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Structural revision of shatavarins I and IV, the major components from the roots of *Asparagus racemosus*

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Abstract—The two major steroidal saponins from the roots of *Asparagus racemosus* were isolated by RP-HPLC and their structure determined by extensive NMR studies. Their structures did not match those reported previously for shatavarins I and IV and were found to be 3-*O*-{[β-D-glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranosyl}-26-*O*-(β-D-glucopyranosyl)-(25*S*)-5β-furostan-3β,22α,26-triol and 3-*O*-{[β-D-glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranosyl}-(25*S*)-5β-spirostan-3β-ol.

Asparagus racemosus (among 300 species classified in the family Asparagaceae) was thought to possess therapeutic properties in traditional medicine, such as the Himalayan medicine system (Ayurveda), Tibetan medicine and by the early Romans.[1] The root of this herb has been used to treat a wide range of ailments including dysentery, tumours, inflammations, neuropathy, nervous disorders, bronchitis, hyperacidity, certain infectious diseases,[1] conjunctivitis,[2] spasm, chronic fevers and rheumatism.[3] Apart from that, *A. racemosus* is also consumed as a post-partum tonic to increase lactation in women (galactogogue) and to normalize the uterus and hormone changes that have occurred during pregnancy.[3] Only a few reports on the saponin content of *A. racemosus* roots have been published. In 1987[4] and 1988,[5] Sukh et al. reported the presence of four steroidal saponins from the roots of *A. racemosus*, shatavarin I (3-*O*-{[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→4)]-β-D-glucopyranosyl}-26-*O*-(β-D-glucopyranosyl)-(25*S*)-5β-furostan-3β,22α,26-triol), shatavarin II (no reported structure), shatavarin IV (3-*O*-{[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→4)]-β-D-glucopyranosyl}-(25*S*)-5β-spirostan-3β-ol) and glycoside-AR-4 (incomplete structure elucidation) with the two major ones being named shatavarins I and IV. A more recent report described the isolation of another saponin called immunoside (3-*O*-{[α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranosyl}-(25*S*)-5β-spirostan-3β-ol) patented as an immunomodulor.[6] Subsequently, a variety of properties were ascribed to these two steroidal saponins including specific and competitive blockage of oxytocin-induced contraction of rat, guinea pig and rabbit uteri (in vitro and in vivo) for shatavarin I.[7] Shatavarin IV has been reported to display significant activity as an inhibitor of Core 2 GlcNAc-transferase in cell free assays,[8] and recently to exhibit immunomodulation activity against specific T-dependent antigens in immunocompromised animals (0.15 mg/kg p.o.).[9]

While the steroidal saponins found in the fruits of *A. racemosus*, the racemosides, have been recently fully characterized,[10] only the structure of immunoside, from the roots of *A. racemosus*, has been rigorously proven by 1D and 2D NMR.[6,11] The structure of the major saponins, shatavarin IV and shatavarin I, and their glycosidic linkages were characterized only by proton NMR (90 MHz in $CDCl_3$) and FAB MS. We now report the revision of the structures of shatavarin I and IV, identified here through a combination of 1D (^1H, ^{13}C, DEPT, TOCSY) and 2D (COSY, HSQC, HMBC) NMR (Fig. 1).

Extraction and isolation: Powdered roots of *A. racemosus* were extracted (90% acetonitrile/water) using

