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Steroidal saponins from the roots of *Asparagus adscendens* Roxb and *Asparagus racemosus* Willd

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Phytochemical constituents of *Asparagus adscendens* and *Asparagus racemosus* are being reported in the present communication. Two new sarsasapogenin glycosides have been isolated, one from each of the species. Structure elucidation of **1** and **10** has been accomplished through the extensive use of 1D- and 2D-NMR experiments including ^{1}H-^{1}H (DQF-COSY), ^{1}H-^{13}C (HMQC, HMBC) spectroscopy along with mass spectroscopy. The reported glycosides have been given NMR assignments using 1D- and 2D-NMR experiments. Further, compound **6** and **9** have been found in *A. racemosus* for the first time.

Keywords: *Asparagus adscendens, Asparagus racemosus*, steroidal glycosides

IPC: **Int.Cl.**[8] **A61K**

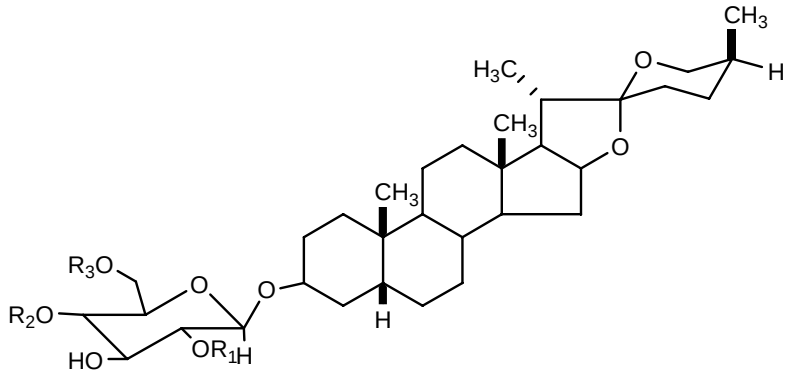
As a part of the ongoing investigations on saponins from Indian medicinal plants, studies on the phytochemistry of roots of *Asparagus adscendens* Roxb. and *Asparagus racemosus* Willd. (Liliaceae)[1,2], were initiated. These are known as shatavar bhed and shatavari respectively in Ayurvedic medicine and are used in treatment of female disorders[3]. These two plants closely resemble in their characteristics and hence were selected simultaneously for the current investigations.

Earlier studies on roots of *A. adscendens* have reported various saponins[4-6] and stigmasterol glycosides[7]. Whereas in case of roots of *A. racemosus* saponins[8,9], polycyclic alkaloid[10], isoflavone[11] and a 9,10-dihydrophenanthrene[12] have been reported. Traditionally *A. adscendens* is used in diarrhoea, dysentery, leucorrhoea, and general debility. It is useful as nutritive, tonic, galactagogue and demulcent[13]. It possesses antifilarial activity[14]. Roots of *A. racemosus* are antidiarrhoeal, diuretic, nutritive, tonic, galactagogue and used in gonorrhoea, leucorrhoea, threatened abortion, nervous disorders and dyspepsia[13]. It is shown to have immunomodulatory[15] and estrogenic activity[16,17], and is useful in dyspepsia and duodenal ulcers[18].

Results and Discussion

The ethanolic extract of *A. adscendens* on repeated column chromatography gave compounds **1-5** (**Figure 1**). These gave positive test with Komarowsky reagent, indicating presence of steroidal glycosides.

Compound **1** gave molecular ion peak at m/z 711(M^+) corresponding to molecular formula of $C_{38}H_{62}O_{12}$ based on ESIMS and NMR. The hydrolysis product was fractionated and chloroform fraction showed presence of sarsasapogenin as aglycone by co-TLC with standard sample and NMR[19]. IR spectrum showed bands at 1069 and 988 cm^{-1} characteristic of glycosidic linkage. Anomeric signals of two sugar units were observed in the ^{1}H NMR spectra of **1** δ_H 4.35 (J = 7.8 Hz), 4.30 (J = 6.7 Hz) indicating β-, β-linkages respectively. ^{13}C NMR showed two anomeric carbons at δ_C 102.5 (CH) and 105.3 (CH) assigned to above protons using DEPT, HMQC and HMBC experiments. DEPT experiment showed presence of 4 methyl, 12 methylene, 19 methine and 3 quaternary carbon atoms. The hydrolysis experiment showed presence of glucose and arabinose (1:1) by HPLC. Anomeric proton at δ_H 4.35 with a doublet (J = 7.8 Hz) indicating β-linkage



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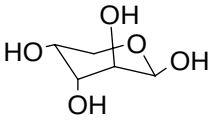
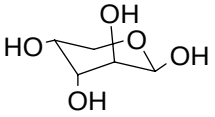
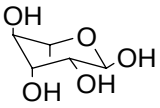
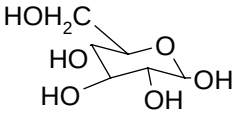
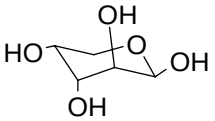
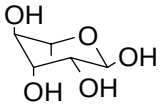
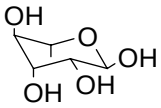
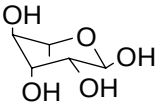
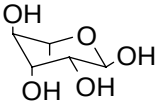
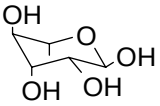
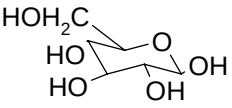
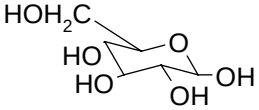
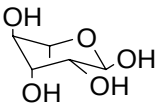
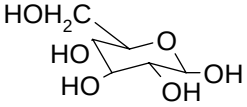
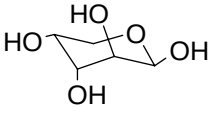
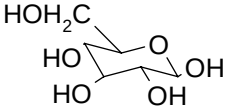
1	H		H
2	H		
3			
6	H		H
7			H
8			H
9			H
10			

Figure 1—Compounds **1-3** and **6-10**

Table I – ^{13}C NMR data (δ) of **1-11** in CD_3OD:$CDCl_3$ (1:1) and CD_3OD[#]

