A Diosgenin Tetrasaccharide from *Cestrum nocturnum*

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A new monodesmosidic spirostanol glycoside extracted from the fresh leaves of *Cestrum nocturnum* was identified as spirost-5-ene-3-ol, [3β,25*R*]-3-O-[{α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)}-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside. The name proposed for this saponin is nocturnoside B. The structure elucidation was done on the basis of 1D and 2D spectroscopic studies [DEPT, HeteroCOSY, HMBC, COSY-45°, HOHAHA] and chemical analysis.

Introduction

In a previous paper [1], we reported the isolation and structure elucidation of a new spirostanol saponin, nocturnoside A, from the fresh leaves of *Cestrum nocturnum.* This is an ornamental plant cultivated in Pakistan for its fragrant flowers [2]. As its flowers are fragrant during the night only, locally it is known as "raat ki raani" (lady of the night). The studies on the odoriferous volatile constitutents of the flowers have been carried out. Olfactory characterization of the head space volatiles showed that the main contributors to the characteristic flower fragrance are phenylacetaldehyde and linaloöl [3].

In our continuing survey of the constituents of *Cestrum* species, we report the isolation and characterization of a new saponin, a diosgenin tetrasaccharide named nocturnoside-B (**1**). A combination of different spectroscopy techniques have been applied to arrive at a conclusive structure; especially by the use of 2D-NMR techniques – 2D-*J*-Resolved, ^{1}H–^{1}H COSY, HOHAHA (also called TOCSY), ^{1}H–^{13}C COSY (correlation *via* one bond coupling) and HMBC (correlation *via* two or three bond coupling, *i.e.* heteronuclear multiple bond connectivity) experiments. The structure was further confirmed by chemical analysis. **1** was subjected to acid hydrolysis. The analysis of the hydrolysate indicated the presence of rhamnose and glucose; whereas the non-aqueous part yielded the algycone which was identified as (25*R*)-spirost-5-ene-3β-ol, *i.e.* diosgenin. ^{1}H and ^{13}C NMR spectral assignments have been made partly through a comparison of chemical shifts with the published data for similar compounds [4–8] and partly through DEPT, ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY experiments. These were then confirmed by the HOHAHA and HMBC experiments.

Results and Discussion

The methanolic extract of fresh leaves of *C. nocturnum* was suspended in water and successively extracted with ethyl acetate and *n*-butanol. The *n*-butanol soluble phase was fractionated by vacuum liquid chromatography on silica gel, Merck Art No. 7747. Slightly impure **1** was obtained from chloroform-methanol (85:15). This was further purified by recrystallization with cold methanol. Finally, fine needle like white crystals (69.4 mg) were obtained. The positive ion FAB-mass spectrum of **1** showed molecular ion peak at *m/z* 1015.5520 (calculated for $C_{51}H_{83}O_{20}$, 1015.5477) $[M+H]^+$. The fragment ions at *m/z* 869 $[M+H-146]^+$, 723 $[M+H-(2\times146)]^+$, 577 $[M+H-(3\times146)]^+$ indicated the successive loss of three deoxyhexose units which are either linked in a straight chain to an inner hexose or a branched chain with at least two deoxyhexoses in terminal positions. The fragment at *m/z* 577 is the genin + one hexose moiety. The fragment ion at *m/z* 415 $[M+H-(3\times146)-162]^+$ shows the loss of the inner most hexose along with the three deoxyhexoses;

