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STEROID SAPONINS FROM *SOLANUM LAXUM*

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Key Word Index—*Solanum laxum*; Solanaceae; glycoside; spirosten saponin.

Abstract—Two new steroid saponins, named laxumins A and B, were isolated from the ethanolic extract of the aerial parts of *Solanum laxum*. These compounds were characterized, using mainly NMR spectroscopy, mass spectrometry and chemical methods, as (23*S*,25*S*)-spirost-5-en-3β,15α,23-triol 3-*O*-{β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-galactopyranoside} and 3-*O*-{β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)]-β-D-galactopyranoside}, respectively.

INTRODUCTION

The Solanaceae family is a rich source of active secondary metabolites. In particular, the genus *Solanum* produces a great variety of steroidal saponins and glycoalkaloids of importance in the natural resistance of these plants against several pests [1]. In our continued study of the glycosides produced by Uruguayan *Solanum* plants [2], we now report our findings on saponins produced by *S. laxum* Steud. *S. laxum* is a bindweed native to Uruguay and Argentina [3] that grows associated with the vegetation of riversides. In our studies of the glycosides produced by this plant, two new steroidal saponins were isolated from the aerial parts. This paper describes the isolation and structural elucidation of these compounds.

RESULTS AND DISCUSSION

The material obtained after extraction of the aerial parts of *S. laxum* with ethanol, and evaporation, was partitioned between water and chloroform. In this procedure a layer between the water and chloroform was also formed. This was separated and the residue obtained after concentration was fractionated by medium pressure liquid chromatography (MPLC) using first reverse and then normal stationary phases. By this procedure, two major saponins, named laxumin A (**1**) and laxumin B (**2**), were isolated.

Compound (**1**) was obtained as a yellow powder, $[\alpha]_D^{20}$ −54° (methanol; *c* 0.3). The IR spectrum of **1** exhibited, among other signals, absorption bands due to hydroxyl groups (3417 and 1069 cm^{-1}) [4]. The sugars obtained after acid hydrolysis of **1** were analysed as their alditol acetates by GC–mass spectrometry using authentic samples as references [5]. Glucose, rhamnose and galactose in the relative proportions 2:1:1 were the only sugars detected. The D-configuration of the glucose and galactose and the L-configuration of rhamnose were determined by GC of the trimethylsilylated (+)-2-butyl glycosides [6].

To find the linkage position of the monosaccharide residues, the glycoside was methylated using sodium methylsulphinyl anion and methyl iodide according to Hakomori [7]. The product was hydrolysed, and the partially methylated sugars were reduced with $NaBH_4$ and acetylated. Analysis of the products on GC–mass spectrometry yielded the acetates of 2,3,4-tri-*O*-methyl-L-rhamnitol, 2,3,4,6-tetra-*O*-methyl-D-glucitol, 3,4,6-tri-*O*-methyl-D-glucitol and 3,6-di-*O*-methyl-D-galactitol [8]. These results indicated the presence of terminal rhamnose and glucose groups, a 2-substituted glucose and a 2,4-disubstituted galactose residue.

From the ^{1}H and ^{13}C NMR spectra of **1** information on the respective sugar residues and the anomeric configurations could be obtained. Using different 1D and 2D experiments (H–H COSY, relay and double relay H–H COSY), most of the ^{1}H NMR signals could be assigned and the $J_{1,2}$ values determined (Table 1).

The ^{1}H NMR spectrum of **1** in methanol-d_4 at 30° showed signals corresponding to four anomeric protons at δ 5.14 (J = 1.6 Hz), 4.80 (J = 7.9 Hz), 4.62 (J = 7.6 Hz) and 4.47 (J = 7.6 Hz), confirming the presence of a tetrasaccharide moiety in accordance with the sugar and methylation analysis.

HMQC experiments allowed the assignment of the corresponding carbon signals and the measurement of the $^1J_{C,H}$ values for the respective anomeric carbons (Table 1). This information, together with published

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Table 1. ^{1}H and ^{13}C chemical shifts of laxumins A and B in CD_3OD solutions at 30°