Carbon	**1**[#]	**2**[#]	**3**	**4**[#]	**5**[#]	**6**[#]	**7**	**8**	**9**	**10**[#]	**11**[#]
1	30.8	30.6	30.2	28.1	27.9	29.4*	30.5	30.2	29.6	31.6	28.4
2	31.4	27.6	30.3	32.8	32.3	30.9	29.8*	29.9	30.0	27.3	32.9
3	75.9	74.0	74.9	77.3	76.5	74.2	74.8	75.1	74.7	76.3	76.2
4	28.4	28.4	35.4	30.5	36.4	29.4*	29.8*	30.5	35.2	31.4	31.0
5	36.1	36.1	36.2	36.4	36.2	35.2	36.7	36.6	36.0	37.5	36.7
6	26.7	26.9	26.9	27.4	27.3	25.8	26.5*	26.7*	26.2*	27.6*	27.7*
7	26.9	26.6	26.7	27.0	26.9	25.9	26.5*	26.7*	26.2*	27.6*	27.7*
8	32.6	36.7	35.4	35.9	35.7	34.3	35.4	35.2	34.6	36.7	36.1
9	41.3	41.3	40.7	41.1	41.0	40.0	40.2	40.3	39.9*	41.3	40.9
10	36.7	36.1	35.0	36.5	37.2	34.7	35.0	35.5	34.8	63.1	36.9
11	22.0	22.0	20.9	21.8	21.6	20.2	20.9	21.0	20.5	21.9	22.0
12	37.8	41.4	39.7	41.6	41.5	39.5	40.3	40.4	39.9*	41.8	41.4
13	41.8	41.8	40.7	41.7	41.6	39.6	40.8	40.9	40.3	41.8	41.4
14	57.6	57.6	56.5	57.5	57.2	55.8	56.5	56.6	56.2	57.6	57.5
15	27.7	31.0	31.8	31.1	31.0	29.7	31.7	31.8	31.8	31.0	31.5
16	82.5	82.5	81.2	82.2	82.1	80.6	81.3	81.4	80.9	82.3	82.4
17	63.6	63.5	63.3	62.9	62.9	61.4	62.0	62.1	61.8	63.6	64.2
18	16.9	16.3	16.3	17.4	17.4	15.6	16.5	16.6	15.5	16.9	17.0
19	24.2	24.2	24.0	24.4	24.3	22.9	23.6	24.0	23.0	24.4	24.5
20	41.4	43.4	42.0	43.1	43.0	41.5	42.2	42.3	41.9	43.4	42.3
21	14.7	14.7	14.9	14.7	16.9	13.3	14.3	14.3	13.3	14.7	16.0
22	111.2	111.2	109.6	111.0	110.9	109.4	110.2	110.3	109.5	111.0	111.9
23	27.0	27.2	25.8	26.7	26.6	25.2	25.9	26.0	25.9	26.9	27.0
24	24.2	27.6	26.0	24.4	26.3	24.9	25.7	25.8	25.2	26.7	27.3
25	27.4	27.3	26.6	26.5	27.4	26.5	27.1	27.2	27.0	28.4	34.9
26	67.8	66.1	66.7	76.0*	76.1	64.5	65.3	65.4	64.6	66.0	76.0
27	16.4	16.9	16.6	16.3	17.9	15.1	16.0	16.0	14.8	16.4	17.4
	glu	glu	glu	glu	glu	glu	glu	glu	glu	glu	glu
1′	102.5	101.8	100.0	100.4	100.4	100.5	100.3	100.1	99.5	101.1	100.9
2′	74.3	74.3	79.7	76.0*	79.0	72.9	79.3	71.5	79.4	80.1	79.3
3′	72.5	72.4	81.0	76.7	77.1	74.8	77.3	78.7	81.9	76.4	77.1
4′	74.8	76.2	77.5	80.6	80.5	78.8	80.2	80.2	77.8	80.0	80.8
5′	76.3	76.7	76.1	78.3	78.5	74.3	76.3	75.5	75.3	75.0	78.4
6′	61.9	63.5	61.7	63.2	62.8	60.3	61.1	61.0	60.5	68.5	61.9
	ara	ara	ara	glu	glu	rha	rha	rha	rha	glu	glu
1″	105.3	103.0	103.3	104.0	104.3	101.1	101.7	101.8	101.3	104.4	104.3
2″	66.1	67.7	66.7	74.2	75.9	70.3*	71.3	70.6	70.6	74.3	75.11
3″	74.8	74.8	74.8	76.8	76.6	70.3*	71.5	70.9	70.9	77.7	77.8
4″	69.9	69.9	69.7	78.3	77.8	71.8	73.0	72.6	72.3	71.6	72.0
5″	66.1	66.1	66.7	69.4	78.1	68.9	69.5	69.6	69.1	78.1	76.3
6″	-	-	-	63.9	63.0	16.4	17.3	17.2	16.4	62.7	62.8
		rha	rha	ara	ara		rha	glu	glu	glu	rha
1‴		105.4	104.7	104.5	105.0		101.1	103.7	102.9	104.6	102.7
2‴		72.2	71.8	72.9	67.4		70.5	75. 0	74.8	75.1	72.1

—*Contd*

Table I – ^{13}C NMR data (δ) of **1-11** in CD_3OD:$CDCl_3$ (1:1) and $CD_3OD^{\#}$ — *Contd*

Carbon	**1**#	**2**#	**3**	**4**#	**5**#	**6**#	**7**	**8**	**9**	**10**#	**11**#
3‴		69.9	71.0	74.6*	74.4		71.0	76.8	77.4	77.8	72.4
4‴		74.0	73.1	69.5	69.3		72.8	71.0	70.8	72.0	73.8
5‴		69.7	69.3	67.5	66.5		68.5	75.6	76.9	78.3	70.6
6‴		18.1	18.2	-	-		17.5	62.2	62.2	63.2	17.8
			glu	rha	rha					ara	glu
1⁗			104.0	101.8	101.2					105.1	104.6
2⁗			74.8	71.5	71.9					72.5	75.1
3⁗			76.3	73.7	73.6					74.3	78.1
4⁗			71.0	69.4	65.9					70.0	71.7
5⁗			76.4	71.3	71.4					67.7	77.8
6⁗			62.2	18.0	18.0						63.2
				glu	glu						
1′′′′′				100.8	100.8						
2′′′′′				75.1	75.0						
3′′′′′				79.4	78.2						
4′′′′′				71.8	71.7						
5′′′′′				74.6*	74.0						
6′′′′′				64.0	62.9						
OMe				41.2							

*values interchangeable in a column

showed HMBC correlation with C-3 at δ_C 75.9. Anomeric proton at δ_H 4.30 with β-linkage exhibited HMBC correlation with δ_C 74.8 for C-4′ of glucose. Using DQFCOSY, HMBC, HMQC and DEPT remaining assignments were done (**Table I**). Compound **1** was identified as 25*S*-5*β*-spirostan-3*β*-yl-*O*-[*O*-*β*-D-arabinopyranosyl (1→4)]-*β*- D-glucopyranoside. This is being reported for the first time.