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and this fragment is due to the [genin + H]$^+$ moiety. There is also a fragment ion at *m/z* 397 [genin + H−(H_2O)]$^+$; which depicts the loss of a water molecule from the genin fragment. The negative ion FAB-mass spectrum, however, exhibited only three peaks, the pseudomolecular ion peak at *m/z* 1013.5330 (calculated for $C_{51}H_{81}O_{20}$, 1013.5321) [M−H]$^-$, and two fragment ions at *m/z* 867 [M−H-146]$^-$ and *m/z* 721 [M−H−(2×146)]$^-$. These showed the loss of one and two deoxyhexoses respectively. Thus the presence of three deoxyhexoses, either in a straight chain or branched chain with two terminal ones, and an inner most hexose was confirmed. Two saponins corresponding to the molecular formula $C_{51}H_{82}O_{20}$ have been reported in literature [5, 9, 10]. They are diosgenin 3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside, m.p. 203–206° (decomp) [5, 9], $[\alpha]_D$ = −153.2°, $[\alpha]_D$ = −113.4° (MeOH, *c* 0.57), $[\alpha]_D$ = −136.2° (pyridine, *c* 0.89) [5], $[\alpha]_D$ = −128.0° (MeOH; *c* 0.50) [9] and kallstroemin-D which is diosgenin 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside, m.p. 275–276 °C $[\alpha]_D$ = −94.5° (pyridine) [10]. Both have the same aglycone and identical sugar groups as in nocturnoside-B, but the difference lies in the sequence of the oligosaccharide moiety. The IR spectrum exhibited the presence of hydroxyl group(s), as a very strong band at 3410 cm^{-1} was observed. The bands at 890 and 910 cm^{-1} indicated the presence of spirostan skeleton. The relative intensities of the two IR bands, 890 > 910 cm^{-1} revealed the 25*R* stereochemistry [11]. The C=C absorption was observed at 1440 cm^{-1}. The ^{1}H NMR spectrum revealed the presence of two tertiary methyl groups (δ_H 0.82 and 1.04) and five secondary methyl groups (δ_H 0.69 d, *J* = 5,77 Hz; 1.12 d, *J* = 6.95 Hz; 1.57 d, *J* = 6.3 Hz; 1.59 d, *J* = 6.3 Hz and 1.77 d, *J* = 6.3 Hz), one trisubstituted olefinic proton (δ_H 5.31 dist. t) and four anomeric protons doublets (δ_H 4.93, *J* = 6.5 Hz; 5.81, *J* = 2.8 Hz; 6.26, *J* = 3.6 Hz and 6.37, *J* = 3.6 Hz). The spin coupling constant values indicated one β-linkage and three α linkages for anomeric configurations. In the ^{13}C NMR spectrum of **1** (Table I), there were signals corresponding to two olefinic carbons (δ_c 140.7 and 121.6) and four anomeric carbons between 100.21 and 103.12; one spiroketal quaternary carbon was also discernible at δ 109.1. In the DEPT experiment, seven methyl carbons could be distinguished. Among them, there were two tertiary methyl groups (δ_c 16.1 and 19.2), whereas five were secondary methyls (δ_c 14.8, 17.1, 18.2, 18.4 and 18.6). Thus the use of ^{13}C NMR, BB and DEPT experiments helped in the assignment of the multiplicities of the intact saponin. A total of 27 carbon signals were from the aglycone moiety and 24 carbons were from the oligosaccharide moiety. The 27 carbons comprising four methyl, eight methylene, eleven methine and four quaternary carbons were distinguished. These signals were in good agreement with those of diosgenin *i.e.* (25*R*)-spirost-5-en-3β-ol [4–7]. The sugar moiety is attached to the hydroxyl group at C-3 position, and this was supported by the ^{13}C NMR glycosidation shifts. The signal attributable to C-3 position in diosgenin (δ_c 71.5) [6] was shifted downfield by 7.3 ppm in **1.** Thus C-3 resonated at δ_c 77.8 due to the linkage of oligosaccharide moiety at this point. Simultaneously an upfield shift of 3.8 ppm was observed at C-4 (δ_c 38.8), whereas C-2 (δ 29.9) was shielded by 1.7 ppm. In the aglycone diosgenin, C-2 and C-4 resonate at δ_c 42.2 and 31.6 respectively. A greater effect on C-4 was due to the pro-*S* relationship with the sugar as compared with C-2 pro-*R* relationship [12]. Thus **1** is a monodesmosidic saponin. The characteristic shieldings for C-23, C-24, C-25, C-26 and C-27 were at δ_c 31.6, 29.0, 30.4, 66.7 and 17.1 respectively. The *R*-orientation of the C-27 methyl is confirmed by its signal at δ_c 17.1 [13]. It is observed that the shielding of all carbons in ring F are in accordance with the equatorially oriented C-27 methyl group. For the identification of monosaccharides, acid hydrolysis of **1** was performed with 20% HCl. The sugars identified were glucose and rhamnose, and the aglycone as diosgenin. The latter was identified by co-tlc with an authentic sample as well as by EI-MS, *m/z* 396 [M−H_2O]$^+$. The four anomeric carbon signals at δ_c 103.12, 102.08, 102.02 and 100.21 confirmed the presence of a tetrasaccharide moiety. The remaining 20 carbon signals comprised three methyl, one methylene and sixteen methine carbons. Then completion of the structure assignment required ascertaining the linkage points and sequence of the monosaccharide units. Initially in the ^{13}C

