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^{1}H- AND ^{13}C-NMR ASSIGNMENTS AND STRUCTURAL DETERMINATION OF A NOVEL GLYCOALKALOID FROM *SOLANUM PLATANIFOLIUM*

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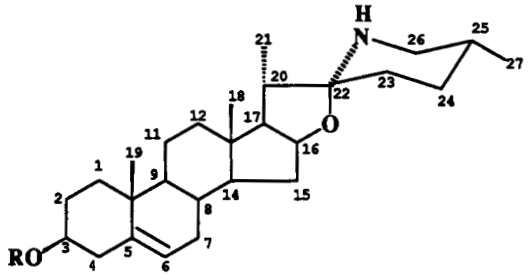
ABSTRACT.—Four steroidal glycoalkaloids, solasonine [**2**], solamargine [**3**], khasianine [**4**], and ravifoline [**5**], with solasodine [**1**] as the aglycone moiety, have been isolated from *Solanum platanifolium*. The present study involved the characterization of the novel compound **5** by one- and two-dimensional nmr techniques and has also led to the unambiguous and total ^{13}C- and ^{1}H-nmr assignments of all of these steroidal glycoalkaloids and the direct elucidation of their glycosidic linkages.

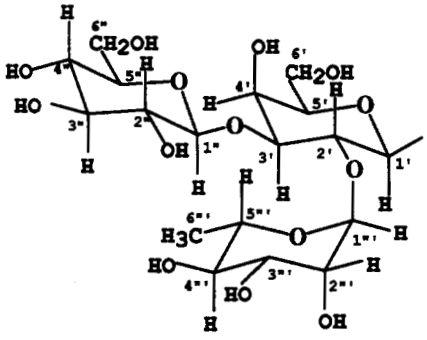
Solanum platanifolium Sims (Solanaceae) has been found to be a potential source of the steroidal hormone precursor solasodine [**1**] (1). A further phytochemical investigation on a petroleum ether extract of the berries of the plant showed the presence of *n*-alkanes, *n*-alkanols, sterols, and unknown triterpenoids (2). Comparison of the concentration levels of alkanes, alkanols, and sterols found in different organs of this plant showed interesting features (3,4). The current phytochemical study on an alcoholic extract of the berries of *S. platanifolium* has revealed the presence of solasonine [**2**], solamargine [**3**], khasianine [**4**], and a novel glycoalkaloid designated ravifoline [**5**]. The structures of **2** and **3** were confirmed earlier (5).

A survey of the literature has revealed limited ^{13}C- and ^{1}H-nmr information on these compounds. Earlier structural investigations of steroidal saponins by nmr involved acid hydrolysis, yielding an aglycone and a glycoside, which were then investigated separately (6). The previous ^{13}C-nmr assignments of the aglycone portion of these glycoalkaloids were based on comparison with model compounds, paramagnetic broadening, chemical shift considerations, specific deuterium labeling, and relatively simple nmr techniques (7). The complex region (28–35 ppm) of these spectra has been assigned by obtaining qualitative assessment of spin-lattice relaxation times of carbon nuclei by an inversion recovery method based on the assumption of molecular tumbling and the mechanism of isotropic relaxation (8). The assignment of the oligosaccharide residues has been accomplished by employing glycosylation shift rules (carbon resonance displacements of both sugar and aglycone moieties on glycoside formation) derived from previous ^{13}C-nmr studies (9,10). Furthermore, the complete ^{1}H-nmr assignments for these compounds are unavailable and previous reports on the proton assignments of steroidal molecules, especially those of more complex glycoalkaloids, have been rather limited. With the advancement of two-dimensional (2D) nmr and the availability of high magnetic fields (>7 T), both the structure elucidation and total assignment of the nmr spectra of such complex glycoalkaloids has become feasible. It is desirable to obtain *de novo* and direct information from 2D correlations for these glycoalkaloids as intact molecules.

