Androstane-Type Steroidal Glycoside from the Roots of *Asparagus curillus* BUCH.-HAM. ex ROXB.

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The novel androstane-type steroidal glycoside **1** was isolated from the roots of *Asparagus curillus* BUCH.-HAM. ex ROXB. Its structure was elucidated as (2*α*,3*β*,5*α*,17*β*)-17-(1-methoxyethoxy)-17-methylandrostane-2,3-diol 3-(*β*-D-digitoxopyranoside) by means of chemical and advanced spectral analysis.

Introduction. – *Asparagus curillus* (BUCH.-HAM.) ex Roxb., belonging to the family Liliaceae, is a reputed medicinal shrub distributed in the tropical and temperate climate (1000 to 2250 meter altitude) of central Himalaya and is known for its wide spectrum of medicinal utility including diabetes. Traditionally, ripe fruits from the plant are often used for abortion. The extract obtained from the plant is also used to increase the appetite and secretion of milk in lactating women [1][2]. Previous phytochemical reports on the plant have lead to the isolation of oligospirostanosides, oligofurostanosides, spirostanol, furostanol glycosides, and other steroidal saponins [3–6]. Recently, the plant was also screened for the concentration level of nine trace elements (Zn, Cu, Mn, Fe, Co, Na, K, Ca, and Li) by atomic absorption spectroscopy [7]. In the present study, we report a novel androstane type steroidal glycoside **1** (*Fig. 1*), isolated for the first time from the EtOH extract of *Asparagus curillus* roots.

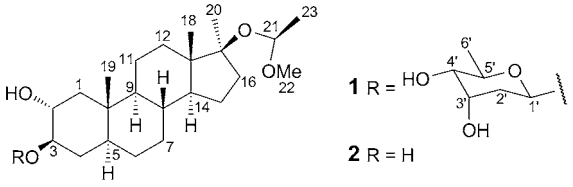


Fig. 1. *Steroidal glycoside* **1**, *isolated from* Asparagus curillus. Arbitrary atom numbering for C(20) to C(23).

Result and Discussion. – Compound **1** was isolated as white granules, and a molecular formula $C_{28}H_{48}O_7$ was inferred from the molecular-ion peak at *m*/*z* 510.6985 in its HR-ESI-MS as well as from elemental analysis. Its steroidal glycosidic nature was recognized by means of chemical tests with *Salkowski*, *Liebermann–Burchard*, and *Molisch*'s reagents. The IR bands at 3448 and 1126 cm^{-1} were assigned to O–H

stretching and asymmetric C–O–C streching (ethers), respectively. The ^{1}H-NMR signals at δ(H) 3.61 – 3.63 (H–C(2)) and 3.81 – 3.83 (H–C(3)) suggested the presence of oxygenated substituents at C(2) and C(3) whereas a signal at δ(H) 3.38 (Me(22)) was indicative of a MeO group (*Table*). A *dd* at δ(H) 5.46 (J = 9.6, 1.8 Hz) was due to a

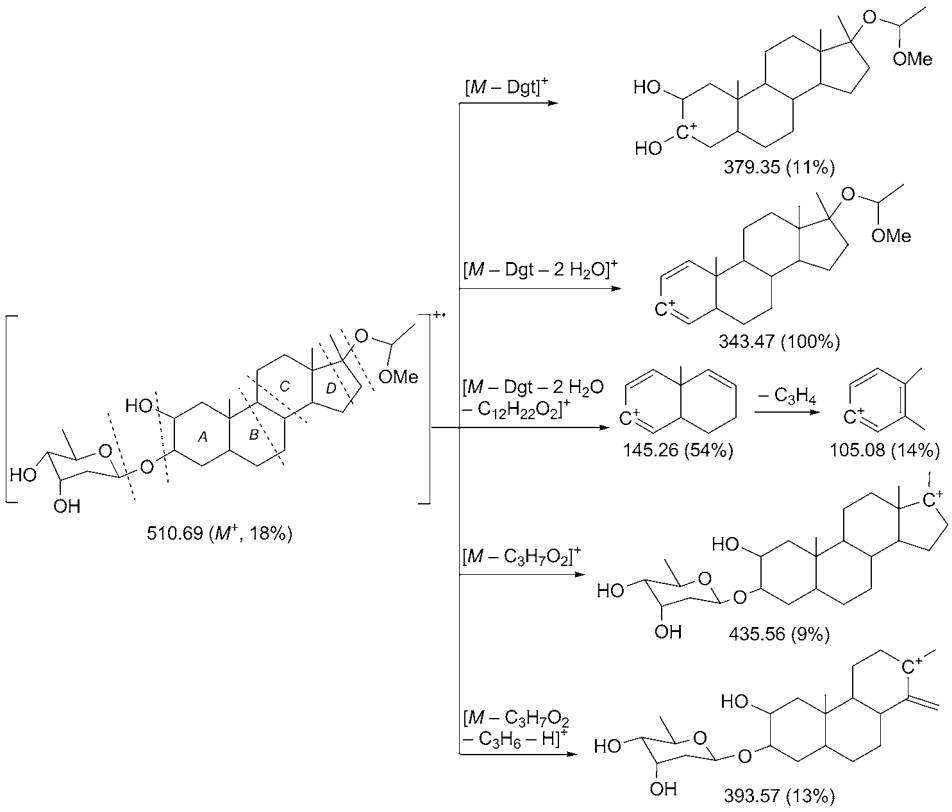
Table. *^{1}H-, ^{13}C-, and 2D-NMR Data* (MeOD) *of* **1**[a])