Compound **2-5** were elucidated using spectral techniques and were found to be spirostanol glycosides asparanin C and D and furostanol glycosides asparoside C and D reported earlier on the basis of derivatized sugar analysis[4]. However, these compounds were fully characterized by NMR and mass spectral studies. The NMR assignments of compound **2-5** are reported for the first time (**Table I**).

Butanolic fraction of ethanolic extract of *A. racemosus* was also found to be rich in saponins, and was fractionated by repeated column chromatography. This process gave compounds **6-11** (**Figures 1** and **2**), reacting positive to Komarowsky reagent.

Compound **6** was obtained as white crystals and on hydrolysis gave aglycone sarsasapogenin and sugars rhamnose and glucose, identified by co-TLC and HPLC with available standard (1:1). The aglycone was identified as sarsasapogenin by co-TLC and by comparison with literature data[19]. Further, compound **6** gave a sodium adduct at m/z 747 and a pseudo-molecular ion peak at m/z 725 (M+1). Fragmentation pattern showed loss of 145 amu followed by 162 amu suggesting rhamnose as terminal sugar. Anomeric signals of two sugar units were observed in the ^{1}H NMR spectra of **6** at δ_H 4.77 (s) and 4.25 (J = 7.8 Hz) indicating α-, β-linkages respectively. ^{13}C NMR showed two anomeric carbons at δ_C 101.1 (CH) and 100.5 (CH). Using 2D NMR experiments, HMQC, HMBC and DQFCOSY and DEPT the linkages were determined to assign the structure of **6** as 25*S*-5*β*-spirostan-3*β*-yl-*O*-[*O*-α-L-rhamnopyranosyl (1→4)]-*β*-D-glucopyranoside. This is a known glycoside that is being reported for the first time from *A. racemosus*. This compound has been earlier reported as tuberoside M from *Allium tuberosum*[20].

Compound **7** was crystallized from chloroform:methanol. Hydrolysis of compound **7** gave aglycone sarsasapogenin and the sugars rhamnose and glucose, identified by co-TLC and HPLC with available standard (2:1). Further, compound **7** gave a

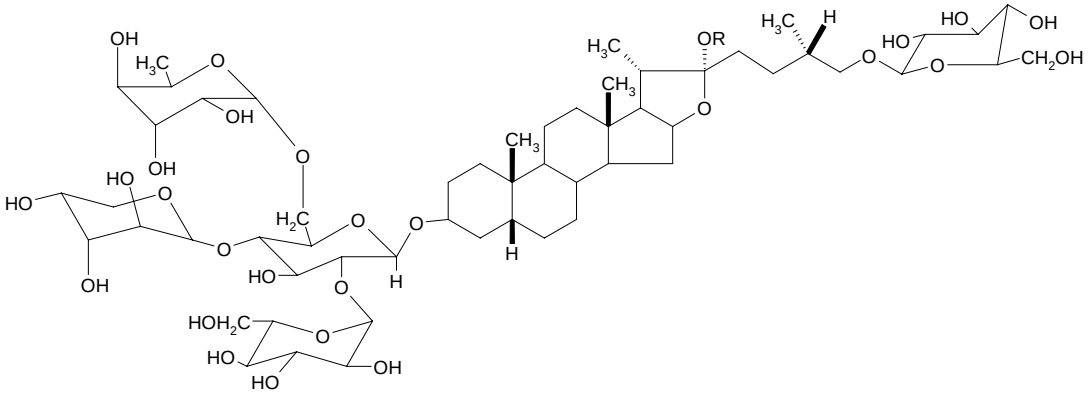
sodium adduct at m/z 893. High-resolution mass of **7** showed molecular ion peak at 893.4838 m/z for $C_{45}H_{74}O_{16}Na+$ (calculated 893.4875). Anomeric signals of three sugar units were observed in the ^1H NMR spectra of **7** at δ_H 5.33 (s), 4.85 (s) and 4.36 (J = 7 Hz) indicating α-, α- β-linkages respectively. ^{13}C NMR showed three anomeric carbons, which were assigned to anomeric carbons at δ_C 101.1 (CH), 101.7 (CH) and 100.3 (CH) respectively using 2D NMR experiments. With the help of DEPT, HMQC, HMBC and DQFCOSY the linkages were determined to assign the structure of **7** as 25*S*-5*β*-spirostan-3*β*-yl-*O*-*α*-L-rhamnopyranosyl (1→2)-*O*-[*O*-*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside. This glycoside has been recently patented as *Immunoside* for its immunomodulatory properties[21].

Compound **8** formed adduct with sodium at m/z 909 (M+ Na^+) and gave a molecular ion peak at m/z 886 with molecular formula of $C_{45}H_{74}O_{17}$ based on ESIMS and NMR. The hydrolysis product was fractionated and chloroform fraction showed presence of sarsasapogenin by co-TLC with standard sample. Using DQFCOSY, HMBC, HMQC and DEPT techniques NMR assignments were done and **8** was identified as Shatavarin IV reported by Ravikumar *et al.*[9] NMR assignments of this compound are being done for the first time (**Table I**).