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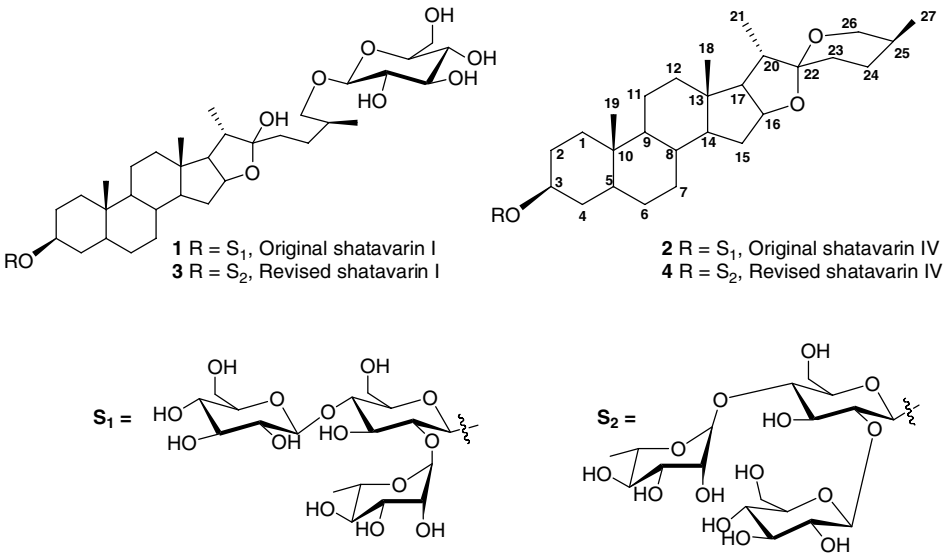

Figure 1. Structures of revised shatavarin I **3** and IV **4** isolated from *Asparagus racemosus*, and the structures of shatavarin I **1** and IV **2** as originally reported.[4,5]

Table 1. ^{1}H and ^{13}C spectral data (δ in parts per million) of the aglycon moiety of **3** (750 MHz, pyridine-d_5, J in hertz) and **4** (500 MHz, pyridine-d_5, J in hertz)

	Aglycon **3**, shatavarin I		Aglycon **4**, shatavarin IV	
	δ_H	δ_C	δ_H	δ_C
1	1.75–1.83 m	30.7	0.94 m	27.1
			1.24 m	
2	1.14 m	26.7	1.45 m	26.5
	1.78 m		2.05 ddd (5.9, 7.6, 12.6)	
3	4.24 m	75.3	4.26 m	75.4
4	1.43 m	30.8	1.82 m	30.7
	1.78 m		1.82 m	
5	2.16 m	36.7	2.21 m	36.8
6	1.47 m	27.0	1.53 m	26.8
	1.82 m		1.91 m	
7	1.20 m	26.8	1.17 m	26.8
	1.90 m		1.79 m	
8	1.46 m	35.5	1.48 m	35.3
9	1.24 m	40.2	1.28 m	40.3
10		35.3		35.6
11	1.20 m	21.2	1.20 m	21.2
	1.30 m		1.32 m	
12	1.08, m	40.4	1.10 m	40.4
	1.71 m		1.71 m	
13		41.2		40.9
14	1.04 m	56.4	1.06 m	56.5
15	1.40 ddd (6.1, 6.1, 12.8)	32.4	1.45 m	32.2
	2.00 m		1.98 m	
16	4.97 dt (7.1, 7.1)	81.3	4.63 dt (7.2, 7.2)	81.4
17	1.95 m	64.0	1.88 dd (6.7, 8.5)	63.0
18	0.86 s	16.7	0.85 s	16.6
19	0.94 s	24.0	0.97 s	24.0
20	2.22 dq (6.8, 6.8)	40.7	1.96 dq (6.8, 6.8)	42.5
21	1.31 d (6.8)	16.5	1.19 d (6.9)	15.0
22		110.7		109.8
23	1.95 m	37.2	1.47 m	30.9
	2.06 m		1.84 m	
24	1.66 m	28.4	1.40 m	26.3
	2.06 m		2.17 dt (4.4, 12.7)	
25	1.91 m	34.5	1.61 m	27.6
26	3.46 m	75.4	3.43 br d (10.6)	65.2
	4.08 m		4.11 dd (2.7, 11.2)	
27	1.01 d (6.8)	17.5	1.10 d (7.1)	16.3

sonication. The two major constituents **3** and **4** (representing 19% and 44% of the total saponin content of the *A. racemosus* extract, respectively) were purified by preparative HPLC performed on a Shimadzu LC-10AT Liquid Chromatograph equipped with a Shimadzu ELSD-LT detector (flow rate: 2 mL/min, 52 °C, 200 kPa) and a ChromSep OmniSpher C_{18} column (150 mm × 4.6 mm ID, Alltech). An acetonitrile/water gradient from 8% CH_3CN to 100% in 50 min was used. (Retention times: 19.5 min for compound **3** and 34.8 min for compound **4**.)