	Laxumin A			Laxumin B		
	δ_H			δ_H		
	α	β	δ_C	α	β	δ_C
1	1.08	1.87	38.6	1.07	1.86	38.7
2	1.91	1.60	30.7	1.89	1.57	30.6
3	3.59		80.0	3.57		80.0
4	2.45	2.29	39.3	2.44	2.28	39.5
5			141.1			141.2
6	5.38		123.2	5.38		123.2
7	1.77	2.38	33.2	1.73	2.36	33.4
8		1.84	30.7		1.84	30.8
9	1.00		51.5	1.00		51.5
10			37.0			36.6
11	1.52	1.56	21.8	1.49	1.60	21.8
12	1.25	1.71	41.7	1.24	1.70	41.7
13			41.7			41.7
14	1.25		61.4	1.24		61.4
15		4.02	80.0		4.00	80.2
16	4.14		91.3	4.14		91.3
17	1.88		61.1	1.88		60.4
18		0.89	17.9		0.88	17.9
19		1.06	19.8		1.05	19.8
20		2.56	36.7		2.56	37.0
21	0.96		14.3	0.96		14.3
22			112.4			112.4
23		3.68	64.0		3.68	64.0
24	1.95	1.69	36.0	1.94	1.69	36.0
25	1.89		30.7	1.89		30.8
26	3.20	3.89	65.1	3.20	3.89	65.1
27		1.10	17.6		1.10	17.6
α-L-Rhamnopyranosyl (1→2						
A						
1	5.14		102.4	5.15		102.4
	(1.6)		(170)	(1.6)		(172)
2	3.94		72.4	3.91		72.2
3	3.68		72.4	3.66		72.4
4	3.40		74.0	3.39		74.0
5	4.18		69.9	4.18		69.8
6	1.28		18.0	1.24		17.9
→2,4)-β-D-Galactopyranosyl (1→						
B						
1	4.47		100.8	4.46		100.9
	(7.6)		(160)	(7.7)		(161)
2	3.75		78.8	3.65		77.6
3	3.68		72.3	3.67		72.4
4	4.01		80.0	4.01		80.2
5	3.53		75.1	3.53		75.3
6/6′	3.57*	3.91*	63.2*	3.62*	3.86*	63.2
→2)-β-D-Glucopyranosyl (1→4				β-D-Glucopyranosyl (1→4		
C						
1	4.62		104.5	4.48		106.4
	(7.6)		(162)	(7.7)		(163)
2	3.67		81.7	3.30		75.7
3	3.62		79.0	3.59		76.5
4	3.22		72.0	3.38		66.8
5	3.38		78.4	3.51		75.3
6/6′	3.60*	3.90*	63.2	3.62	3.86	61.3
β-D-Glucopyranosyl (1→2						
D						
1	4.80		104.3			
	(7.9)		(164)			
2	3.24		71.9			
3	3.69		76.7			
4	3.38		66.8			
5	3.53		75.1			
6/6′	3.63	3.90	61.1			

*Approximate values.

chemical shift data for the sugars [9] and the correlations of the $^{1}J_{C,H}$ values with anomeric configuration [10] allowed the assignment of the different spin-systems to specific sugar residues and the respective anomeric configurations. The data showed that the rhamnose residue was an α-pyranoside whereas the other residues were β-pyranosides.

Information on the sequence of the sugar residues was obtained by inter-residue NOEs (Table 2), i.e. NOE between the anomeric proton of one residue and the proton on the substituted carbon of the next residue, observed as cross-peaks in a NOESY spectrum. Inter-residue NOEs between H-1 (δ 5.14) of the rhamnosyl group and H-2 (δ 3.75) of the 2,4-disubstituted galactose residue, H-1 (δ 4.80) of the terminal glucosyl group and H-2 (δ 3.67) of the 2-substituted glucose residue, indicated that the terminal L-rhamnose and D-glucose groups were substituted at the 2-position of the 2,4-disubstituted D-galactose and the 2-position of the 2-substituted D-glucose residue, respectively.

The FAB mass spectrum of **1** gave a $[M + Na]^+$ ion at *m/z* 1101 and a $[M + H]^+$ ion at *m/z* 1079, indicating that the molecular weight was 1078. The high energy collision induced dissociation (CID) mass spectrum of the $[M + H]^+$ ion at *m/z* 1079 of **1** using He as the collision gas, showed, among others, fragments at *m/z* 917 and 755, corresponding to the successive loss of two hexoses, and an ion at *m/z* 933 corresponding to the loss of the rhamnose group (Fig. 1).

The ^{1}H NMR spectrum of **1** showed, in addition to the signals of the oligosaccharide, signals corresponding to one olefinic proton at δ 5.38, two methyl groups substituted at quaternary carbons at δ 0.89 (*s*) and δ 1.06 (*s*) and three methyl groups substituted at methine carbons at δ 0.96 (*d*, $J = 6.8$ Hz), δ 1.10 (*d*, $J = 6.4$ Hz) and δ 1.28 (*d*, $J = 7.0$ Hz). The ^{13}C NMR spectrum showed signals for 51 carbons, 27 of which arose from the aglycone moiety. The spectrum displayed, among others, signals corresponding to two olefinic carbons at δ 141.1 and 123.2, and a carbon atom at δ 112.4. These data, and comparison with data in the literature [2, 11], suggested that **1** consisted of a Δ^5-spirosten aglycone, which is a structure commonly found in *Solanum* plants, substituted with a tetrasaccharide.