Position	δ(H)	COSY	NOESY	δ(C)	HMBC
H_a–C(1)	2.08 – 2.10 (*m*)	H–C(2)	Me(19)	36.7	
H_b–C(1)	1.34 – 1.35 (*m*)		H–C(3)		
H–C(2)	3.61 – 3.63 (*m*)	H–C(1), H–C(3)	H_a–C(4)	76.3	C(10)
H–C(3)	3.81 – 3.83 (*m*)	H–C(2), H–C(4)	H_b–C(1)	82.4	C(1′)
H_a–C(4)	1.87 – 1.89 (*m*)	H–C(3), H–C(5)	H–C(2)	32.7	
H_b–C(4)	1.25 – 1.27 (*m*)				
H–C(5)	1.42 – 1.46 (*m*)	H–C(4), H–C(6)		43.4	
H_a–C(6)	1.38 – 1.40 (*m*)	H–C(5), H–C(7)	Me(19)	31.7	
H_b–C(6)	0.96 – 0.97 (*m*)				
H_a–C(7)	2.12 – 2.13 (*m*)	H–C(6), H–C(8)		26.7	
H_b–C(7)	1.77 – 1.79 (*m*)		H–C(9)		
H–C(8)	1.43 – 1.46 (*m*)	H–C(7), H–C(9), H–C(14)	H_a–C(11)	36.1	
H–C(9)	1.32 – 1.33 (*m*)	H–C(8), H–C(11)	H_b–C(7)	48.4	
C(10)				24.2	
H_a–C(11)	1.21 – 1.23 (*m*)	H–C(9), H–C(12)	H–C(8)	28.5	
H_b–C(11)	1.19 – 1.20 (*m*)				
H_a–C(12)	1.28 – 1.29 (*m*)	H–C(11)		27.6	
H_b–C(12)	1.30 – 1.31 (*m*)		H–C(14)		
C(13)				41.8	
H–C(14)	1.36 – 1.37 (*m*)	H–C(8), H–C(15)	H_b–C(12), Me(20)	49.3	
H_a–C(15)	1.90 – 1.92 (*m*)	H–C(14)	H_a–C(16), Me(18)	26.9	C(17)
H_b–C(15)	1.10 – 1.12 (*m*)				
H_a–C(16)	2.03 – 2.05 (*m*)	H–C(15)	H_a–C(15)	41.4	C(17)
H_b–C(16)	1.13 – 1.15 (*m*)		Me(20)		
C(17)				63.7	
Me(18)	0.79 (overlap)		H_a–C(15), Me(23)	16.4	C(13), C(17)
Me(19)	0.99 (overlap)		H_a–C(1), H_a–C(6)	16.9	C(1), C(5), C(9)
Me(20)	1.02 (overlap)		H–C(14), H_b–C(16)	22.3	C(13), C(16), C(21)
H–(21)	4.68 (*s*)	Me(23)		111.1	C(17)
Me(22)	3.38 (overlap)			57.6	
Me(23)	0.81 (overlap)	H–C(21)	Me(18)	14.7	C(22)
H–C(1′)	5.46 (*dd*, J = 9.6, 1.8)	H–C(2′)	H–C(5′)	105.5	C(3)
H_a–C(2′)	2.14 – 2.16 (*m*)	H–C(1′), H–C(3′)	H–C(4′)	38.1	
H_b–C(2′)	1.99 – 2.01 (*m*)				
H–C(3′)	4.18 – 4.20 (*m*)	H–C(2′), H–C(4′)		66.1	
H–C84′)	3.83 – 3.84 (*m*)	H–C(3′), H–C(5′)	H–C(2′)	73.8	
H–C(5′)	4.46 – 4.48 (*m*)	H–C(4′), Me(6′)	H–C(1′)	72.1	
Me(6′)	0.96 (overlap)	H–C(5′)		16.9	C(4′)