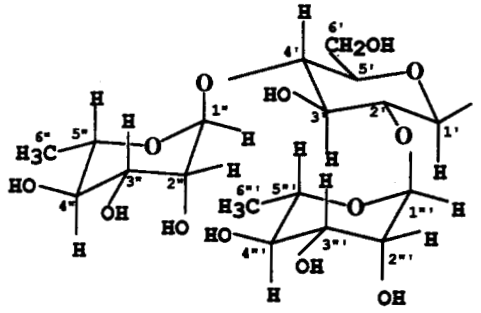
In an earlier communication, we reported the total ^{1}H- and ^{13}C-nmr assignments for solasodine [**1**] and a related molecule, diosgenin, via 2D nmr (11). The present study was undertaken to establish total ^{1}H- and ^{13}C-nmr assignments of additional glycoalkaloids on their intact molecules and to elucidate the structure of the unknown glycoalkaloid, **5**, via 2D nmr techniques. The results of the assignments of **2**, **3**, and **4** were then applied to determine the structure and the total assignment of the unknown [**5**]. Various 2D nmr



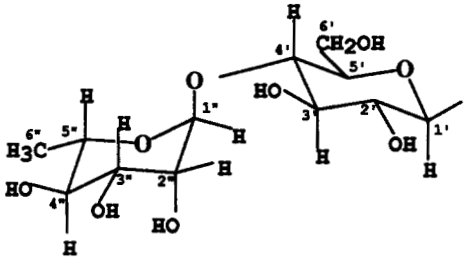
1 H=R



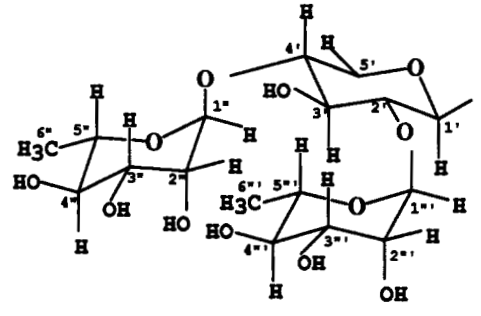
2 glc–gal– (rha) =R



3 rha–glc– (rha) =R



4 rha–glc– =R



5 rha–xyl– (rha) =R

correlation experiments (COSY, TOCSY, DQF-COSY, and ^{13}C-^{1}H HMQC) were used to obtain explicit resonance assignments for these steroidal saponins. NOESY was then used to determine the glycosidic linkages of the sugar moieties.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps are uncorrected. Tlc was performed on Merck Si gel G using $CHCl_3$-EtOH-NH_4OH (2:2:1), and Dragendorff's reagent was employed as visualization reagent. All solvents were evaporated under reduced pressure at 40°.

PLANT MATERIAL.—*Solanum platanifolium* Sims (Solanaceae) used in this investigation was collected from Dharmsala and surrounding hills (1000–1500 meters) in the Kangra Valley, Himachal Pradesh, India. The type specimen of the plant has been deposited at the herbarium at the Royal Botanic Gardens, Kew, UK.

EXTRACTION AND ISOLATION OF GLYCOALKALOIDS.—Solasonine [**2**], solamargine [**3**], khasianine [**4**], and ravifoline [**5**] were isolated essentially by following the protocols in an earlier communication (5). Powdered, oven-dried (50°) berries (500 g) of *S. platanifolium* were defatted with *n*-hexane and then extracted with 90% EtOH. The EtOH extract, on removal of the solvent under vacuum, gave a dark-brown semi-solid mass (90 g). This residue was extracted with HOAc (5%, 3×200 ml) and then filtered. The combined extract was basified with concentrated NH_4OH at pH 9.5–10. The precipitates were collected and the process repeated three times. Finally the glycoalkaloids were dried (30 g) and extracted with MeOH. Solvent was removed under vacuum and the residue chromatographed over neutral Al_2O_3. Successive elution with *n*-BuOH saturated with H_2O yielded four crystalline compounds [**2–5**].

Khasianine [**4**], crystallized from MeOH (80%), exhibited: mp 228–230°; $[\alpha]^{25}D$ −90° (MeOH); *anal.*, found C 64.82, H, 8.71, N 1.90%, $C_{39}H_{63}O_{11}N$ requires C 64.93, H 8.80, N 1.94%; ir ν max (KBr) 3450, 2940, 1280, 1190, 980, 915, 895, 815 cm^{-1}.

Ravifoline [**5**], crystallized from MeOH (80%), exhibited: mp 280–282°; $[\alpha]^{25}D$ −104° (pyridine); *anal.*, found C 63.24, H 8.50, N 1.69%, $C_{44}H_{71}O_{14}N$ requires C 63.04, H 8.60, N 1.71%; ir ν max (KBr) 3448, 2941, 1626, 1470, 1282, 1190, 1081, 980, 917, 893, and 813 cm^{-1}.