Compound **9** was found to have a molecular formula of $C_{45}H_{74}O_{17}$ based on HRESIMS and NMR studies. Anomeric signals of three sugar units were observed in the ^1H NMR spectra of **9** at δ_H 4.85 (s), 4.70 (d, J = 7.5 Hz) and 4.45 (d, J = 7.5 Hz) indicating α-, β-, β-linkages respectively. ^{13}C NMR showed three anomeric carbons, which were assigned to anomeric carbons at δ_C 101.3 (CH), 102.9 (CH) and 99.5 (CH) respectively using DEPT, HMQC and HMBC experiments. DEPT experiment showed presence of 5 methyl, 13 methylene, 24 methine, and 3 quaternary carbon atoms. The aglycone of **9** was determined to be sarsasapogenin by comparison of NMR data with reported data and by co-TLC with the standard sample. The hydrolysis experiment showed presence of glucose and rhamnose (2:1) by HPLC. FAB mass spectra showed that loss of two sugar units one with m/z 145 and other with m/z 162 gave ion peak at m/z 579 indicating that glucose is directly attached to the aglycone. Anomeric proton at δ_H 4.45 with a doublet (J = 7.5 Hz) indicating β-linkage showed HMBC correlation with C-3 at δ_C 74.7. Anomeric proton at δ_H 4.85 and 4.70 with α- and β-linkage exhibited HMBC correlation with 77.8 and 79.4 for C-4′ and C-2′ of glucose respectively. Using DQFCOSY, HMBC, HMQC and DEPT remaining assignments were done. 25*S*-5*β*-spirostan-3*β*-yl-*O*-*β*-D-glucopyranosyl (1→2)-*O*-[*O*-*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside. This compound has been earlier reported from *Asparagus adscendens* as asparanin B using hydrolysis studies along with GC analysis of derivatized sugars[5]. NMR assignments are being given for the first time (**Table I**).

Compound **10** has the molecular formula $C_{50}H_{82}O_{22}$ based on HRESIMS and NMR studies. Anomeric signals of four sugar units were observed in the ^1H NMR spectra of **10** at δ_H 4.69 (d, J = 7.5 Hz), 4.47 (d, J = 7.5 Hz), 4.45 (d, J = 5 Hz) and 4.41 (d, J = 7.5 Hz) indicating β-, β-, α- and β-linkages respectively. ^{13}C NMR showed four anomeric carbons, which were assigned to anomeric carbons at δ_C 104.4 (CH), 101.1 (CH), 105.1 (CH) and 104.6 (CH) respectively using DEPT, HMQC and HMBC experiments. SEFT experiment showed presence of 4 methyl, 15 methylene, 28 methine, and 3 quaternary carbon atoms. The aglycone of **10** was determined to be sarsasapogenin by comparison of NMR data with reported data[19] and by co-TLC with the standard sample. The hydrolysis experiment showed presence of glucose and arabinose (3:1) identified by HPLC. Anomeric proton at δ_H 4.47 with a doublet (J = 7.5 Hz) indicating β-linkage showed HMBC correlation with C-3 at δ_C 76.3. Anomeric proton at δ_H 4.41, 4.45 and 4.69 with α-, β- and β-linkage exhibited HMBC correlation with 68.5 (C-6′), 80.0 (C-4′) and 80.1(C-2′) of glucose attached to the aglycone respectively. Using DQFCOSY, HMBC, HMQC and DEPT remaining assignments were done. HRESIMS showed a mass of 1057.5130 indicating the formula $C_{50}H_{82}O_{22}Na+$ (calculated mass 1057.5195). Thus compound **10** was established as 25*S*-5*β*-spirostan-3*β*-yl-*O*-*β*-D-glucopyranosyl (1→2)-O-{[*O*-β-D-glucopyranosyl-(1→4)]-*O*-[*α*-D-arabinopyranosyl (1→6)]}-*β*-D-glucopyranoside. This compound is being reported for the first time.

Compound **11** was obtained using reverse phase column chromatographic separation. It gave positive Komarowsky and Ehrlich test indicating furostanol glycoside. IR spectrum showed bands at 1122, 1074, and 1037 cm^{-1}. ESIMS of the compound gave an ion peak at m/z 1089 for sodium adduct of $C_{51}H_{86}O_{23}$. Molecular formula was confirmed from the HRMS showing ion peak at m/z 1089.5305 for $C_{51}H_{86}O_{23}Na^+$