Table I. ^{13}C (100 MHz) and ^{1}H (400.13 MHz) spectral data of nocturnoside-B from one and two-dimensional experiments (in pyridine-d_5).

	C	^{13}C (δ)	DEPT	^{1}H (δ) chemical shift	$^{1}H-^{1}H$ connectivity	HOHAHA[a]
	1	37.3	Ch_2	1α 0.99	(1β) 1.70, (2α) 1.84, (2β) 2.05,	
				1β 1.70 m	(1α) 0.99, (2α) 1.84, (2β) 2.05	
	2	29.9	CH_2	2α 1.84	(1α) 0.99, (2β) 2.05	
				2β 2.05	(1α) 0.99, (1β) 1.70, (2α) 1.84	
	3	77.9	CH	3α 3.85 m	(2α 1.84, (2β) 2.05, (4α) 2.72, (4β) 2.79, (H-6) 5.3	5.3, 3.85, 2.79, 2.72, 2.05, 1.70, 0.99
	4	38.8	CH_2	4α 2.72	(3α) 3.85, (4β) 2.79, (H-6) 5.3	
				4β 2.79	(3α) 3.85, (4α) 2.72, (H-6) 5.3	
	5	140.7	C	–	–	
	6	121.6	CH	5.3 dist. t	(4α) 2.72, (7α) 1.82, (7β) 1.87, (8β) 1.50	5.3, 3.85, 1.87, 1.82, 1.50, 1.04, 0.92
	7	32.1	CH_2	7α 1.82	(H-6) 5.3, (7β) 1.87, (8β) 1.50	
				7β 1.87	(H-6) 5.3, (7α) 1.82, (8β) 1.50	
	8	31.5	CH	8β 1.50		
	9	50.1	CH	9α 0.92	(7α) 1.82	
	10	36.9	C	–		
	11	20.9	CH_2	1.42		
	12	39.7	CH_2	12α 1.65		
	13	40.3	C	–	–	
	14	56.4	CH	14α 1.02	(15α) 2.01, (15β) 1.45	
	15	32.04	CH_2	15α 2.01	(14α) 1.02. (15β) 1.45, (16α) 4.53	
				15β 1.45	(14α) 1.02, (15α) 2.01, (16α) 4.53	
	16	80.9	CH	16α 4.53 q like	(15α) 2.01, (15β) 1.45, (17α) 1.78	4.53, 2.01, 1.94, 1.78, 1.45, 1.12, 1.02
	17	62.7	CH	17α 1.78	(16α 4.53	
	18	16.1	CH_3	0.82 s		
	19	19.2	CH_3	1.04 s		
	20	41.8	CH	20β 1.94 t (J = 6.69 Hz)	(H_3-21) 1.12	
	21	14.8	CH_3	1.12 d (J = 6.95 Hz)	(20β) 1.94	
	22	109.1	C	–	–	
	23	31.6	CH_2	23α 1.67	(23β) 1.55	
				23β) 1.55	(23α) 1.67	
	24	29.0	CH_2	1.52	(25β) 1.61	
	25	30.4	CH	25β 1.61	(H_2-24) 1.52, (26α) 3.58, (26β) 3.48, (H_3-27) 0.69	
	26	66.7	CH_2	26α 3.58	(H_2-24) 1.52, (25β) 1.61, (26β) 3.48	3.58, 1.61, 1.55, 1.52, 0.69
				26β 3.48	(H_2-24) 1.52, (25β) 1.61, (26α) 3.58	
	27	17.1	CH_3	0.69 d (J = 5.77 Hz)	(25β) 1.61, (26β) 3.48	
Glc	1	100.2	CH	4.93 d (J = 6.5 Hz)	(H-2) 4.41	4.93, 4.41, 4.38, 4.19, 4.16, 4.03, 3.6
	2	80.2	CH	4.41 t (J = 9.9 Hz)	(H-1) 4.93	
	3	77.5	CH	4.19		
	4	77.6	CH	4.38 br s		
	5	76.8	CH	3.6		
	6	61.0	CH_2	H_a 4.03 dd (J = 3.25, 12.18 Hz)	(H-5) 3.6, (H_b-6) 4.16	
				H_b 4.16	(H-5) 3.6 (H_a-6) 4.03	
Rha-I	1′	103.1	CH	6.26 d (J = 3.6 Hz)	(H-2′) 4.87	6.26, 4.87, 4.62, 4.36, 4.26, 1.59
	2′	72.7[b]	CH	4.87 t (J = 1.64 Hz)	(H-1′) 6.26	
	3′	73.1	CH	4.62 dd (J = 3.41, 9.25 Hz)		
	4′	77.8	CH	4.36		
	5′	70.2	CH	4.26	(H_3-6′) 1.59	
	6′	18.2	CH_3	1.59	(H-5′) 4.36	