Structure elucidation: Acid hydrolysis[12] of the powdered roots of *A. racemosus* by heating under reflux for 15 h in 2 N HCl in 70:30 isopropanol/water yielded only sarsasapogenin, which had identical spectroscopic (^{1}H and ^{13}C NMR) properties as in the literature reports.[13,14]

Compound **3** was isolated as an amorphous solid ($[\alpha]_D$ −53.6 (*c*, 9.8, pyridine)). Positive-ion ESI-HRMS gave an ion at 1089.548 ($[M+Na^+]$) indicating a molecular formula of $C_{51}H_{86}O_{23}$. The ^{1}H NMR spectrum (in pyridine-d_5) revealed the presence of two quaternary methyl groups (δ 0.86 and 0.94 ppm) corresponding to the angular methyl groups of a steroidal sapogenin as well as two tertiary methyl groups [δ 1.01 (*J* 6.8 Hz) and δ 1.31 (*J* 6.8 Hz)]. The presence of another tertiary methyl group at 1.67 ppm (d, *J* 6.3 Hz) and 4 anomeric protons signals at δ 4.85 (d, *J* 7.8 Hz), δ 5.46 (d, *J* 7.8 Hz), δ 5.92 (br s) and δ 4.81 (d, *J* 7.6 Hz) suggested the presence of four monosaccharides including one deoxyhexose (Table 1). The nature of the sugars and the glycosidic linkage between them and to the steroidal nucleus were determined by HMBC (heteronuclear multiple bond correlation) and 1D-TOCSY (one-dimensional total correlation spectroscopy) experiments. The different vicinal coupling constants (see Table 2) for the various sugar moieties were obtained via 1D-TOCSY experiments and indicated the presence of three β-D-glucopyranoses and one α-rhamnopyranose. A cross-peak observed in the HMBC spectrum (see Fig. 2) between the ^{1}H NMR signal at δ 4.81 (H1‴, C-26 terminal glucose) and δ 75.4 (C-26, aglycon) proved that these two moieties were connected. Likewise, the correlation between the proton signal at δ 4.85 (H1′, 2,4-disubstituted glucose) and the carbon signal at δ 75.3 (C-3, aglycon) revealed that this glucose was attached to C-3 of the aglycone. The linkages of the terminal glucose and rhamnose to the 2,4-disubstituted glucose were determined from cross-peaks between the anomeric proton at δ 5.46 (H1″, terminal glucose) and the carbon signal at δ 82.7 (C-2′ of the

Table 2. ^{1}H and ^{13}C spectral data (δ in parts per million) for the sugars of **3** (750 MHz, pyridine-d_5, *J* in hertz) and **4** (500 MHz, pyridine-d_5, *J* in Hz)