4 R=Me
5 R=H

Figure 2—Compounds **4** and **5**

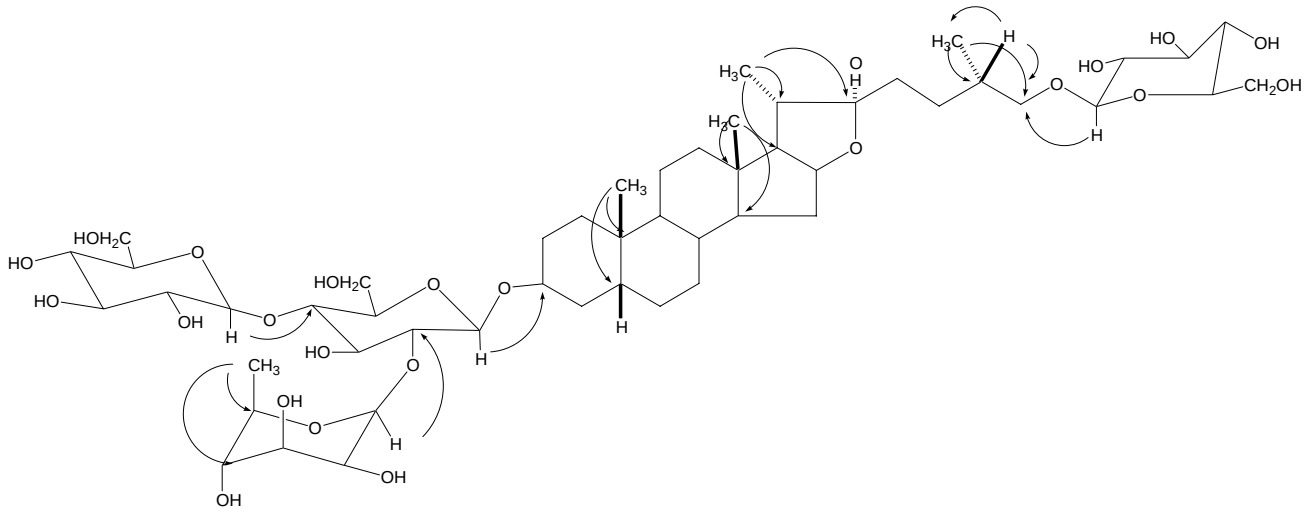


Figure 3—Selected HMBC correlations in **11**

(calculated 1089.5458) and from NMR studies. NMR studies showed that anomeric protons at δ_H 4.23 (J = 7.5 Hz), 4.45 (J = 7.5 Hz), 4.69 (J = 7.5 Hz) and 4.83 attached to the anomeric carbons δ_C 104.6 (CH), 100.9 (CH), 104.3 (CH), and 102.7 (CH) showed HMBC correlations (**Figure 3**) with δ_C 76.0, 76.2, 80.8 and 79.3. Methyl of C-27 showed HMBC correlation with δ_C 76.0 and 34.9. Proton attached to 76.0 (δ_H 1.76) showed HMBC correlation to C-27 methyl confirming the furostanol nucleus. Using 1D and 2D NMR experiments compound **11** was identified as Shatavarin I from the plant[8]. NMR assignments of this compound are reported in the present communication (**Table I**).

Experimental Section

NMR spectra in $CDCl_3$: CD_3OD (1:1) or CD_3OD were obtained using a Bruker DRX-300 and Bruker DRX-500 spectrometer, operating at 300 MHz and 500 MHz for ^1H, and 75 MHz and 125 MHz for ^{13}C. 1D-experiments: ^1H, ^{13}C, DEPT (distortionless enhancement of polarization transfer). 2D-experIments: ^1H-^1H DQF-COSY (double quantum filtered correlation spectroscopy), ^1H-^{13}C HMQC (heteronuclear multiple quantum coherence), HMBC (heteronuclear multiple bond connectivity) were obtained using XWIN NMR software. ESIMS were performed on a Finnigan Matt, LCQ with Xcalibur software. Samples were dissolved in MeOH and infused in the

ESI source using a syringe pump. High resolution mass spectra were measured on Bruker, Daltonics. Optical rotations were obtained on Rudolph Autopol IV with a cell volume of 0.4 mL and 100 mm path length with light source consisting of sodium D-line (589 nm). Sugars were identified by comparative TLC on silica gel plates (GF_{254}) using chloroform: methanol:water (64:32:4) as mobile phase. Individual sugars were separated on semi preparative Waters Novapak HR C-18 column (7.6 × 300 mm; elution with water at a flow rate of 1 mL min^{-1}) with fixed wavelength UV detection (190 nm) using Waters HPLC. Optical rotation of the collected elutes was compared with the authentic sugars.

Plant material. Tuberous roots of *Asparagus adscendens* Roxb. and *Asparagus racemosus* Willd. were obtained from NIPER medicinal plant garden, and dried in shade. Samples were identified by the botanist of the institute and are maintained in the department of natural products herbarium (42-A-4, 31-A-4 resp.).

Extraction and isolation of the compounds. ***Asparagus adscendens* Roxb**. Tuberous roots of *A. adscendens* were powdered (1 kg), defatted with *n*-hexane (2 × 3 L) and subsequently extracted with 70% ethanol (2 × 5 L) at RT by maceration. Ethanolic extract (150 g) was resuspended in water (1 L) with the help of methanol (10%). It was successively partitioned with chlorofom (3 × 500 mL), ethyl acetate (3 × 500 mL) and *n*-butanol (3 × 500 mL). The *n*-butanol layer rich in saponins was concentrated on a rotary evaporator to give *n*-butanolic fraction (25 g). It was chromatographed on a column of silica gel (500 g, 60-120 mesh) using isocratic elution with mixture of *n*-butanol:methanol:water (7:2:1). A total of 80 fractions each of approximately 100 mL volume were collected. Fraction 9-23 (90 mg) was chromatographed on silica gel column (230-400 mesh) and ethyl acetate:methanol:water (8:1:1) for isocratic elution on Buchi MPLC system to get **1** (white crystalline compound, 16 mg). Fraction 24-56 (500 mg) and fraction 57-70 (800 mg) were chromatographed on silica gel (60-120 mesh) column isocratically eluting with ethyl acetate:methanol: water (7:2:1) to give **2** (30 mg), **3** (33 mg) and **4** (27 mg), **5** (30 mg).

Compound 1. White crystalline solid, m.p. 142-43°C, $[\alpha]_D^{20}$ -55.5° (*c* 0.104, methanol); IR (KBr): 3407, 2931, 1449, 1378, 1069, 986 cm^{-1}; ^{1}H NMR (CD_3OD): δ 4.40 (1H, C, *J* = 6.6 Hz), 4.35 (1H, d, *J* = 7.8 Hz, H-1′), 4.30 (1H, d, *J* = 6.7 Hz, H-1″), 3.94 (1H, m, H-3), 3.84 (1H, m, H_a-26), 3.83 (1H, t, *J* = 6.4 Hz), 3.55 (1H, m, H-3″), 3.53 (1H, t, *J* = 6.6 Hz, H-4′), 3.52 (1H, m, H-3′), 3.39 (1H, m, H-5″), 3.35 (1H, m, H-2″), 3.25 (1H, m, H-2′), 3.22 (1H, m, H-26), 1.95 (1H, m, H-23), 1.65 (1H, m, H_a-12), 1.56 (1H, m, H-8), 1.35 (1H, m, H-11), 1.18 (1H, m, H-14), 1.14 (1H, m, H_b-12), 1.08 (3H, d, *J* = 7.4 Hz, H-27), 0.98 (3H, d, *J* = 6.2 Hz, Me-21), 0.94 (3H, s, Me-19), 0.78 (3H, s, Me-18); ^{13}C NMR (CD_3OD): **Table I**; ESIMS: m/z 711 (M^+).