NMR SPECTRA.—All nmr spectra of the compounds were obtained on a Bruker AMX-500 spectrometer at a probe temperature of 27°. Spectra were obtained from solutions of ca. 8 mg of the compound in 0.5 ml of solvent. Compounds **2**, **4**, and **5** were dissolved in DMSO-d_6 and **3** in dioxane-d_8, because the latter was not sufficiently soluble in DMSO. The chemical shifts are expressed in ppm downfield from TMS.

Proton spectra.—The proton 90° pulse was 9.2 μsec. Typical conditions: block size 32k and 32 scans, spectral width, 4000 Hz. A "reverse detection" 5 mm probe was used.

2D Homonuclear correlated spectra.—The 2D proton spectra by COSY (12), DQF-COSY (13), TOCSY (14), and NOESY (15), were recorded with 2k and 512 data points in the t_2 and t_1 dimensions, respectively, on the reverse-detection probe. The data sets were processed with zero-filling in t_1 to give a 1k×1k contour map. Magnitude calculation COSY was used, while NOESY, TOCSY, and DQF-COSY were recorded in pure phase mode using the TPPI technique (16). A mixing time of about 120 μsec was used typically in the TOCSY experiment.

^{13}C-Nmr spectra.—The 90° pulse was 7.9 μsec, block size 64k and 256 scans, and the spectral width was 22,500 Hz. Composite pulse decoupling (CPD), GARP (17), was used to decouple protons on a 5-mm broadband probe.

2D Heteronuclear correlated spectra.—Because of the relatively low concentrations of the samples, one-bond ^{13}C-^{1}H nmr chemical shift correlations were obtained using the proton-detected HMQC technique (18). The data were accumulated with 2k and 512 points in the t_2 and t_1 dimensions, respectively. Zero filling in the t_1 dimension during processing gave a 1k×1k contour map. The experiments were run in the phase-sensitive mode using the TPPI method. Bilinear rotation (BIRD) pulses (19) were used at the beginning of the HMQC pulses to remove the proton signals arising from protons attached to ^{12}C (18).

1D TOCSY.—The pulse sequence of Kessler *et al.* (20) was used to generate the 1D TOCSY spectra. Selective Gaussian pulse of 35 msec (90° pulse) was generated from the Bruker waveform memory unit. A Z-filter (21) was used in this experiment to produce absorption lineshape.

RESULTS AND DISCUSSION

Solasonine [**2**] and solamargine [**3**] have been isolated from *Solanum* species (22), while khasianine [**4**] has been reported in *S. khasianum* (6). Their initial characterization was carried out by hydrolyzing the compounds and separately identifying the glycoside and aglycone moieties (6,9,10). Limited information is available on complete ^{1}H-nmr assignments for these compounds. Total nmr assignments of these glycoalkaloids along with the direct elucidation of glycosidic linkages by 2D NOESY is reported for the first time in the present investigation. The unknown **5** has also been characterized following this technique. Results from 1D and 2D experiments were used collectively in making total nmr assignments of these glycoalkaloids.

Aglycone nmr assignments were established using homonuclear correlation techniques (COSY and TOCSY) for the assignment of ^{1}H-nmr spectra, and HMQC was then used for the assignment of the ^{13}C-nmr spectra. After recording ^{1}H, ^{13}C and 1D DEPT spectra, the 2D DEPT-COSY technique was used to correlate the ^{13}C-, and ^{1}H-nmr chemical shifts. NOESY was used to differentiate the α and β protons on rings A–E. The

TABLE 1. ^{13}C- and ^{1}H-Nmr Chemical Shifts (δ in ppm) of Solasodine [**1**], Solasonine [**2**], Solamargine [**3**], Khasianine [**4**], and Ravifoline [**5**].