The CID mass spectrum of the $[M + H]^+$ ion at *m/z* 1079 produced by FAB ionization of **1**, showed, *inter alia*, an ion at *m/z* 447, corresponding, according to the nomenclature of Domon and Costello [12], to Y_0 (Fig. 1), i.e. the protonated aglycone moiety. Thus, the molecular weight of the aglycone should be 446, which corresponds to $C_{27}H_{42}O_5$, suggesting the presence of a Δ^5-spirosten skeleton substituted with three hydroxyl groups.

The proton spin-system of the A, B and C rings could be assigned by different H–H correlation experiments (H–H double quantum filtered phase sensitive COSY and relay and double relay COSY). These data, together with the proton–carbon correlation from a ^{13}C-decoupled HMQC experiment and comparison with literature data, allowed the assignment of the carbon signals. H-3 (δ 3.59) was readily identified by its coupling with H-4α (δ 2.45), H-4β (δ 2.29), H-2α (δ 1.91) and H-2β (δ 1.60). The C-2, C-3 and C-4 signals (δ 30.7, 80.0 and 39.3, respectively) are similar to the corresponding values for diosgenin 3-*O*-β-D-galactoside (δ 30.5, 78.4 and 39.6, respectively) [11]. The observed NOE between the anomeric proton of the galactose residue (δ 4.47) and H-3 confirmed that the

Table 2. Selected nuclear Overhauser enhancements (NOE) obtained for laxumins A and B in CD_3OD solutions at 30°

Irradiated proton (δ)	Enhanced protons (δ)
Aglycone (as observed for laxumin A)	
H-7β (2.38)	H-7α (1.77), H-8 (1.84)
H-17 (1.88)	H-14 (1.25), H-16 (4.14), H_3-21 (0.96)
H-18 (0.89)	H-8 (1.84), H-12β (1.71), H-15 (4.02), H-20 (2.56)
H-19 (1.06)	H-1β (1.87), H-2β (1.60), H-4β (2.29), H-8 (1.84), H-11β (1.56)
H-20 (2.56)	H_3-21 (0.96)
H-23 (3.68)	H-24β (1.69), H_3-27 (1.10), H-20 (2.56), H_3-21 (0.96)
H-24β (1.69)	H-24α (1.95)
H-25 (1.89)	H_3-27 (1.10)
Oligosaccharide	
Laxumin A	
A, H-1 (5.14)	**A**, H-2 (3.94); **B**, H-2 (3.75)
B, H-1 (4.47)	**B**, H-2 (3.75); **B**, H-3 (3.68), Aglycone, H-3 (3.59)
C, H-1 (4.62)	**C**, H-3 (3.62); **B**, H-4 (4.01)
D, H-1 (4.80)	**C**, H-2 (3.67)
Laxumin B	
A, H-1 (5.15)	**A**, H-2 (3.91); **B**, H-2 (3.65)
B, H-1 (4.46)	**B**, H-3 (3.67); Aglycone, H-3 (3.57)
C, H-1 (4.48)	**C**, H-3 (3.59); **B**, H-4 (4.01)