	Shatavarin I, **3**			Shatavarin IV, **4**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
3-O-α-D-Glucose						
1′	4.85 d (7.8)	101.9	C3, C3′, C5′	4.87 d (7.6)	101.9	C3
2′	4.28 dd (8.0, 9.0)	82.7	C1″	4.30 dd (7.6, 9.0)	81.4	C1″, C3′
3′	4.26 dd (9.0, 9.0)	76.4	C1′	4.26 dd (9.0, 9.0)	76.5	C2′
4′	4.48 dd (9.1, 9.1)	77.2	C1‴	4.34 dd (9.0, 9.0)	77.4	C1‴
5′	3.59 ddd (2.3, 3.1, 9.2)	77.1	C1′	3.61 ddd (2.1, 3.6, 9.6)	77.1	
6′	4.08 dd (3.1, 12.0)	61.2		4.04 dd (3.6, 12.2)	61.3	
	4.22 br d (12.0)			4.19 dd (2.1, 12.2)		
2′-O-β-D-Glucose						
1″	5.46 d (7.8)	105.6	C2′, C5″, C2″	5.48 d (7.8)	105.6	C2′, C2″
2″	4.08 dd (8.0, 9.2)	77.1	C1″, C4‴	4.07 dd (8.4, 8.4)	76.5	C1″
3″	4.26 dd (9.2, 9.2)	77.9	C2″, C1″	4.27 dd (8.8, 8.8)	78.0	
4″	4.34 dd (9.2, 9.2)	71.8		4.23 dd (8.8, 8.8)	71.9	
5″	3.97 ddd (3.2, 4.5, 9.2)	78.6		3.99 ddd (2.9, 5.5, 8.8)	78.6	C6″
6″	4.49 dd (4.5, 11.8)	62.9		4.43 dd (5.8, 12.0)	63.0	C5″
	4.58 dd (3.2, 11.8)			4.61 dd (2.9, 12.0)		
4′-O-α-Rhamnose						
1‴	5.92 br s	102.4	C2‴, C4′, C5‴	5.78 d (1.4)	102.5	C4′, C2‴, C5‴
2‴	4.67 dd (1.5, 3.3)	72.6	C1‴, C4⁗	4.68 dd (1.4, 3.2)	72.6	C1‴, C4‴
3‴	4.53 dd (3.3, 9.3)	72.8	C4‴, C5″	4.56 dd (3.2, 9.4)	72.8	C1‴
4‴	4.32 dd (9.3, 9.3)	74.0	C3‴, C2‴	4.36 dd (9.4, 9.4)	74.0	C2‴
5‴	4.99 dq (6.3, 9.3)	70.3	C3‴, C1‴	4.91 dq (6.1, 9.3)	70.3	C1‴
6‴	1.67 d (6.3)	18.5		1.67 d (6.2)	18.6	
26-O-β-D-Glucose						
1⁗	4.81 d (7.8)	105.2	C26, C2⁗, C5⁗			
2⁗	4.03 dd (7.8, 9.0)	75.3				
3⁗	4.25 m	78.6				
4⁗	4.25 m	71.7				
5⁗	3.95 ddd (2.7, 5.1, 9.3)	78.6				
6⁗	4.40 dd (5.1, 11.8)	62.8				
	4.56 br d (2.7, 11.8)					

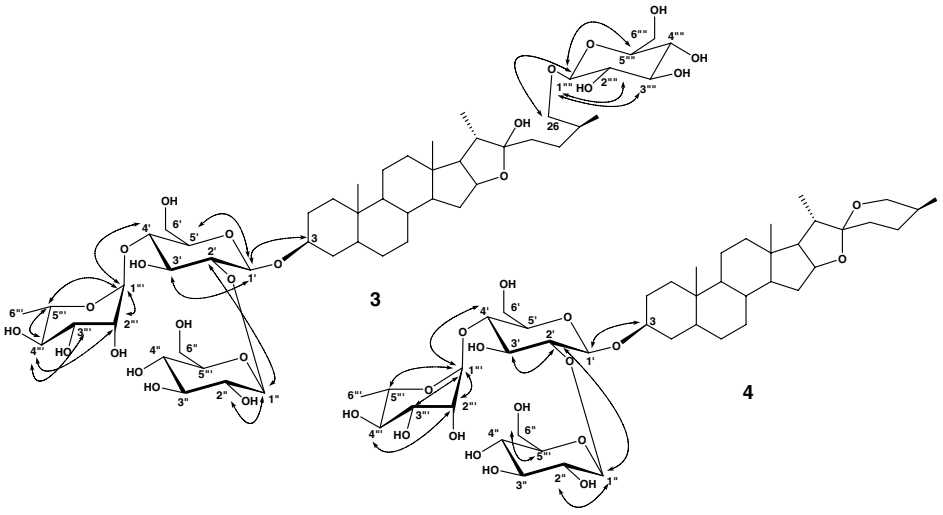

Figure 2. HMBC correlations of revised shatavarin I **3** (750 MHz) and revised shatavarin IV **4** (500 MHz) in pyridine-d_5.