Asparanin C 2. White crystalline solid, m.p. 230-31°C (reported 229-32°C [4]; $[\alpha]_D^{20}$ –41.8° (*c* 0.1, methanol); reported $[\alpha]^{18}_D$ –56.6° (*c* 1.5, pyridine) [4]; IR (KBr): 3404, 2930, 1450, 1376, 1068, 986 cm^{-1}; ^{1}H NMR (CD_3OD): δ 5.35 (1H, d, *J* = 7.8 Hz, H-1′), 4.69 (1H, d, *J* = 3 Hz, H-1″), 4.42 (1H, d, *J* = 3 Hz, H-1‴), 4.04 (1H, m, H-4′), 3.93 (1H, m, H-5‴), 3.85 (2H, d, *J* = 6.7 Hz, H-6′), 3.79 (1H, m, H-3‴), 3.59 (1H, m, H-3″), 3.51 (1H, m, H-3′), 3.41 (1H, m, H-4‴), 3.38 (1H, m, H-5′), 3.36 (2H, d, *J* = 7.8 Hz, H-5″), 3.32 (1H, m, H-2″), 3.28 (1H, m, H-2′) 3.25 (1H, m, H-26), 1.92 (1H, m, H-23), 1.62 (1H, m, H-12), 1.55 (1H, m, H-8), 1.27 (3H, d *J* = 6.4 Hz, Me-6‴), 1.26 (1H, m, H-11), 1.16 (1H, m, H-14), 1.12 (1H, m, H_b-12), 1.07 (3H, d, *J* = 7.4 Hz, H-27), 0.98 (3H, d, *J* = 6.4 Hz, Me-21), 0.94 (3H, s, Me-19), 0.78 (3H, s, Me-18); ^{13}C NMR (CD_3OD): **Table I**; ESIMS: m/z 879 $(M+Na)^+$.

Asparanin D 3. White crystalline solid, m.p. 234-35°C (decomp) (reported 234-37°C [4]; $[\alpha]_D^{20}$ –45.8° (*c* 0.106, methanol); [reported $[\alpha]_D^{20}$ –54.0° (pyridine) [4]]; IR (KBr): 3406, 2929, 1446, 1378, 1066, 982 cm^{-1}; ^{1}H NMR ($CDCl_3$: CD_3OD, 1:1): δ 5.17 (1H, d, *J* = 7.8 Hz, H-1′), 4.85 (1H, d, *J* = 3 Hz, H-1⁗), 4.56 (1H, d, *J* = 3 Hz, H-1‴), 4.40 (1H, d, *J* = 7.5 Hz, H-1″), 4.08 (1H, m, H-4′), 3.94 (1H, m, H-5⁗), 3.84 (2H, d, *J* = 6.8 Hz, H-6′), 3.81 (1H, m, H-3⁗), 3.38 (2H, m, H-5′), 3.36 (2H, m, H-5‴, H-2‴), 3.34 (1H, m, H-2″), 3.24 (1H, m, H-2′), 3.22 (1H, m, H-26), 1.94 (1H, m, H-23), 1.62 (1H, m, H-12), 1.54 (1H, m, H-8), 1.29 (3H, overlap, Me-6⁗), 1.27 (1H, m, H-11), 1.17 (1H, m, H-14), 1.13 (1H, m, H_b-12), 1.05 (3H, d, *J* = 7.6 Hz, H-27), 0.98 (3H, d, *J* = 6.8 Hz, Me-21), 0.97 (3H, s, Me-19), 0.75 (3H, s, Me-18); ^{13}C NMR (CD_3OD, **Table I**); ESIMS: m/z 1041 $(M+Na)^+$.

Asparoside C 4. White amorphous solid, m.p. 167-70°C (decomp.) (reported 167-72°C [4]; $[\alpha]_D^{20}$

–29.9° (*c* 0.105, methanol); [reported $[\alpha]_D^{20}$ –75° (*c* 1.0) [4]]; IR (KBr): 3436, 2926, 1452, 1378, 1068, 1048, 986 cm^{-1}; ^1H NMR (CD_3OD): δ 4.81 (1H, d, *J* = 7.0 Hz, H-1″), 4.55 (1H, s, H-1‴″), 4.53 (1H, d, *J* = 7.0 Hz, H-1‴″), 4.43 (1H, s, H-1‴), 4.20 (1H, d, *J* = 7.0 Hz, H-1′), 3.92 (1H, m, H-2‴), 3.83 (1H, m, H-4″), 3.78 (2H, m, H-6‴″), 3.28 (3H, s, 22- α-OMe), 1.85 (1H, m, H-23), 1.54 (1H, m, H-8), 1.27 (3H, overlap, Me-6‴″), 1.08 (3H, overlap, H27), 0.98 (3H, *J* = 6.8 Hz, Me-21), 0.96 (3H, s, Me-19), 0.78 (3H, s, Me-18); ^{13}C NMR (CD_3OD, **Table I**); ESIMS: m/z 1235 $(M+Na)^+$.

Asparoside D 5. White amorphous solid, m.p. 165-67°C (decomp.) (reported 161-66°C); $[\alpha]_D^{20}$ –44.5° (*c* 0.098, Methanol); [reported $[\alpha]_D^{20}$ –66° (*c* 1.5, pyridine) [4]; IR (KBr): 3436, 2928, 1450, 1374, 1068, 1048, 986 cm^{-1}; ^1H NMR (CD_3OD): δ 4.86 (1H, d, *J* = 7.6 Hz, H-1″), 4.50 (1H, s, H-1‴″), 4.46 (1H, d, *J* = 7.0 Hz, H-1‴″), 4.43 (1H, s, H-1‴), 4.20 (1H, d, *J* = 7.0 Hz, H-1′), 3.92 (1H, m, H-2‴), 3.84 (1H, m, H-4″), 3.42 (2H, m, H-5‴), 3.34 (1H, m, H-4‴), 3.30 (1H, m, H-2″), 3.28 (1H, m, H-2′), 1.85 (1H, m, H-23), 1.54 (1H, m, H-8), 1.27 (3H, overlap, Me-6‴″), 1.10 (1H, m, H_b-12), 1.07 (3H, d, *J* = 7.5 Hz, H-27), 0.99 (3H, *J* = 6.8 Hz, Me-21), 0.96 (3H, s, H-19), 0.78 (3H, s, Me-18); ^{13}C NMR (CD_3OD,**Table I**); ESIMS: m/z 1237$(M+K)^+$.

