Rha	102.4^b		102.7	Glc	105.7
2″			72.5^c		72.5		74.8^a
3″			72.8^c		72.5		78.7^b
4″			73.9		73.7^b		71.4
5″			70.3		70.3^c		78.3^b
6″			18.5		18.3		61.8^c
1‴				Rha	101.8		102.0
2‴					72.5		72.4^d
3‴					72.5		72.7^d
4‴					73.9^b		74.0
5‴					69.3^c		69.3
6‴					18.5^c		18.5

a, b, c, d and e may be reversed on each vertical column.
Glc = glucose; Gal = galactose; Rha = rhamnose.

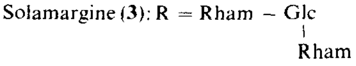
Table 2. ^{13}C NMR chemical shifts of methyl-β-D-glucopyranoside, β-D-galactopyranoside and α-L-rhamnopyranoside in pyridine-d_5

Methyl glycosides	C-1	C-2	C-3	C-4	C-5	C-6
Methyl-β-D-glucopyranoside (**5**)	105.5	74.9	78.3	71.6	78.3	62.7
Methyl-β-D-galactopyranoside (**6**)	105.5	72.0	74.6	69.8	76.2	62.0
Methyl-α-L-rhamnopyranoside (**7**)	102.4	72.6	72.0	73.7	69.4	18.5

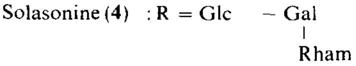


Solasodine (**1**) : R = H

Khasianine (**2**) : R = Rham − Glc

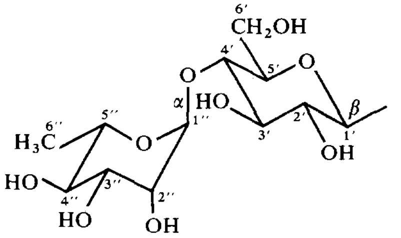


Solamargine (**3**): $R = \mathrm{Rham} - \underset{\underset{\textstyle \mathrm{Rham}}{|}}{\mathrm{Glc}}$

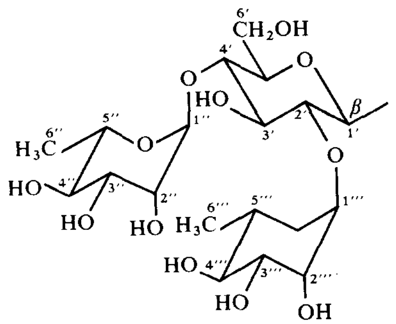


Solasonine (**4**) : $R = \mathrm{Glc} - \underset{\underset{\textstyle \mathrm{Rham}}{|}}{\mathrm{Gal}}$

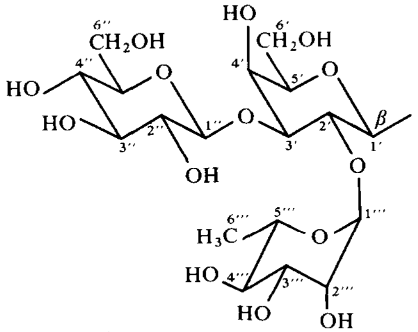
Rham—Glc =



Rham—Glc
|
Rham =



Glc—Gal
|
Rham =



carbon). The results are in good agreement with those obtained by glucosylation and mannosylation of *S*-alcohols [20–22]. Interestingly, the γ $-\mathrm{C}\leqslant$ signals are shifted upfield by *ca* −1.3 ppm.

EXPERIMENTAL

Mps are uncorr. TLC was performed on Si gel using *n*-BuOH satd with H_2O–$(C_2H_5)_2NH$–MeOH (40:5:1). ^{13}C NMR spectra were taken in Py-d_5 soln (0.05–0.4 mM) on a JEOL-PFT-100 spectrometer (25.15 MHz); temp. 20°, pulse width 32 μsec (90°), repetition time 1 sec, accumulation time 2000, spectral width 4 kHz, acquisition time 0.4 sec, data points: 4096. A 10 mm sample tube was used. The chemical shifts are expressed as δ ppm from an int. ref. TMS.

Isolation of glycoalkaloids. Air-dried powdered berries (650 g) collected from Jorhat, Assam (India) were successively extracted with petrol (60–80°) and 90% EtOH. The EtOH extract on removal of the solvent gave a dark-brown, semi-solid mass (105 g). The residue was extracted with 5% HOAc (3 × 300 ml) and then filtered. The combined extract was basified with conc NH_4OH, the pptd glycoalkaloids were collected by centrifuge, washed with H_2O, dried (37 g) and then chromatographed on a Si gel column (800 g). Successive elution with $CHCl_3$, $CHCl_3$–MeOH (95:5), (90:10), (85:15) and (80:20) afforded solasodine (traces), khasianine (30 mg), solamargine (2.1 g) and solasonine (70 mg) as their silicate salts. These were deionized by passing their MeOH soln through a column of ion exchange resin, Amberlite IRA 410 ($-NH_3^+$ OH^- form) and then concd to dryness.

Khasianine (**2**). The silicate salt of **2** obtained from column chromatography crystallized from MeOH as needles, mp 250–252°. This was deionized and then crystallized from aq. MeOH as micro-needles, mp 226–228°, $[\alpha]_D^{25}$ − 95° (MeOH). (Found: C, 64.86; H, 8.76; N, 1.91. $C_{39}H_{63}O_{11}N$ requires: C, 64.93; H, 8.80; N, 1.94%).

Solamargine (**3**). Its silicate salt crystallized from MeOH as needles mp 213–215° (dec). After deionization it crystallized from MeOH as needles, mp 310° (dec), $[\alpha]_D^{25}$ − 106° (EtOH). (Found: C, 62.21; H, 8.50; N, 1.59. Calc. for $C_{45}H_{73}O_{15}N$: C, 62.26; H, 8.47; N, 1.61%).

Solasonine (**4**). The silicate salt of **4** was crystallized from MeOH–$CHCl_3$ as micro-needles, mp 202–204° (dec). On deionization and crystallization from MeOH it afforded needles of solasonine, mp 301° (dec), $[\alpha]_D^{25}$ − 73° (MeOH). (Found: C, 59.86; H, 8.32; N, 1.52. Calc. for $C_{45}H_{73}O_{16}N.H_2O$: C, 59.94; H, 8.38; N, 1.55%).

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