2,4-disubstituted glucose), as well as between the anomeric proton signal at δ 5.92 (H1‴, rhamnose) and the carbon signal at δ 77.2 (C-4′ of the 2,4-disubstituted glucose). Shatavarin I was thus shown to be 3-*O*-{[β-D-glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranosyl}-26-*O*-(β-D-glucopyranosyl)-(25*S*)-5β-furostan-3β,22α,26-triol **3**. This structure has also been assigned to asparoside B,[†] isolated previously from the fruits of *A. currillus*[16] and *A. adscendens*[17] (but not from *A. racemosus)*. However, no spectroscopic data for **3** were available from these reports and we are thus unable to confirm the identity of asparoside B.[16,17]

Compound **4** was isolated as a white solid (mp: 305–307 °C (decomp.), $[\alpha]_D$ −70.8 (*c*, 3.3, pyridine)). Positive-ion ESI-HRMS gave an ion at 909.4827 ([M+Na$^+$]) indicating a molecular formula of $C_{45}H_{74}O_{17}$. The ^{1}H NMR spectrum (in pyridine-d_5) revealed the presence of two quaternary methyl groups (δ 0.85 and 0.97 ppm) corresponding to the angular methyl groups of a steroidal sapogenin as well as two tertiary methyl groups [δ 1.10 (*J* 7.1 Hz) and δ 1.19 (*J* 6.9 Hz)]. The presence of another tertiary methyl group at 1.67 ppm (d, *J* 6.2 Hz) and three anomeric protons signals at δ 4.87 (d, *J* 7.6 Hz), δ 5.48 (d, *J* 7.8 Hz), δ 5.78 (d, *J* 1.4 Hz) suggested the presence of three monosaccharides including one deoxyhexose. The vicinal coupling constants of the sugars were determined through 1D-TOCSY experiments, which indicated the presence of two β-D-glucopyranoses and one α-rhamnopyranose (see Table 2). In the HMBC spectrum (see Fig. 2), the glycosylation of the aglycone at C-3 was deduced from a cross-peak between the proton signal at δ 4.87 (H1′, 2,4-disubstituted glucose) and the carbon signal at δ 75.4 (C-3, aglycon). Similarly, the anomeric protons at δ 5.48 (H1″, terminal glucose), δ 5.78 (H1‴, rhamnose) showed cross-peaks with the carbon signals at δ 81.4 (C-2′ of the 2,4-disubstituted glucose) and the carbon signal at δ 77.4 (C-4′ of the 2,4-disubstituted glucose), respectively. Shatavarin IV was therefore identified as 3-*O*-{[β-D-glucopyranosyl(1→2)][α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-(25*S*)-5β-spirostan-3β-ol **4**. This structure has also been assigned to asparanin B, which has been reported from the fruits of *A. officinalis*,[18,19] *A. currillus*[16] and *A. adscendens*.[17] Spectroscopic data were only available for the most recent report[19] and appears consistent with the data obtained here. However, given that significant biological data have been obtained for shatavarin IV from *A. racemosus*[8,9] in the absence of structural data, we suggest that the name be retained with the corrected structure **4**.

The two major saponins contained in the roots of *A. racemosus*, shatavarin I and shatavarin IV, have been isolated and their correct structures have been unambiguously shown to be **3** and **4**, respectively, through a combination of NMR (1D and 2D) and mass spectrometry. This information will clearly be of importance in understanding and assigning the compounds responsible for the reported bioactivity of *A. racemosus*.

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[†] The name asparoside B has been attributed to another saponin, 26-*O*-β-glucopyranosyl-5β-furost-20(22)-ene-3β,26-diol-3-*O*-[β-D-xylopyranosyl(1→4)]-β-D-glucopyranoside, from *A. meioclados*.[15]

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