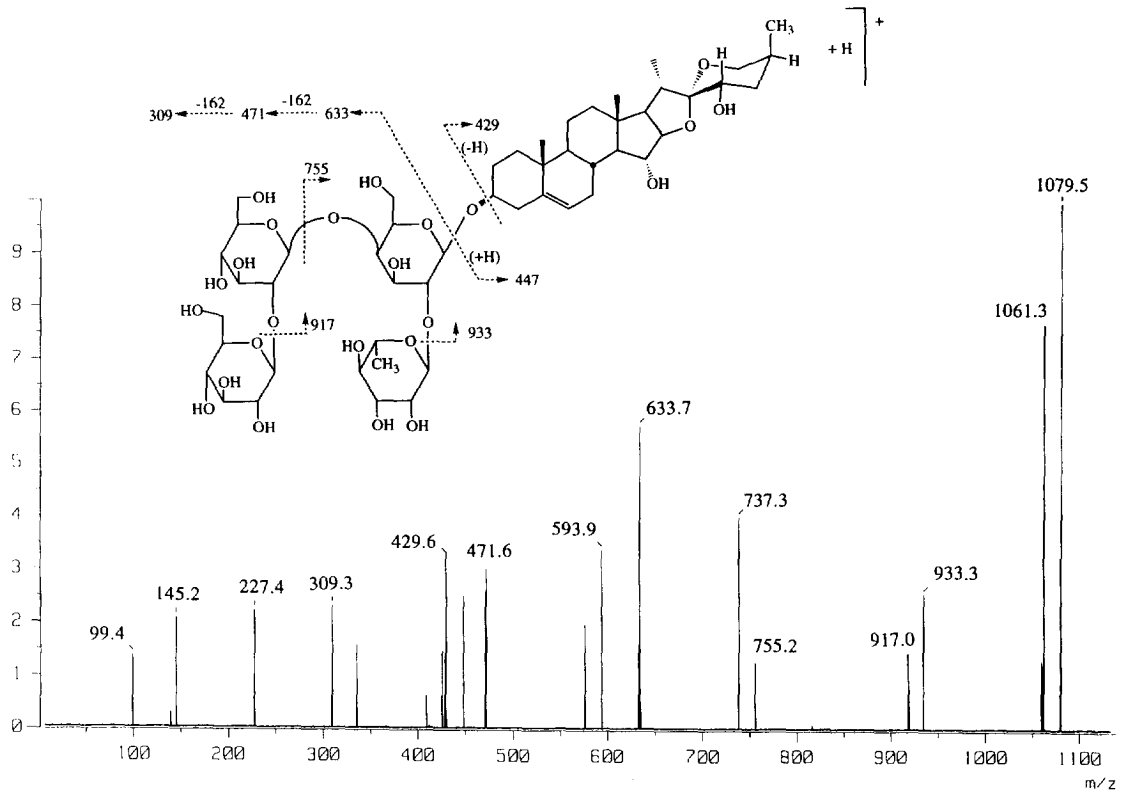


Fig. 1. Collision-induced fragment ions from laxumin A observed in an MS/MS experiment using the $[M + H]^+$ ion at *m/z* 1079, obtained by positive FAB-MS.

tetrasaccharide was linked to the 3-OH of the aglycone. The signals for H-1α (δ 1.08) and H-1β (δ 1.87) were assigned by their coupling with H-2α and H-2β.

The coupling between the signal for the vinylic H-6 (δ 5.38) and H-7β (δ 2.38) an H-7α (δ 1.77) allowed their identification. H-8 (δ 1.84) was identified by its couplings with both H-7 and a cross peak between its signal and the H-6 signal in the relay H–H COSY spectrum. The H-9 signal at δ 1.00 was readily assigned by its coupling with H-8. In a similar way, all the ^{1}H and ^{13}C NMR signals for the A, B and C rings could be assigned (Table 1) and were characteristic for a Δ^5-spirosten system [2, 11]. Thus, the signals for the C-18 and C-19 methyl groups and the quaternary carbons C-5, C-10 and C-13 were assigned by comparison with literature data for similar compounds [2, 11]. The observed NOE between the C-19 methyl protons and H-1β (δ 1.87), H-2β (δ 1.60), H-4β (δ 2.29), H-8 (δ 1.84) and H-11β (δ 1.56) established their β-position.

Furthermore, the spin-system of the protons in the D and E rings could be assigned. The signal at δ 1.25 was assigned to H-14 according to its coupling with H-8. The coupling between H-14 and a proton at δ 4.02 identified the latter as H-15. The chemical shifts for the H-15 and C-15 (δ 80.0) signals indicated the latter to be hydroxylated. The signal at δ 4.14 (*dd*, $J = 9.2$ and 6.4 Hz) was assigned to H-16. This proton is coupled with the protons at δ 4.02 and 1.88, corresponding to H-15 and H-17, respectively. The multiplet at δ 2.56 corresponds to H-20, according to its coupling with H-17 and the C-21 methyl group at δ 0.96.

The stereochemistry of the D and E rings could be determined by the observed NOE between protons, indicating short inter-atomic distances (Table 2 and Fig. 2). From the NOE between the protons of the β-oriented C-18 methyl group and H-8, H-15, H-12β (δ 1.71) and H-20, the β-orientation of these protons was determined. The NOE between the α-oriented C-21 methyl group and H-17 established its α-orientation, and the NOE between the latter and H-16 and

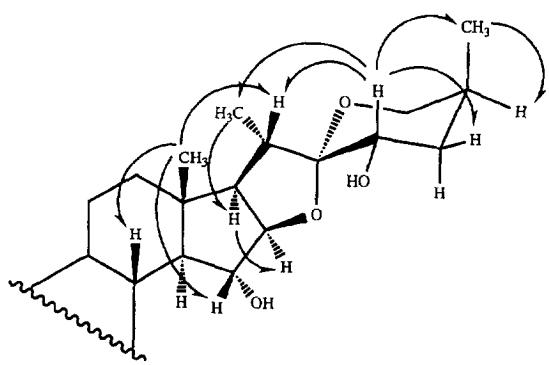


Fig. 2. Selected nuclear Overhauser enhancements (NOE) observed between protons in rings C, D, E and F.

H-14 confirmed their position on the α-side of the molecule.

The continuity of the proton-spin system is broken between rings E and F in the spirostan systems due to the presence of the quaternary C-22 (δ 112.4). The coupling between the proton at δ 3.68 and the protons at δ 1.95 and 1.69 suggested the former to be H-23 and the latter two as H-24α and H-24β, respectively. The H-23 and C-23 chemical shifts (δ 3.68 and 64.0, respectively) indicated that C-23 is substituted with a hydroxyl group. The signal at δ 1.89 was assigned to H-25 due to its coupling with both H-24 and the protons of the C-27 methyl group (δ 1.10). The protons with signals at δ 3.20 and 3.89 are coupled with H-25, and thus were identified as H-26α and H-26β, respectively. These assignments were in accordance also with the geminal coupling and the chemical shift of the corresponding C-26 (δ 65.1) [11], and the chemical shifts indicate that C-26 is substituted with an oxygen.