Position	Compound											
	1[a]			**2**			**3**			**4**			**5**		
	^{13}C	^{1}H		^{13}C	^{1}H		^{13}C	^{1}H		^{13}C	^{1}H		^{13}C	^{1}H	
		α	β		α	β		α	β		α	β		α	β
1	37.16	1.00	1.80	36.82	0.98	1.80	37.90	1.02	1.80	36.75	0.99	1.80	36.74	1.00	1.80
2	31.99	1.78	1.40	29.03	1.78	1.48	29.90	1.82	1.55	29.17	1.80	1.47	28.96	1.79	1.48
3	71.65	3.42		75.84	3.50		78.05	3.50		77.04	3.46		76.22	3.48	
4	42.22	2.24	2.16	37.47	2.41	2.18	38.86	2.41	2.27	38.27	2.40	2.10	37.55	2.42	2.20
5	140.91			140.00			141.12			140.43			140.30		
6	121.40	5.31		121.24	5.33		122.05	5.35		121.31	5.23		120.27	5.33	
7	32.08	1.48	1.97	31.75	1.50	1.95	32.47	1.50	2.01	31.60	1.50	1.96	31.55	1.52	1.92
8	31.54	1.58		31.01	1.53		31.84	1.63		30.99	1.55		30.96	1.57	
9	50.03	0.90		49.62	0.90		51.04	0.92		49.57	0.90		49.58	0.90	
10	36.57			36.42			37.55			36.37			36.35		
11	20.79	1.48, 1.41[b]		20.40	1.50		21.48	1.50		20.38	1.49		20.35	1.52	
12	39.85	1.10	1.68	40.00	1.11	1.70	40.61	1.14	1.75	39.35	1.11	1.69	39.30	1.11	1.70
13	40.45			40.48			41.00			40.00			39.99		
14	56.46	1.02		55.94	1.02		57.21	1.08		55.92	1.02		55.91	1.05	
15	32.08	1.95	1.25	31.60	1.88	1.11	32.73	1.89	1.23	31.71	1.89	1.16	31.70	1.78	1.16
16	78.91	4.20		77.67	4.19		78.92	4.20		77.69	4.18		77.68	4.19	
17	62.73	1.65		62.31	1.70		63.91	1.65		62.31	1.69		62.30	1.65	
18	16.29	0.75		16.18	0.72		16.66	0.81		16.12	0.72		16.11	0.75	
19	19.30	0.95		18.95	0.98		19.76	1.10		19.09	1.00		18.91	0.98	
20	41.20	1.81		40.81	1.75		41.89	1.82		40.80	1.75		40.77	1.77	
21	15.13	0.88		15.28	0.85		15.61	0.89		15.23	0.85		15.21	0.88	
22	98.25			97.41			98.47			97.40			97.39		
23	33.92	1.56		30.30	1.50		31.14	1.45		30.25	1.52		33.71	1.55	
24	30.12	1.55	1.35	33.76	1.57	1.40	30.11	1.89	1.23	33.72	1.55	1.44	30.24	1.52	1.28
25	31.34	1.48		30.65	1.52		32.13	1.47		30.64	1.48		30.58	1.42	
26	47.51	2.58	2.54	47.13	2.52	2.38	48.22	2.52	2.38	47.10	2.51	2.39	47.09	2.52	2.40
27	19.17	0.80		19.50	0.78		19.51	0.80		19.46	0.79		19.44	0.78	

[a]Data from Puri *et al.* (11).

[b]The two protons 11 are not equivalent. However, their chemical shifts are too close to be resolved. See Puri *et al.* (11).

chemical shifts for the aglycone part of these glycoalkaloids (Table 1) exhibited little difference from those of the parent compound, solasodine [**1**], reported in our earlier communication (11). The major variations of approximately 2–6 ppm in the ^{13}C-nmr chemical shifts occurred in positions C-2, C-3, and C-4 as expected.

After determining the 27 signals in the ^{13}C-nmr spectra due to each aglycone, the remaining signals were due to the carbons constituting the sugar moieties. For **2** and **3**, 18 such signals were found, whereas for **4** and **5** there were 12 and 17 signals, respectively. The majority of resonances for the glycosides were found in the 60–105 ppm range for ^{13}C and 3–5 ppm for ^{1}H, as would be expected for saccharides. Structure assignments to the glycosides involved both the identification of the sugar residues and the establishment of their linkages. Identification of the sugar residues utilized some characteristic resonances of the different types of sugar rings. For example, in **4**, the key resonance in starting this process appeared at 17.7 ppm, which represents the C-6 signal of 6-deoxy sugars. This signal was identified as a methyl carbon (C-6″) from the 135° DEPT spectrum and the corresponding ^{1}H-nmr chemical shift was determined for this signal from an HMQC nmr spectrum which was at 1.1 ppm. This was used as a starting point in the homonuclear correlated spectra (COSY, TOCSY, and DQF-COSY) to determine all the protons on the same sugar ring. Thus, the methyl protons were correlated to H-5″ at 3.82 ppm, which was further coupled to H-4″ and so on. With the help of the HMQC data the corresponding carbon chemical shifts were assigned for the 6-deoxy-hexose unit. Two such 6-deoxy-hexose moieties were found for **3** and **5**, while one each was found in **2** and **4**. From the *J*-coupling patterns of the ring protons (Table 2) it was clear that this 6-deoxy hexose is a rhamnose. Thus, for **3–5**, only one more sugar residue had to be interpreted while **2** required the elucidation of two other sugar residues because it contained only one deoxy-hexose. Again, using **4** as an example, the 135° DEPT spectrum showed the presence of a methylene carbon at ca. 60 ppm not associated