Table I (continued).

	C	^{13}C (δ)	DEPT	^{1}H (δ) chemical shift	$^{1}H-^{1}H$ connectivity	HOHAHA[a]
Rha-II	1″	102.08	CH	6.37 d (J = 3.6 Hz)	(H-2″) 4.83	6.37, 4.95, 4.90, 4.83, 4.34, 1.77
	2″	72.7[b]	CH	4.83 dd (J = 1.54, 3.39 Hz)	(H-1″) 6.37	
	3″	72.4	CH	4.90		
	4″	74.0	CH	4.34 br s		
	5″	69.3	CH	4.95 q	(H_3-6″) 1.77	
	6″	18.4	CH_3	1.77 d (J = 6.3 Hz)	(H-5″) 4.95	
Rha-III	1‴	102.02	CH	5.81 d (J = 2.8 Hz)	(H-2‴) 4.52	5.81, 4.89, 4.52, 4.43, 1.57
	2‴	72.7 b	CH	4.52	(H-1‴) 5.81	
	3‴	72.3	CH	4.80		
	4‴	73.8	CH	4.43 d (J = 9.3 Hz)		
	5‴	68.1	CH	4.89 q	(H_3-6‴) 1.57	
	6‴	18.6	CH_3	1.57 d (J = 6.3 Hz)	(H-5‴) 4.89	

[a] HOHAHA Experiment was performed at 500.13 MHz; [b] repeated assignment.

NMR spectrum of **1,** it was obvious that all the carbon signals due to the sugar moiety as well as the aglycone were almost the same as for the published data [9]. However, the 2D NMR experiments helped in deciphering the finer differences in nocturnoside-B as compared to the two known saponins [5, 10]. The $^{1}H-^{13}C$ COSY spectrum enabled the one bond correlation between most protons and carbons in **1.** Subsequently, the COSY-45° experiment (Table I), helped in the confirmation of these assignments. The signal for the vinylic H-6 proton appeared at δ_H 5.3 and this was coupled to H-7α and H-7β at δ_H 1.82 and 1.87 respectively. Also homoallylic couplings with H-8β at δ_H 1.50 as well as with the geminal protons H-4α, H-4β at δ_H 2.72 and 2.79 respectively, could be easily discerned. The latter showed couplings with H-3α at δ_H 3.85. The H-3α in turn exhibited cross-peaks with the geminal protons H-2α and H-2β at δ_H 1.84 and 2.05 respectively (values interchangeable). The H-16α at δ_H 4.53 showed cross-peaks with H-17α at δ_H 1.78 and H-15α at δ_H 2.01, as well as with H-15β at δ_H 1.45. The H-17α in turn showed connectivity with H-15β (δ 1.45) and H-20β (δ 1.94). The latter correlated with H_3-21 (δ 1.12); thus confirming their respective assignments. A set of COSY-45° geminal interactions was observed between the H_2-26 methylenic protons at δ_H 3.58 (H-26α) and δ_H 3.48 (H-26β); both of which in turn showed cross-peaks with H_3-27 at δ_H 0.69, H-25β at δ_H 1.61 and H-23β at δ_H 1.55. The H-26α (δ_H 3.58) and H_3-27 (δ_H 0.69), both showed correlations with H_2-24 (δ_H 1.55). In the oligosaccharide moiety, the four anomeric protons at δ_H 4.93 (glc H-1), 5.81 (rha III H-1‴), 6.26 (rha I H-1′) and 6.37 (rha II H-1″) showed cross-peaks with their vicinal protons glc H-2, rha III H-2‴, rha I H-2′ and rha II H-2″ at δ_H 4.41, 4.52, 4.87 and 4.83, respectively. Similarly, methylenic protons glc H_2-6 at δ 4.03 and 4.16 exhibited geminal coupling as well as cross-peaks with glc H-5 (δ_H 3.60). A set of very diagnostic connectivities were observed between the proton resonances assigned to rha I H_3-6′ (δ_H 1.59), rha III H_3-6‴ (δ_H 1.57) and rha II H_3-6″ (δ_H 1.77) and their respective H-5 protons at δ_H 4.36, 4.89 and 4.95. Similarly, other cross-peaks, where ever distinguished have been recorded (Table I). These assignments were further delineated by the Homonuclear Hartmann-Hahn sequence (HOHAHA) (Table I).