[a]) All H-atom assignments were based on COSY, NOESY, and HMBC data, and could be slightly vary; H_a and H_b mean above and below the plane, resp.

coupling with adjacent axial and equatorial H-atoms and was recognized to arise from the anomeric H-atom (H–C(1′)) of the sugar moiety with β-D-configuration. The presence of six Me, nine CH_2, and eleven CH groups and of three quaternary C-atoms was confirmed by the ^{13}C-NMR and DEPT (135°) spectra. The ^{13}C-NMR signals at δ(C) 111.1 (C(21)) and 105.5 (C(1′)) were suggestive of C-atoms linked to two oxygenated moieties (O–C–O), the later value being typical for an anomeric C-atom of a sugar residue. Other selected signals at δ(C) 82.4 (C(3)), 76.3 (C(2)), and 57.6 (C(22)) were assigned to O-substituted C-atoms (C–O), the signal at δ(C) 57.6 being typical for an MeO group. The index of H-atom deficiency for $C_{28}H_{48}O_7$ (*i.e.*, 5) showed the presence of five rings including the sugar residue and the absence of any multiple bond (confirmed by IR and NMR). The sugar moiety was confirmed by hydrolysis and comparison of the hydrolysate with an authentic sample of D-digitoxose (=2,5-dideoxy-D-*ribo*-hexose; Dgt) and was further supported by the mass fragment at *m*/*z* 379 $[M - \text{Dgt}]^+$ in the ESI-MS of **1**, which produced an another fragment at *m*/*z* 343 as base peak by the loss of two H_2O molecules (*Scheme*). An informative mass fragment at *m*/*z* 435 $[M - C_3H_7O_2]^+$ corroborated the presence of a methoxyethoxy group in **1** as side chain which was also supported by the IR band at 1126 cm^{-1} and the ^{13}C-NMR

Scheme. *Proposed Fragmentations in the ESI-MS of* **1**



signal at δ(C) 111.1 (C(21)). Other important fragments at *m*/*z* 393, 145 and 105 were typical for a steroid [8] (*Scheme*). The exact positions of all substituents were confirmed by detailed COSY, HMBC, and NOESY data. The ^{1}H,^{1}H-COSY displayed the correlations for all adjacent H-atoms in the structure (*Fig. 2*). The HMBCs δ(H) 5.46 (H–C(1′))/δ(C) 82.4 (C(3)), δ(H) 3.61 (H–C(2))/δ(C) 24.2 (C(10)) and δ(H) 4.68 (H–C(21))/δ(C) 63.7 (C(17)) confirmed the position of the sugar residue at C(3), an OH group at C(2), and the methoxyethoxy moiety at C(17), respectively. Similarly, the correlations δ(H) 0.99 (Me(19))/δ(C) 36.7, 43.4, and 48.4 (C(1), C(5), and C(9), resp.), δ(H) 0.79 (Me(18))/δ(C) 41.8 and 63.7 (C(13) and C(17), resp.), and δ(H) 1.02 (Me(20))/δ(C) 41.8, 41.4, and 111.1 (C(13), and C(16), C(21), resp.) established the position of Me groups at C(10), C(13), and C(17), respectively (*Fig. 2*). The configuration of **1** was confirmed by the NOESY plot in which the correlation δ(H) 3.61 – 3.63 (H–C(2))/δ(H) 1.87 – 1.89 (H_a–C(4)) suggested the α-position of OH–C(2), whereas δ(H) 1.34 – 1.35 (H_b–C(1))/δ(H) 3.81 – 3.83 (H–C(3)) suggested the β-position of the sugar residue at C(3). Furthermore, the correlations δ(H) 0.99 (Me(19))/δ(H) 2.08 – 2.10 and 1.38 – 1.40 (H_a–C(1) and H_a–C(6), resp.); δ(H) 0.79 (Me(18))/δ(H) 1.90 – 1.92 and 0.81 (H_a–C(15) and Me(23), resp.) and δ(H) 1.02 (Me(20))/δ(H) 1.36 – 1.37 and 1.13 – 1.15 (H–C(14) and H_b–C(16), resp.) furnished the information about the β-position of the Me groups at C(10), C(13), and C(21), and the α-position of Me–C(17) (*Fig. 3*). On the basis of the above discussion and our previous research on similar compounds [9][10], the structure of **1** was established as (2α,3β,5α,17β)-17-(1-methoxyethoxy)-17-methylandrostane-2,3-diol 3(β-D-digitoxopyranoside).