TABLE 2. Vicinal Coupling Constants (*J* in Hz) of the Glycones in Glycoalkaloids [**2–5**].[a]

	Compound			
	2	**3**	**4**	**5**
$J_{1'2'}$	7.6	7.5	7.8	7.9
$J_{2'3'}$	9.7	9.6	8.8	8.6
$J_{3'4'}$	3.0	8.0	8.0	8.0
$J_{4'5'}$	<2	8.8	7.4	4.9, 9.6
$J_{5'6'}$[b]	5, 9	4, 8	5, 8	$J_{5'a5'e}$ 11
$J_{6'6'}$	10.9	11	11	
$J_{1''2''}$	7.8	1.2	1.2	1.6
$J_{2''3''}$	9.5	<2	<2	<2
$J_{3''4''}$	8.7	9	10	10
$J_{4''5''}$	9.0	9.8	9.4	9.5
$J_{5''6''}$[b]	5, 8	6.1	6.2	6.1
$J_{6''6''}$	12			
$J_{1'''2'''}$	1.3	1.1		1.4
$J_{2'''3'''}$	<2	<2		<2
$J_{3'''4'''}$	9	9		10
$J_{4'''5'''}$	9.3	9.8		9.5
$J_{5'''6'''}$	6.2	6.4		6.4

[a]The uncertainties are ±0.2 Hz for coupling constants determined from 1D data and ±1 Hz for those determined from 2D data.

[b]$J_{a,e}$ (axial, equatorial).

with the steroidal portion of the compound. Then, using the ^{13}C-^{1}H correlation from the HMQC data, the corresponding proton chemical shifts were determined to be 3.58 and 3.42 ppm, respectively. By using these methylene proton signals and their counterparts for **2**, **3**, and **5** as the starting points, the sugar intra-residue connectivity was deciphered for the remaining sugars in the same manner as for the deoxy-hexose. Chemical shifts for the glycosidic moieties are listed in Table 3.

The combined use of 1D TOCSY and NOESY (or ROESY) to decipher the structure and linkage for oligosaccharides has been applied by Wessel *et al.* (23), and by Willker *et al.* (24) in the study of tomatidine. 1D TOCSY in this case was particularly useful in tracing the propagation of the correlation through the ring by varying the mixing time. As shown in Figure 1, the well isolated signal of 1‴ in **5** at 5.03 ppm was selectively excited and the propagation of correlation to H-2‴, 2‴-OH, H-3‴, and so on can be monitored by gradually increasing the mixing time (from A to D 27, 50, 72, and 120 msec, respectively). In this manner, the assignment of the various protons on the ring can be ascertained. Such propagation of correlation is very efficient for sugars with primarily axial-axial proton configurations ($^{3}J_{HH}$>7 Hz), such as glucose and xylose (which occur in **3**, **4**, and **5**, respectively). It is less efficient for rhamnose and galactose with one or more equatorial protons, thus slowing down propagation of the correlations due to smaller J couplings (ca. 3 Hz).

Identification of the sugar residues in these molecules was made in the next step using J-coupling constants. It is well known that protons of a hexose in a chair conformation will have different coupling constants depending on their orientation in space. Axial-axial proton couplings will be the largest (7–10 Hz), whereas equatorial-axial or equatorial-equatorial couplings will be smaller (<2–3 Hz) (25). The vicinal coupling constants for the glycosides in **2** and **5** determined from their 1D spectra and from DQF-COSY are given in Table 2. Vicinal coupling constants between the OH proton and the CH at the carbon site to which the hydroxy group is attached range between 4–5.5 Hz and are not included in this table. However, because of these additional couplings with the OH protons, some of the vicinal couplings of the ring protons are difficult to determine. The uncertainties due to this complication are noted

TABLE 3. ^{13}C- and ^{1}H-Nmr Chemical Shifts (δ in ppm) of the Glycosidic Moiety of Solasonine [**2**], Solamargine [**3**], Khasianine [**4**], and Ravifoline [**5**].