***Asparagus racemosus* Willd.** Tuberous roots of *A. racemosus* were powdered (1.5 kg), defatted with *n*-hexane (2 × 3 L) and subsequently extracted with 70% ethanol (2 × 5 L) at RT by maceration. Ethanolic extracts were combined and concentrated under reduced pressure to form a dark brown syrupy residue (210 g). This extract (90 g) was resuspended in water (1 L) with the help of methanol (10%). It was successively partitioned with chloroform (3 × 500 mL), ethyl acetate (3 × 500 mL) and *n*-butanol (3 × 500 mL). The *n*-butanol extract (25 g) was chromatographed on a column packed with silica gel (60-120 mesh) with initial isocratic elution using ethyl acetate:methanol:water (8:1:1, 7L) and subsequently eluted with ethyl acetate:methanol:water (7:2:1, 5 L). Fractions 10-15 were concentrated and crystallized from methanol to give **6** (18 mg). Fractions 18-60 were concentrated and repeatedly purified on silica gel column (60-120 mesh) with ethyl acetate: methanol: water (8:1:1) as the mobile phase to give **7** (60 mg), **8** (360 mg), **9** (40 mg). Fractions 61- 80 were complex mixture of saponins and further chromatographed on reverse phase silica gel (RP-18) using isocratic elution with water : acetonitrile: methanol (6:2:2) as the mobile phase giving **10** (12 mg) and **11** (9 mg).

Compound 6. White crystalline solid; m.p. 148-50°C (decomp); $[\alpha]_D^{20}$ –65.1° (*c* 0.360, methanol); IR (KBr): 3416 (OH), 2931(CH), 1450 and 1379 (CH_3), 1070, 1048, 986 cm^{-1}; ^1H NMR ($CDCl_3$-CD_3OD: 1:1): δ 4.77 (1H, s, H-1″), 4.32 (1H, C, *J* = 7.3 Hz, H-16), 4.25 (1H, d, *J* = 7.8 Hz, H-1′), 3.95 (1H, s, H-3), 3.84 (1H, s, Ha-26), 3.78 (1H, m, H-3″), 3.55 (1H, m, H-2″), 3.44 (1H, t, *J* = 9 Hz, H-4′), 3.34 (1H, m, H-4″), 3.22 (1H, m, H-26), 3.21 (1H, m, H-2′), 1.93 (1H, m, H-23), 1.67 (1H, m, H-12), 1.54 (1H, m, H-8), 1.38 (1H, m, H-11), 1.22 (3H, d, *J* = 6.2 Hz, Me-6″), 1.13 (1H, m, H-14), 1.10 (1H, m, H_b-12), 0.99 (3H, d, *J* = 7 Hz, Me-27), 0.90 (3H, d, *J* = 6.9 Hz, Me-21), 0.88 (3H, s, Me-19), 0.68 (3H, s, Me-18); ^{13}C NMR (CD_3OD, **Table I**); ESIMS: m/z 725 [M+1].

Compound 7. White crystalline solid; m.p. 278-79°C; $[\alpha]_D^{20}$ –18.8° (*c* 0.193, methanol); IR (KBr): 3411 (OH), 2929(CH), 1449 and 1379 (CH_3), 1070, 1041, 986 cm^{-1}; ^1H NMR ($CDCl_3$-CD_3OD: 1:1): δ 5.33 (1H, s, H-1‴), 4.85 (1H, s, H-1″), 4.42 (1H, m, H-16), 4.36 (1H, d, *J* = 7Hz, H-1′), 4.08 (1H, H-3), 3.91 (1H, d, *J* = 11Hz, H_a-26), 3.83 (1H, m, H-17), 3.33 (1H, d, *J* = 11Hz, H_b-26), 1.89 (1H, m, H-20), 1.87 (1H, m, H_a-23), 1.86 (1H, m, H-5), 1.40 (1H, m, H_b-23), 1.28 (6H, m, Me-6″, Me-6‴), 1.08 (3H, d, *J* = 6.9 Hz, Me-27), 0.99 (3H, d, *J* = 6.7 Hz, Me-21), 0.96 (3H, s, Me-19), 0.76 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$-CD_3OD: 1:1, **Table I**); ESIMS: m/z 893.4 $[M+Na]^+$; HRESIMS: m/z 893.4838 calculated for $C_{45}H_{74}O_{16}Na$+ (893.4875)

Shatavarin IV 8. White crystalline solid; m.p. 294-95°C (reported 307-9°C [9]; $[\alpha]_D^{20}$ –58.1° (*c* 0.5, methanol)[reported $[\alpha]_D$ –68.6° (*c* 1, pyridine) [4]; IR (KBr): 3408 (OH), 2932(CH), 1449 and 1375 (CH_3), 1073, 1041, 986 cm^{-1}; ^1H NMR ($CDCl_3$-CD_3OD: 1:1): δ 4.86 (1H, s, H-1″), 4.64 (1H, d, *J* = 7.6Hz, H-1‴), 4.42 (1H, d, *J* = 7.6 Hz, H-1′), 4.43 (1H, m, H-16), 4.08 (1H, C-3), 3.91 (1H, d, *J* = 11Hz, H-26a), 3.83 (1H, m, H-17), 3.33 (1H, d, *J* = 11Hz, H-26b), 1.89 (1H, m, H-20), 1.87 (1H, m, H-23a), 1.86 (1H, m, H-5), 1.40 (1H, m, H-23b), 1.29 (3H, d, *J* = 6.2 Hz, Me-6″), 1.08 (3H, d, *J* = 6.9 Hz, Me-27), 0.99 (3H, d, *J* = 6.7 Hz, Me-21), 0.96 (3H, s, Me-19), 0.76 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$-CD_3OD:1:1, **Table I**); ESIMS: m/z 909 $[M+Na]^+$.