The CID mass spectrum of the ion at m/z 447 (Y_0), which corresponds to the protonated aglycone (Fig. 3) [13], shows peaks at m/z 429 and 411, formed by loss of one and two molecules of water, respectively. The presence of a hydroxyl group on C-23 is also supported by the ions at m/z 345 and 327 produced by loss of a C_5H_8O fragment from the protonated aglycone ion minus one and two molecules of water, respectively [14]. This fragmentation is indicative of the presence of a hydroxyl substituent on C-23, and the mechanism when the ions are produced by electron-impact has been discussed [14]. We propose a similar mechanism for the fragmentation of the protonated aglycone ion formed during positive FAB mass spectrometry.

The ^{13}C NMR chemical shifts of C-20 to C-27 of **1** are in agreement with the respective values reported for those of paniculogenin [(23*S*,25*S*)-5α-spirostan-3β,6α,23-triol] [15]. In particular, the chemical shift for the C-20 signal supports the (*S*)-configuration at C-22, since in hispigenin, a (22*R*)-isomer, the C-20 signal (δ 43.4) shows a 6 ppm downfield shift compared to the corresponding value in paniculogenin [15]. The appearance of the C-22 signal at δ 112.4 is indicative of an equatorial position of the hydroxyl at C-23. The chemical shift for the C-25 signal (δ 30.7) is also indicative of the axial position of the C-27 methyl group [15].

The relative *cis*-configuration of H-23 and the C-27 methyl group is confirmed by the observed NOE between them (Table 2 and Fig. 2). The observed NOE from H-23 (δ 3.68) to H-20 (δ 2.56) and H_3-21 (δ 0.96) is also in agreement with this configuration, because it implies short distances between them.

All the above data identified **1** as (23*S*,25*S*)-spirost-5-en-3β,15α,23-triol 3-*O*-{β-D-glucopyranosyl-(1 $\rightarrow$ 2)-β-D-glucopyranosyl-(1 $\rightarrow$ **4**)-[α-L-rhamnopyranosyl-(1 $\rightarrow$ 2)]-β-D-galactopyranoside}.

Compound **2** was a less polar component than **1** as indicated by its higher R_f on silica gel TLC. It was obtained as a white powder with $[\alpha]_D^{20}$ $-74°$ (methanol, *c* 0.3). The IR spectrum exhibited, among others, strong

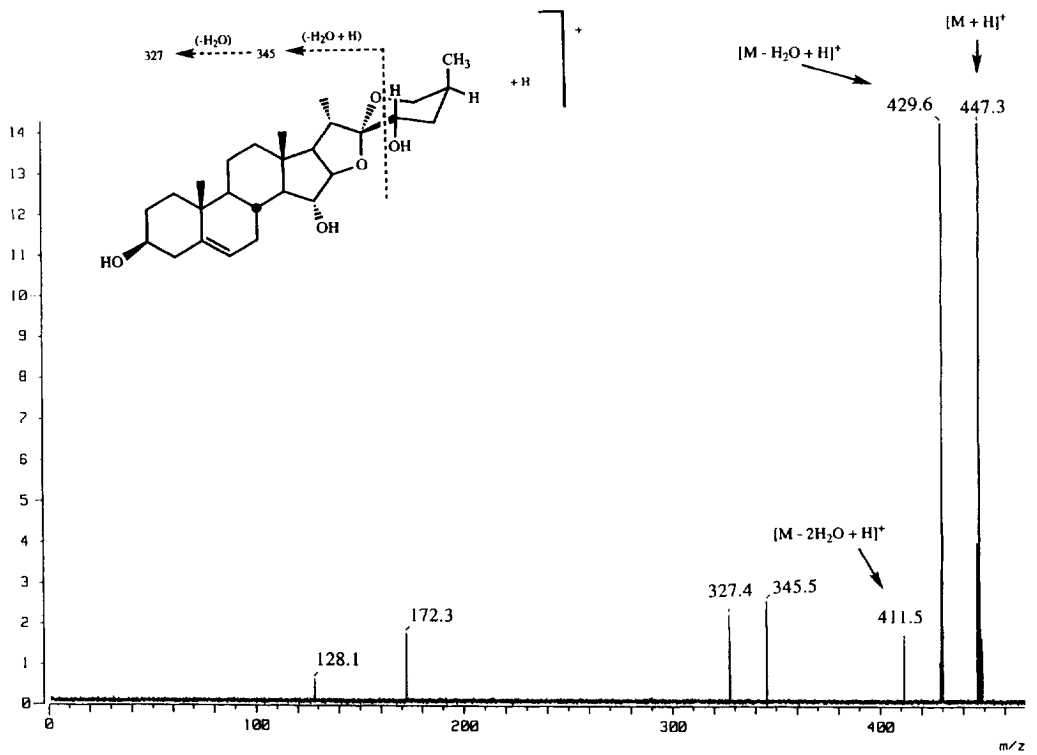


Fig. 3. Collision-induced fragment ions from laxumin A observed in an MS/MS experiment using the $[M + H]^+$ ion at m/z 447, obtained by positive FAB-MS.

absorption bands corresponding to hydroxyl groups (3403 and 1063 cm^{-1}) [4].