The values assigned on the basis of one bond $^{1}H-^{13}C$ COSY and $^{1}H-^{1}H$ COSY-45° experiments were further confirmed in the long range $^{13}C-^{1}H$ (HMBC) experiment. In the aglycone moiety, the protons resonating at δ_H 1.04 (H_3-19) exhibited correlations with δ_c 37.3 (C-1), 140.1 (C-5) and 50.1 (C-9). also the value δ_H 5.31 assigned to the vinylic proton H-6 showed connectivity with δ_c 38.8 (C-4), 32.1 (C-7) and 36.9 (C-10). The δ_H 1.65 (H-12) showed connectivity with δ_c 56.4 (C-14). The δ_H 1.45 (H-15) and 1.12 (H_3-21) showed couplings with δ_c 80.9 (C-16). The δ_H 4.53 (H-16α) manifested couplings with δ_c 40.2 (C-13) and 41.8 (C-20). The δ_H 0.82 (H_3-18) exhibited correlations with δ_c 40.3 (C-13), 56.4 (C-14)

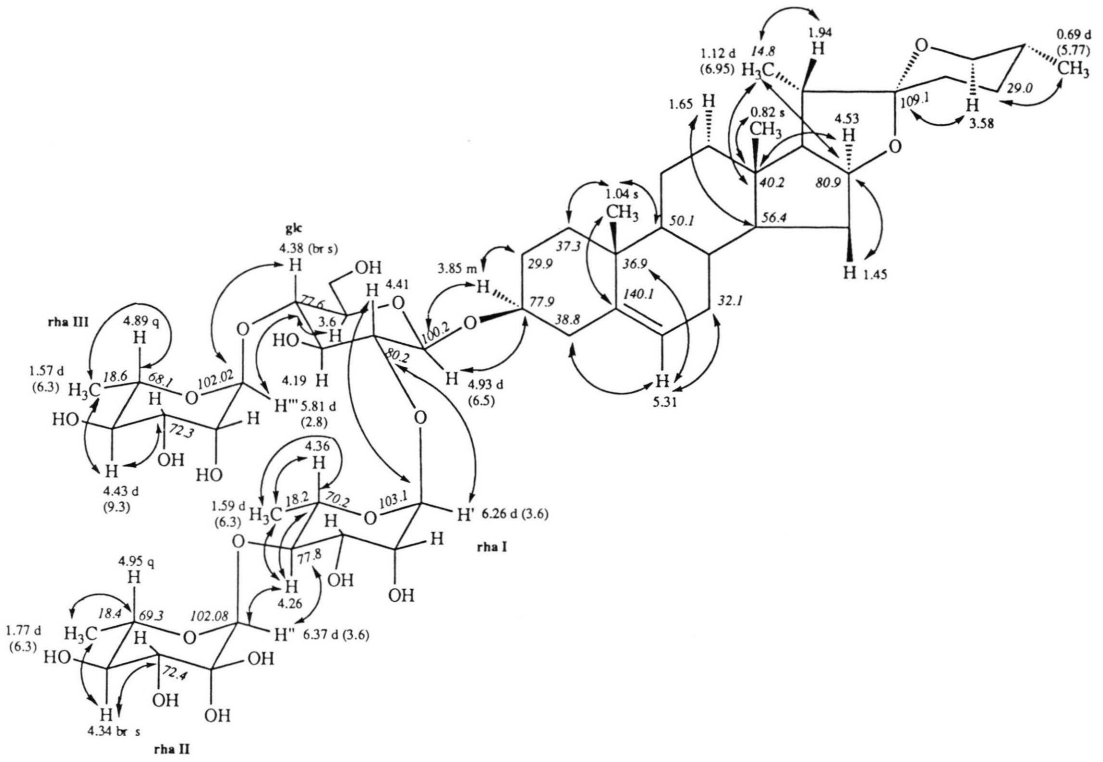

Fig. 1. Heteronuclear multiple bond connectivities of **1** (500.13 MHz, pyridine-d_5). *J* values (Hz) in the ^{1}H NMR are given in parentheses. The ^{13}C NMR chemical shifts are indicated in *italics*.

and 62.7 (C-17). The δ_H 1.94 (H-20β) correlated with δ_c 14.8 (C-21), 40.2 (C-13) and 109.1 (C-22). The δ_H 0.69 (H_3-27) showed connectivity with δ_c 29.0 (C-24). Also δ_H 3.58 (H-26α) exhibited coupling with δ_c 109.1, resonance due to the quaternary C-22. The HMBC experiment proved invaluable not only for further confirming the values assigned to the aglycone (Fig. 1), but also helped especially in ascertaining the linkage points in the oligosaccharide moiety. These correlations were observed between terminal rha II H-1″ (δ_H 6.37) and inner monosaccharide rha I C-4′ (δ_c 77.8), between the second terminal rha III H-1‴ (δ_H 5.81) and inner most monosaccharide glc C-4 (δ_c 77.6) and finally inner rha I H-1′ (δ_H 6.26) exhibited long range connectivity with glc C-2 (δ_c 80.2). Also long range correlation