Compound 9. White crystalline solid, m.p. 188-89°C; $[\alpha]_D^{20}$ +16.6° (*c* 0.067, MeOH); IR (KBr): 3434, 2928, 1451, 1378, 1070, 1048, 986 cm^{-1}; ^1H NMR ($CDCl_3$-CD_3OD, 1:1): δ 4.85 (1H, s, H-1″), 4.70 (1H, d, *J* = 7.5 Hz, H-1‴), 4.45 (1H, d, *J* = 7.5 Hz, H-1′), 4.10 (1H, m, H-3‴), 4.06 (1H, br s, H-3), 3.95 (1H, m, H-5″), 3.83 (1H, m, H-2″), 3.73 (1H, m, H-3′), 3.66 (1H, m, H_a-5′), 3.63 (1H, m, H-4″), 3.54 (2H, overlap, H-4′, 5′), 3.40 (1H, m, H-4‴), 3.26 (2H, d, *J* = 10.5 Hz, H-26), 1.85 (1H, overlap, H-5), 1.84 (1H, m, H-20), 1.72 (2H, m, H-12), 1.42 (2H, m, H-11), 1.25 (3H, d, *J* = 6 Hz, Me-6‴), 1.07 (3H, d, *J* = 7.5 Hz, Me-27), 0.97 (6H, overlap, Me-19, Me-21), 0.77 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$-CD_3OD, 1:1): **Table I**; ESIMS: m/z 925 $[M+K]^+$, 885 (M-H); HRESIMS: m/z 925.4922 for $C_{45}H_{74}O_{17}K^+$ (calculated 925.4563).

Compound 10. White amorphous solid, m.p. 160-62°C; $[\alpha]_D^{20}$ –36.3° (*c* 0.2, MeOH); IR (KBr): 3419, 2929, 1447, 1122, 1074, 1037, 985 cm^{-1}; ^1H NMR (CD_3OD): δ 4.69 (1H, d, *J* = 7.5 Hz, H-1″), 4.47 (1H, d, *J* = 7.5 Hz, H-1′), 4.45 (1H, d, *J* = 5 Hz, H-1⁗), 4.41 (1H, d, *J* = 7.5 Hz, H-1‴), 4.40 (1H, m, H-16), 4.05 (1H, br s, H-3), 3.66 (1H, overlap, H-17), 3.63 (1H, m, H-2′), 3.54 (1H, m, H-2⁗), 3.20 (1H, m, H-2‴), 3.18 (1H, m, H-2″), 1.86 (2H, m, H-1), 1.84 (1H, m, H-20), 1.69 (1H, m, H-25), 1.72 (2H, m, H-12), 1.62 (1H, overlap, Ha-11), 1.49 (2H, m, H-24), 1.44 (1H, m, H_b-11), 1.28 (2H, m,H-15), 1.08 (3H, d, *J* = 7 Hz, Me-27), 0.99 (6H, overlap, Me-19, Me-21), 0.78 (3H, s, Me-18); ^{13}C NMR (CD_3OD, **Table I**); ESIMS: m/z 1057; HRESIMS: m/z 1057.5130 for $C_{50}H_{82}O_{22}Na^+$ (calculated 1057.5195).

Shatavarin I (11). White amorphous solid, m.p. 182-83°C (reported 184-87°C); $[\alpha]_D^{20}$ –31.6° (*c* 1.1, MeOH)[reported $[\alpha]_D$ –34.2° (*c* 1, methanol)[4]; IR (KBr): 3419, 2929, 1731, 1447, 1288, 1122, 1074, 1037 cm^{-1}; ^1H NMR (CD_3OD): δ 4.83 (1H, s, H-1‴), 4.69 (1H, d, *J* = 7.5 Hz, H-1″), 4.45 (1H, d, *J* = 7.5 Hz, H-1′), 4.23 (1H, d, *J* = 7.5 Hz, H-1⁗), 4.06 (1H, br s, H-3), 3.95 (1H, m, H-5⁗), 3.83 (2H, m, H-17, 3⁗), 3.77 (2H, overlap, H-26), 3.76 (1H, m, H-6′), 3.69 (1H, m, H-2), 3.68 (1H, m, H-3′), 3.64 (1H, m, H-2⁗), 3.54 (1H, m, H-4′), 3.39 (1H, m, H-4⁗), 3.38 (1H, m, H-4″), 3.37 (1H, m, H-3‴), 3.31 (1H, m, H-5′), 3.28 (1H, m, H-4‴), 3.20 (1H, m, H-2⁗), 3.19 (1H, m, H-2″), 1.85 (2H, m, H-4), 1.75 (1H, m, H-8), 1.59 (1H, m, H-5), 1.48 (2H, m, H-2), 1.42 (2H, m, H-15), 1.30 (2H, m, H-1), 1.25 (3H, d, *J* = 6.5 Hz, Me-6⁗),1.19 (1H, br s, H-11), 0.99 (3H, overlap, Me-21), 0.98 (3H, s, Me-19), 0.94 (3H, d, *J* = 6.5 Hz, Me-27), 0.80 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$-CD_3OD, 1:1, **Table I**); ESIMS: m/z 1089 $[M+Na]^+$; HRTOFMS: m/z 1089.5305 for $C_{51}H_{86}O_{23}Na^+$ (calculated 1089.5458).

Acid hydrolysis. Compound (3-5 mg) was refluxed with 5 mL of 1 *N* hydrochloric acid for 4 hr. The mass was concentrated to dryness under reduced pressure and then extracted with chloroform. The chloroform layer was washed with water and dried over anhydrous sodium sulphate. The aqueous layer was concentrated and dissolved in methanol.

Identification of sugars. Sugars were identified by comparative TLC with authentic sugars on silica gel plates using chloroform:methanol:water (64:32:4) as the mobile phase and thymol reagent for derivatization. Relative ratios of sugars were determined using Waters HPLC, column: Bondapak NH_2, 4.6 × 250 mm column, mobile phase: water-acetonitrile (30:70) having a flow rate of 0.8 mL min^{-1} at ambient temperature using an evaporative light scattering detector (gain 5, temperature 50°C, air pressure 3.5 bar).

Absolute configuration of sugars. Individual sugars were separated on semi preparative Waters Novapak HR C-18 column (7.6 × 300 mm; elution with water at a flow rate of 1 mL min^{-1}) with fixed wavelength UV detection (190 nm) using Waters HPLC. Optical rotation of the collected elutes was compared with the optical rotation of the authentic sugars like D-arabinose (positive rotation), D-glucose (positive rotation) and L-rhamnose (positive rotation).

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