Position	Compound											
	2			**3**			**4**			**5**		
	^{13}C	^{1}H	OH	^{13}C	^{1}H	OH	^{13}C	^{1}H	OH	^{13}C	^{1}H	OH
1′	98.29	4.31		99.74	4.38		100.65	4.25		98.15	4.40	
2′	72.89	3.57		77.56	3.36	4.42	73.71	2.95	4.90	76.93	3.22	
3′	83.53	3.62		77.61	3.51	4.02	75.16	3.22	4.65	76.68	3.38	4.92
4′	67.81	3.88	4.15	76.20	3.18		76.65	3.32		76.01	3.40	
5′	74.66	3.39		78.98	3.55		75.36	3.15		59.99	3.58,3.42	
6′	60.60	3.49,3.47	4.58	61.39	3.68		60.12	3.58,3.42	4.64			
1″	103.93	4.31		102.22	4.78		100.45	4.68		100.51	4.68	
2″	73.39	3.03	4.91	71.77	3.73	4.30	70.69	3.59	4.65	70.69	3.62	
3″	76.88	3.18	5.07	72.21	3.45		70.59	3.41	4.44	75.55	3.42	
4″	76.70	3.09	4.92	73.33	3.26	4.22	71.83	3.19	4.62	75.21	3.19	4.66
5″	69.74	3.12		69.91	3.84		68.63	3.82		68.63	3.86	
6″	60.89	3.62,3.45	4.40	18.01	1.10		17.72	1.11		17.69	1.11	
1‴	100.37	5.03		101.21	5.15					100.27	5.03	
2‴	70.45	3.72	4.16	71.56	3.74	4.20				70.40	3.68	4.58
3‴	70.41	3.40	4.30	72.05	3.46					70.57	3.42	4.66
4‴	72.02	3.19	4.62	73.57	3.24					71.88	3.20	
5‴	67.93	4.00		68.78	4.04					67.92	3.98	
6‴	17.80	1.08		18.01	1.15					17.74	1.10	