Acid hydrolysis of **2**, reduction of the released sugars with $NaBH_4$, acetylation and GC–mass spectral analysis of the resulting alditol acetates gave galactose, glucose and rhamnose in a 1:1:1 proportion. The D-configuration of the glucose and galactose and the L-configuration of the rhamnose were determined by GC of the trimethylsilylated (+)-2-butyl glycosides [6].

To find the linkage position of the monosaccharide residues, a methylation analysis was performed on **2** as described for **1**. GC–mass spectral analysis yielded the acetates of 2,3,4-tri-*O*-methyl-L-rhamnitol, 2,3,4,6-tetra-*O*-methyl-D-glucitol and 3,6-di-*O*-methyl-D-galactitol [8]. These results indicated the presence of terminal rhamnose and glucose groups and a 2,4-disubstituted galactose residue.

The MALDI-TOF spectrum gave a $[M + Na]^+$ ion at *m/z* 939, and the positive FAB mass spectrum of the saponin gave the $[M + Na]^+$ ion at *m/z* 939 in addition to the $[M + H]^+$ ion at *m/z* 917, confirming the molecular weight of the saponin as 916.

The CID mass spectrum of the $[M + H]^+$ ion at *m/z* 917 (Fig. 4), produced by FAB ionization, showed fragments at *m/z* 771, 755 and 447, corresponding to the loss of a rhamnose group, a glucose group and the protonated aglycone, respectively. It also showed a peak at *m/z* 471, corresponding to the trisaccharide moiety.

From the ^1H and ^{13}C NMR spectra of **2**, information on the respective sugar residues and the anomeric configurations could be deduced. The ^1H NMR spectrum of **2** in methanol-d_4 at 30° showed signals corresponding to three anomeric protons at δ 5.15 (J = 1.6 Hz), 4.48 (J = 7.7 Hz) and 4.46 (J = 7.7 Hz). Analogously, as in the case of **1**, all the ^1H and ^{13}C signals could be assigned as well as the corresponding $^1J_{C,H}$ values for the anomeric carbons (Table 1). These data indicated that the rhamnose group was α-pyranosidic whereas the other residues were β-pyranosides.

Inter-residue NOEs indicated the substitution positions of the rhamnose and glucose groups. Inter-residue NOEs were observed between H-1 (δ 5.15) of the rhamnose group and H-2 (δ 3.65) of the 2,4-disubstituted galactose residue (Table 2).

The ^1H and ^{13}C NMR data showed that the aglycone moiety of **2** was identical to the aglycone moiety of **1** (Table 1). Thus, the total structure of **2** was established as (23*S*,25*S*)-spirost-5-en-3β,15α,23-triol 3-*O*-{β-D-glucopyranosyl - (1 → 4) - [α - L - rhamnopyranosyl - (1 → 2)]-β-D-galactopyranoside.

Δ^5-Spirosten saponins are common in plants of the *Solanum* genus and several carrying a hydroxyl group at C-15 or C-23 have been found [11]. A saponin with a