was observed between glc H-1 (δ_H 4.93) and C-3 of diosgenin (δ_c 77.9). The δ_H 3.85 (H-3α) of diosgenin exhibited three bond coupling with the anomeric carbon of glucose at δ_c 100.2. Further proofs of the sequence were deduced on the basis of connectivities between δ_c 102.02 (rha III C-1‴) and δ_H 4.38 (glc H-4), δ_c 102.08 (rha II C-1″) with δ_H 4.26 (rha I H-4′) and also δ_c 103.1 (rha I C-1′) with δ_H 4.41 (glc H-2). This allowed, to the best of our observation, a correct elucidation of the sequence in the oligosaccharide moiety. The HMBC also revealed cross-peaks between the very diagnostic resonances within the individual monosaccharide units. Thus the two bond connectivities were observed in rha III; H_3-6‴ (δ_H 1.57) exhibited cross-peaks with C-5‴ (δ_c 68.1), and the H-4‴ (δ_H 4.43) with C-6‴ (δ_c 18.6) and also with C-3‴ (δ_c 72.3). Within rha II unit, three bond connectivities were observed between H_3-6″ (δ_H 1.77) with C-5″ (δ_c 69.3). Similarly the H-4″ (δ_H 4.34) showed cross-peak with C-6″ (δ_c 18.4). This proton in turn showed two bond connectivity with C-3″ (δ_c 72.4). The H_3-6′ (δ_H 1.59) of inner rha I showed two bond connectivity with C-5′ (δ_c 70.2); and H-4′ (δ_H 4.26) showed three bond connectivity with

C-6′ (δ_c 18.2). In the light of the above spectroscopic data, the possibility of a straight chain sequence of rhamnose moieties linked to C-6 of inner most glucose [10] or the two rhamnose units linked to C-4 of inner most glucose [5, 9] was precluded. Hence nocturnoside-B (**1**) is identified as spirost-5-en-3-ol, [3β, 25*R*]-3-O-[{α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)}-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside.

Experimental

Fresh leaves and stems of *Cestrum nocturnum* were collected in Islamabad in September 1989.