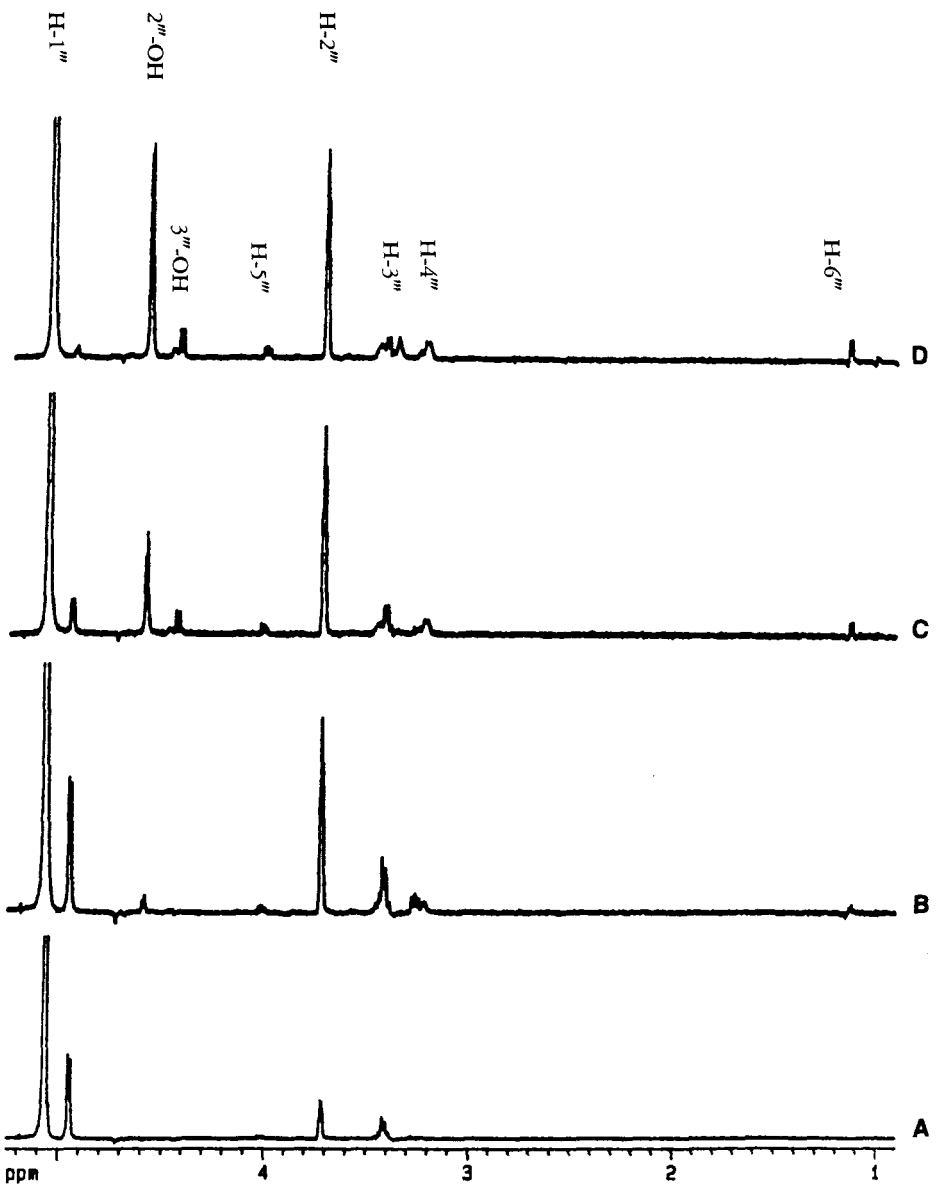


FIGURE 1. ID TOCSY nmr spectra for **5** with selective excitation of proton 1‴ of the rhamnopyranose ring at 5.03 ppm. From A to D, mixing time: 27, 50, 72, and 120 msec, respectively.

in the table. By utilizing the proton coupling constants of the sugar residues, and the propagation of TOCSY correlations in 1D TOCSY experiments (especially in the region with overlapping signals where the *J* couplings cannot be determined) as described above, the sugar residues were identified. Thus, a glucose ring is found in **2**, **3**, and **4**, and a galactose residue is present in **2**. The only pentose residue that exists in **5** was similarly identified as a xylose. Compounds **2** and **3** are confirmed as galactose-glucose-rhamnose and glucose-rhamnose-rhamnose containing glycosides of **1**, respectively (for discussion on the determination of the glycosidic linkage, see the next section). The two glycoalkaloids, khasianine [**4**] and ravifoline [**5**], were found to possess glucose-rhamnose and xylose-rhamnose-rhamnose containing glycosides, respectively. Agree-

ment of the ^{1}H- and ^{13}C-nmr chemical shifts for these sugar residues with those generally found in the literature (25) further supports our identifications of the sugar residues and their linkages.

Having established the identity of the various sugar residues in these molecules, the glycosidic linkage and its point of attachment to the steroidal portion of the compound were then determined. TOCSY correlations do not provide such information because the four-bond H-H coupling across the glycosidic linkage is usually vanishingly small. However, these linkages can be established by the NOESY experiment because NOESY cross-peaks can be observed between protons across glycosidic bonds. Figure 2 shows such an example. The glycosidic linkages between H-1″ (4.68 ppm) and H-4′ (3.40 ppm), and between H-1‴ (5.03 ppm) and H-2′ (3.22 ppm) were clearly observed in the NOESY map. In addition, cross-peaks between H-1′ (4.40 ppm) and H-3 of the aglycone were observed, revealing the point of attachment of the glycoside to the aglycone. Thus, **5** was found to have the linkage rhamnopyranosyl-β(1″→4′α-xyl), rhamnopyranosyl-β(1‴→2′α-xyl)-(1′→3)-β-xylofuranosyl-solasodine and **4** rhamnopyranosyl-β(1″→4′α-glc)-(1′→3)-β-glucopyranosyl-solasodine. The linkages for **2** and **3** determined in the present work are in agreement with the literature, that is,