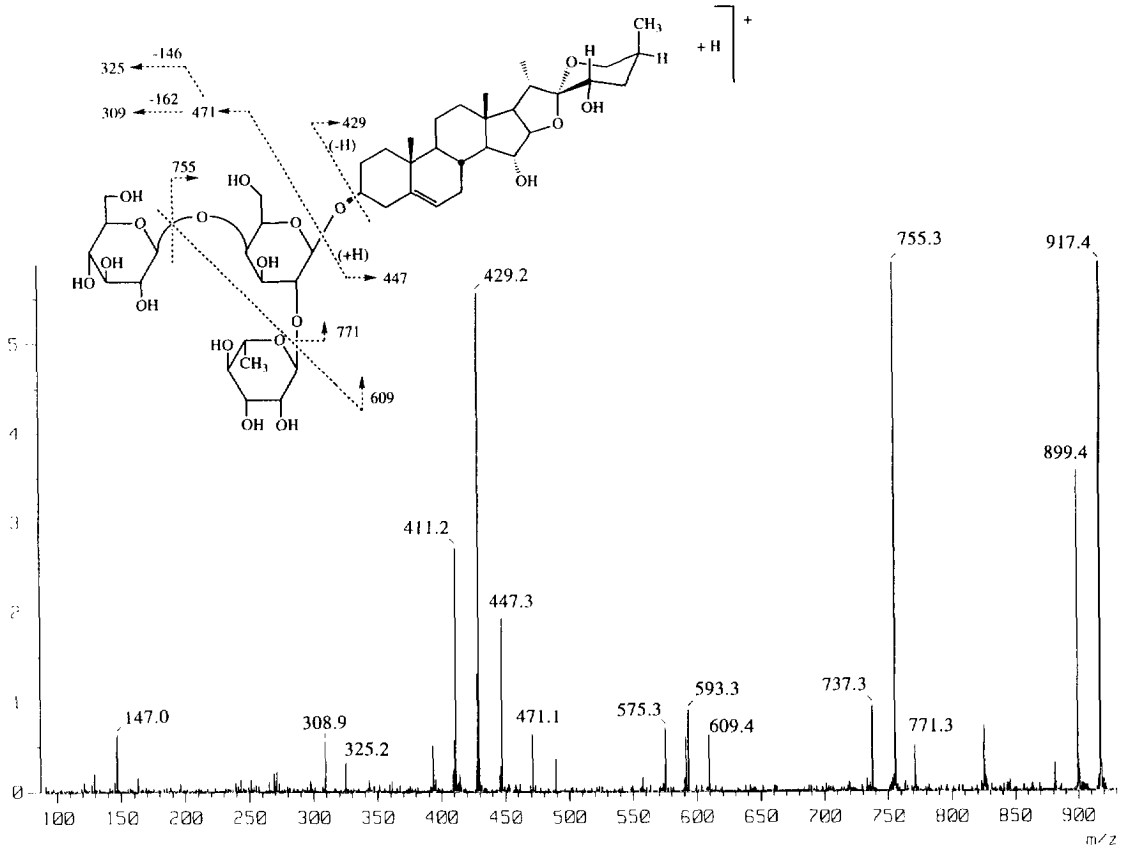
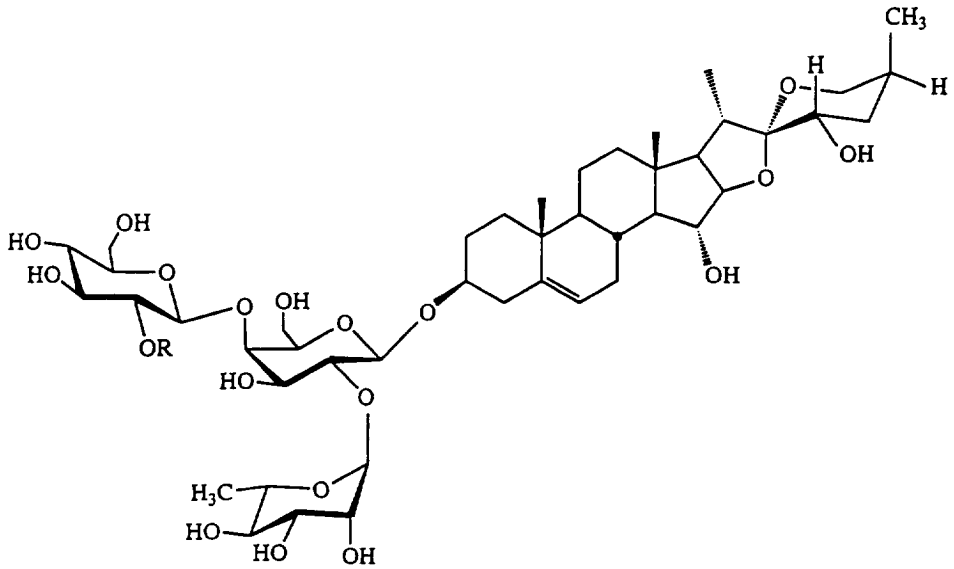


Fig. 4. Collision-induced fragment ions from laxumin B observed in an MS/MS experiment using the $[M + H]^+$ ion at *m/z* 917, obtained by positive FAB-MS.



		R
1:	**Laxumin A**	**β-D-Glucopyranosyl**
2:	**Laxumin B**	**H**

similar aglycone, but with different configuration at C-25 and a different glycosidic moiety, has recently been described [16]. Also, the C-15,C-25 epimer, anosmagenin [(23*S*,25*R*)-spiro-5-en-3β,15β,23-triol], has previously been isolated from *S. vespertillo* [17]. In spite of the amount of research work that has been done on secondary metabolites from *Solanum* plants, they continue to render new structures, assuring its richness as a source of novel natural products.

EXPERIMENTAL

General. Solns were concd under red. pres. at temps not exceeding 40°. For GC, a Hewlett-Packard 5890 instrument fitted with a FID was used. Sepn of the alditol acetates and the partially methylated alditol acetates was performed on an HP-5 fused-silica capillary column, using a temp. programme from 140° (3 min) to 240° at 3° min^{-1}. Electron impact ionization (EI, 70 eV) GC-MS was performed using the column and conditions mentioned above.