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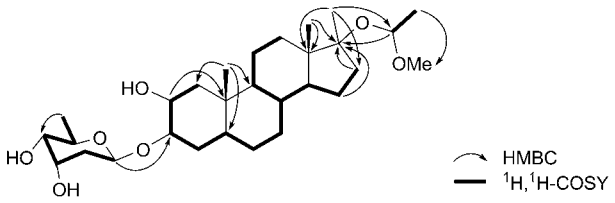

Fig. 2. *Selected HMBC and COSY features of* **12**

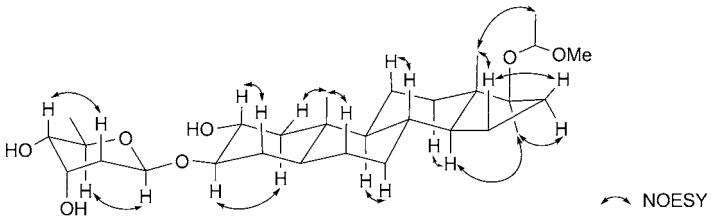

Fig. 3. S*elected NOESY correlations of* **1**

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 60–120 mesh; *Merck*, India). TLC: Aluminium backed plates precoated with silica gel (*Merck*, India); detection by I_2 vapors or spraying with 7% H_2SO_4 soln. followed by heating. M.p.: *Perfit* melting-point apparatus, India. UV: *Perkin-Elmer-Lambda-25* spectrometer; in MeOH. IR: *Perkin-Elmer-Spectrum RX I* FT-IR spectrometer; KBr discs; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-Avance* spectrometer (auto sampler); at 400 (^1H) and 100 MHz (^{13}C) in MeOH; δ in ppm rel. to Me_4Si as internal standard, J in Hz. MS:, *Finnigan-MAT-LCQ* LC/MS^n spectrometer ($n = 9$) with APCI and ESI probe; in m/z (rel.%).

Plant Material. The mature roots (5 kg) of *A. curillus* were collected from Gopeshwar, Chamoli (India), during September 2010 and identified by the Taxonomical Laboratory, Department of Botany, H.N.B. Garhwal University, Srinagar, where a voucher specimen of the plant is available in the herbarium.

Extraction and Isolation. Air dried and powdered roots of the plant were defatted with hexane and finally extracted exhaustively with MeOH at 50–60° in a *Soxhlet* apparatus (*Perfit*, India). The solvent was evaporated to yield 150 g of extract. The extract was pre-adsorbed on SiO_2 (1:1) and the mixture applied on the top of a CC column (SiO_2 400 g) in $CHCl_3$, elution with $CHCl_3$ and then MeOH/$CHCl_3$ up to 30%, 100 ml fractions, TLC monitoring: **1** (with MeOH/$CHCl_3$ 1:4) White granules after recrystallization from MeOH/$CHCl_3$ 1:1.

*(2α,3β,5α,17β)-17-(1-Methoxyethoxy)-17-methylandrostane-2,3-diol 3(β-*D*-Digitoxopyranoside)* (= *(2α,3β,5δ,17β)-2-Hydroxy-17-(1-methoxyethoxy)-17-methylandrostan-3-yl 2,5-Dideoxy-*D*-ribo-hexopyranoside*; **1**): Yield 26 mg (0.017%) MeOH-soluble. M.p. 145°. $[\alpha]_D^{20} = -46$ ($c = 0.5$, MeOH). UV: inactive. IR: 3448, 2935, 2864, 1470, 1218, 1126, 982, 925. ^1H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 510.6985 ($C_{28}H_{18}O_7^+$; calc. 510.7031). ESI-MS: 510.69 (18, M^+), 435.56 (9), 393.57 (13), 379.35 (11), 343.47 (100), 145.26 (54), 105.08 (14). Anal. calc. for $C_{28}H_{48}O_7$: C 68.20, H 9.87, O 21.93; found: C 68.32, H 9.83, O 21.85.

Acid Hydrolysis. Acid hydrolysis of **1** was carried out by following earlier procedures [9][11]: Briefly, **1** (5 mg) was refluxed in 10% aq. HCl soln. (10 ml) for 5 h to afford aglycone **2** and D-digitoxose identified by TLC comparison with an authentic sample (R_f ($CHCl_3$/MeOH/H_2O 12:3:1, lower layer) 0.42).

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