General. Mps are uncorr. Kieselgel 60 PF_{254} (E. Merck Art. No. 7747) was used for VLC. Precoated Kieselgel 60 F_{254} glass sheets (E. Merck Art. No. 5719) was used for TLC. Purity of the compound was checked on HPTLC (E. Merck Art. No. 5556). ^1H and ^{13}C NMR were determined in pyridine-d_5, specific rotation was measured at 24 °C.

Extraction and isolation. Fresh leaves and stems (3.6 kg) were finely chopped and extracted ×3 with MeOH, ×1 with EtOH and finally extracted once with H_2O. The combined extract was evaporated at reduced pressure. The wet residue was partitioned between EtOAc and H_2O. The aqueous layer was extracted ×3 with *n*-BuOH. The *n*-BuOH extract was washed with H_2O and evaporated under reduced pressure to give 60.8 g of solid residue. This solid residue was fractionated by VLC using a gradient of MeOH in $CHCl_3$. Slightly impure saponin was obtained in the fractions eluted with $CHCl_3$–MeOH (85:15). This was purified by repeated solvent fractionation and finally recrystallization from cold MeOH. Its purity was checked on HPTLC using *n*-BuOH–EtOH–H_2O (7:2:4). Liebermann-Burchard reagent was used for the visualization of the spots.

Nocturnoside-B (**1**). M.p. 210–215 °C (sinters) 69.4 mg $[\alpha]_D^{24}$ −72.916 (MeOH; *c* 0.096); FAB-MS positive ion mode *m/z* 1015.5520 $[M+H]^+$, 869 $[M+H\text{-}146]^+$, 723 $[M+H\text{-}(2\times146)]^+$, 577 $[M+H\text{-}(3\times146)]^+$, 415 $[M+H\text{-}(3\times146)\text{-}162]^+$, 397 $[M+H\text{-}(3\times146)\text{-}162\text{-}18]^+$. – FAB-MS negative ion mode *m/z* 1013.5330 $[M-H]^-$, 867 $[M-H\text{-}146]^-$, 721 $[M-H\text{-}(2\times146)]$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2910, 1440, 1042, 970, 910, 890, 890 > 910 (spiroketal str. of the 25*R* series). ^1H NMR (400.13 MHz, pyridine-d_5): see Table I. Anomeric protons δ 4.93 (d, *J* = 6.5 Hz, glc H-1), 5.81 (d, *J* = 2.8 Hz, rha III H-1‴), 6.26 (d, *J* = 3.6 Hz, rha I H-1′) and 6.37 (d, *J* = 3.6 Hz, rha II H-1″). ^{13}C NMR (100 MHz, pyridine-d_5): see Table I.

Acid hydrolysis of **1.** 28.9 mg of the pure saponin nocturnoside-B was hydrolysed with 20% HCl (25 ml) and MeOH–$CHCl_3$ (35:5 ml) at 100 °C for 5 h. MeOH was evaporated under reduced pressure. The mixture then diluted with H_2O (15 ml) and extracted ×3 with $CHCl_3$. The aqueous layer was neutralized with Ag_2CO_3, filtered and concentrated under reduced pressure. The residue obtained was compared with standard sugars on silica gel cards (E. Merck, Art. No. 5554). The solvent system used was *n*-BuOH–EtOAc–*iso*-PrOH–HOAc–H_2O (7:20:12:7:6). The card was developed twice in the same direction. The spots were visualized with aniline phthalate reagent. The sugars identified were L-rhamnose and D-glucose.

Aglycone. The organic layer was concentrated and the aglycone compared with the original saponin 1 (nocturnoside-B) on TLC (E. Merck, Art. No. 5719) using the solvent system $CHCl_3$–MeOH (9:1). The spots were detected by the Liebermann-Burchard reagent. The aglycone was purified by recrystallization ($CHCl_3$ + a few drops of cold MeOH); its purity checked on HPTLC. The aglycone was identified as diosgenin by co-tlc with an authentic sample. EI-MS (probe) 70 eV; *m/z* (rel. int. %): 396 $[M-H_2O]^+$ (72), 381 (3), 354 (7), 324 (20), 282 (100), 267 (19), 253 (21.5), 213 (10), 139 (81.5), 115 (7.5).

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