FAB-MS were recorded on a Jeol JMS-SX/SX-102A tandem mass spectrometer by bombardment of samples (dissolved in glycerol matrix) with Xe atoms of average translational energy 6 keV. The instrument was operated at an accelerating voltage of 10 kV. MS/MS was conducted using the first two sectors (B_1E_1) to select the precursor ions and the second mass spectrometer (B_2E_2) to analyse product ions. A resolution of *ca* 3.000 was used to separate the ^{12}C peak of the $[M+H]^+$ precursor ions. He was used as collision gas at a pressure sufficient to attenuate the precursor ion beam by *ca* 50%. The MALDI-TOF spectra were recorded using a 339 nm N laser and 2,5-dihydroxybenzoic acid as matrix. For better precision of the ions a starch hydrolysate was used as int. standard.

^1H and ^{13}C NMR spectra were obtained at 400 and 100 MHz, respectively, at 30°. CD_3OD was used as solvent. Chemical shifts are reported in ppm, using TMS (δ_H 0.00) and Me_2CO (δ_C 31.00) as int. references. 2D (COSY, relay COSY, NOESY and HMQC) experiments were performed according to standard pulse sequences. A 90° pulse was used in correlation experiments, and in the NOESY experiment mixing times of 0.1 and 0.3 sec.

Plant material. Aerial parts of *S. laxum* Steud. were collected on the Santa Lucía River banks in December 1992. A voucher specimen was deposited at the Facultad de Química Herbarium, Montevideo, under catalogue no. MVFQ 1057 leg. E. Alonso Paz.

Extraction and isolation of the plant material. Aerial parts of *S. laxum* (1.5 kg) were chopped and extracted with EtOH (5 l) at room temp. for 7 days. After filtration and evapn of the solvent to near dryness, a dark green extract (92 g) was obtained.

Part of this extract (70 g) was suspended in H_2O (150 ml), filtered and defatted with $CHCl_3$ (3 × 100 ml). At the H_2O–$CHCl_3$ interface a third layer was detected, which was sepd and evapd to dryness.

Part of this extract (1.0 g) was fractionated by MPLC on a reverse phase column (100 × 5.0 cm, C_{18}; Merck), using the following H_2O–MeOH step gradients: 2:3

(1 l, frs 1–60), 3:7 (1.5 l, frs 61–161) and 100% MeOH (1 l, frs 162–230). After evapn of solvents, the frs were analysed by TLC and ^{1}H NMR spectroscopy. Frs 48–81 gave different mixts of saponins. Frs 48–53 (52 mg) were mixed and redissolved in MeOH, and applied to a MPLC silica gel column (30 × 2 cm) eluted with $CHCl_3$–MeOH–0.5% NH_4OH (14:6:1). A pure saponin, **1** (16 mg), $[\alpha]_D^{20}$ −54° (MeOH, *c* 0.3) was isolated. Frs 54–57 (61 mg) were pooled together, and were subjected to the same MPLC purification procedure as before, yielding pure **2** (23 mg) $[\alpha]_D^{20}$ −74° (MeOH, *c* 0.3).

Hydrolysis of the glycosides and sugar analysis. The saponins (1–3 mg) were hydrolysed with 1 M HCl in MeOH at 60° for 2 hr, then the solvents were evapd and the residues suspended in 0.1 M HCl and heated at 60° for 1 hr. After neutralization with $NaHCO_3$ the products were distributed between H_2O–$CHCl_3$. TLC ($CHCl_3$–MeOH, 9:1) of the $CHCl_3$ phase showed the same product from both saponins.

The aq. layer was freeze-dried, part of the material reduced with $NaBH_4$ (10 mg) in 1 M NH_4OH (1 ml) for 30 min at room temp. Excess $NaBH_4$ was quenched with HOAc, and the boric acid removed by co-distillation with MeOH (3 × 1 ml). The resulting alditols were acetylated with Ac_2O–pyridine (1:1, 1 ml) at 120° for 30 min and analysed by GC-MS using authentic samples as standards [5]. The other part was treated with 1 M HCl in (+)-2-BuOH (200 ml) at 80° for 8 hr in a sealed tube. The mixt. was then evapd to dryness, trimethylsilylated with SIL A (Supelco, 0.2 ml) for 30 min at room temp., concd to dryness, dissolved in *n*-hexane, filtered, and analysed by GC as described [6].

Methylation analysis. Samples (1 mg) dissolved in DMSO were methylated using Na methylsulphinyl anion and CH_3I according to ref. [7], with the procedure suggested in ref. [8]. The permethylated products were isolated using a Sep-Pak C_{18} cartridge [18] and analysed as described [8].

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