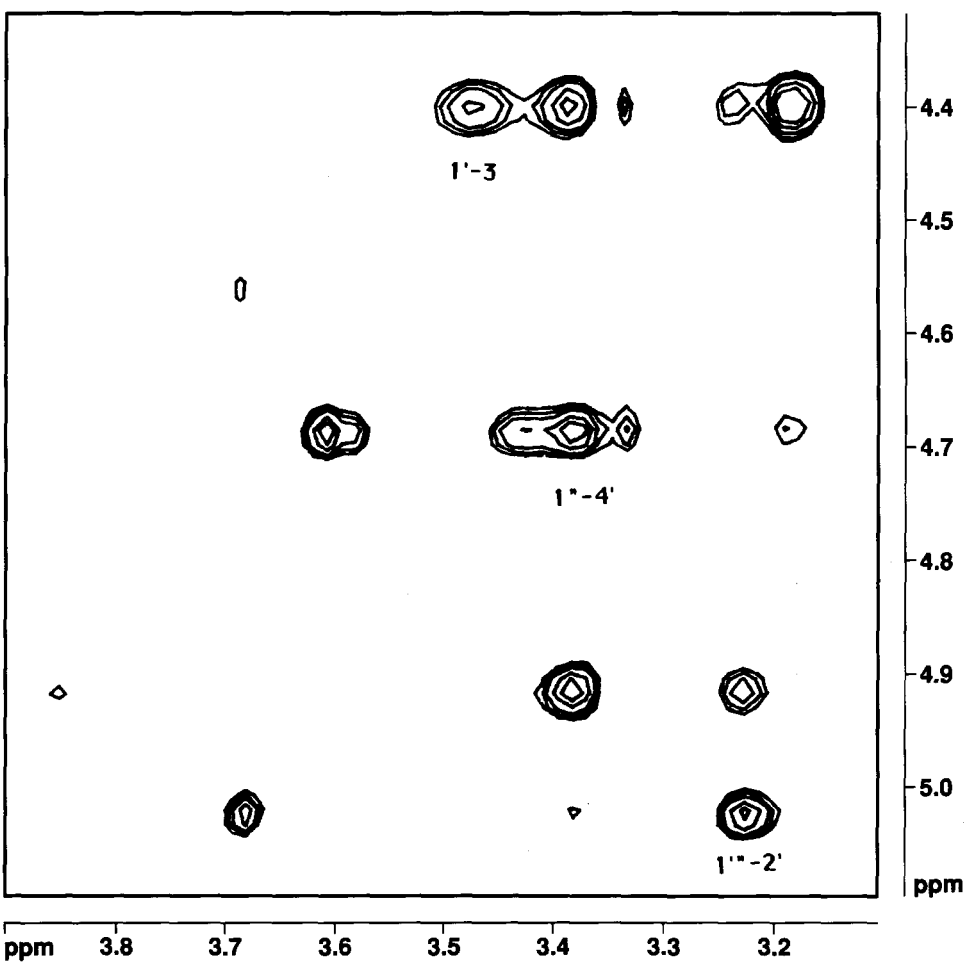


FIGURE 2. A portion of the NOESY correlation for **5**. The nOe correlations across the glycosidic bonds have been shown.

glucopyranosyl-β(1″→3′β-gal), rhamnopyranosyl-β(1‴→2′α-gal)-(1′→3)-β-galactopyranosyl-solasodine and rhamnopyranosyl-β (1″→4′α-glc), rhamnopyranosyl-β(1‴→2′α-glc)-(1′→3)-β-glucopyranosyl-solasodine, respectively.

The proton resonances due to most of the hydroxyl protons were also assigned (Table 3). The proton of each hydroxyl group on the sugar residue showed coupling to the proton attached to its respective carbons in the COSY and TOCSY spectra. The present work also reveals discrepancies in assignments of carbon signals reported by Weston *et al.* (7) for the sugar moieties of **2**, as pointed out by Mahato *et al.* (6). However, certain ^{13}C-nmr assignments made by Mahato *et al.* (6) for the sugar moiety of solasonine also need to be revised: the assignments for galactose C-2′ and C-3′ (the present assignments 72.89 and 83.53 ppm, respectively) and those for glucose C-4″ and C-5″ (the present assignments 76.70 and 69.74 ppm, respectively) need to be reversed. Also, C-3′ of the solamargine [**3**] glycoside was found to have a chemical shift of 78.05 ppm in DMSO-d_6, whereas Mahato *et al.* (10) reported it to be 72.85 ppm in pyridine-d_6. All the other ^{13}C-nmr chemical shifts were within ±1.0 ppm of each other.

The 2D nmr experiments led to unambiguous ^{13}C- and ^{1}H-nmr assignments of the glycoalkaloids solasonine [**2**], solamargine [**3**], khasianine [**4**], and ravifoline [**5**]. The ^{1}H-nmr assignments for these molecules have been reported for the first time. The glycosidic linkage was also confirmed for all compounds through direct nOe correlations observed between the protons across the glycosidic bonds in the 2D NOESY experiment. The uncharacterized glycoalkaloid, designated ravifoline [**5**], has been found to be a rhamnose-xylose-rhamnose glycoside of solasodine [**1**]. Thus, it is evident from the present results that 2D nmr experiments provide a direct and unambiguous technique in the elucidation of intact steroidal saponins. 1D and 2D TOCSY experiments are valuable for assignment of structures to the sugar moieties and, in conjunction with NOESY experiments, for establishing the linkage and conformation of the carbohydrate chain.

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