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Asparinins, asparosides, curillins, curillosides and shavatarins: structural clarification with the isolation of shatavarin V, a new steroidal saponin from the root of *Asparagus racemosus*

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Abstract—A new steroidal saponin, shatavarin V, (3-*O*-{[α-L-rhamnopyranosyl(1→2)][β-D- glucopyranosyl(1→4)]-β-D-glucopyranosyl}-(25*S*)-5β-spirostan-3β-ol), was isolated from the roots of *Asparagus racemosus* by RP-HPLC, and its structure determined by 1D and 2D NMR studies. This data permits clarification of the structures reported for several known saponins: asparinins A and B; asparosides A and B; curillin H; curillosides G and H and shavatarins I and IV.

The genus *Asparagus* (Family Asparagaceae, with about 300 species) is a rich source of sapogenins and saponins, from various parts of the plant,[1] and diverse biological activities (mainly associated with the steroidal saponins) have been reported,[2] with antitumour, immunostimulant, immunoadjuvant, antiinflammatory and antibacterial properties[3] being prominent. Therefore, it is important to characterise fully the different steroidal saponins present in a specific plant, so that the reported bioactivities may be better understood. We have recently reported[4] structural revisions for shatavarin I (**3**) and IV (**6**), the major saponins from the roots of *A. racemosus*. The structures of these saponins were previously based upon proton NMR spectra (90 MHz, $CDCl_3$) and FAB MS,[5,6] but the authors wrongly assigned the glycosidic linkages, reporting the rhamnose moiety linked to the C-2 position and the terminal glucose to the C-4 position of the glucose unit attached to the sarsasapogenin skeleton, giving structures **1** and **5**. We have unambiguously proven that the sugar sequence is in fact 3-*O*-{[β-D-glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranosyl}, by the use of specific 1D and 2D NMR experiments and shown that shatavarins I and IV have structures **3** and **6**, respectively.

Subsequently, a diligent search of the literature (see Fig. 1) revealed that a number of isolated compounds have been assigned[7–9] the same structure as the original[5,6] or revised structures[4] for shatavarins I and IV. In 1982, Sharma and co-workers[7] described the isolation of three saponins from the fruits of *A. adscendens* and assigned the following names/structures: asparinin B/**6**; asparoside A/**4** and asparoside B/**3**. They presented only partial proton NMR data for asparinin B and asparoside A to support their claims. In 1983, the same group[8] reported the isolation of apparently the same compounds from the fruits of *A. curillus* but with no indication of a specific name. Thus they reported: 'fraction III'/**6**; 'fraction IVb'/**4** and 'fraction IVa'/**3**, respectively. Again, only partial proton NMR data were indicated for 'fraction III' to confirm these assignments. Ten years later,[9] their investigation of the leaves of *A. curillus* led to the isolation of a number of other saponins. These were reported as: curillin H/**5**; curilloside G/**2**; and curilloside H/**1**. It was not noted at the time that curilloside H and curillin H were identical with the original, incorrect structures of shatavarin I and shatavarin IV. Again, there was a lack of underpinning spectroscopic data reported for the suggested structures. Finally, during the course of this work, a report on the steroidal

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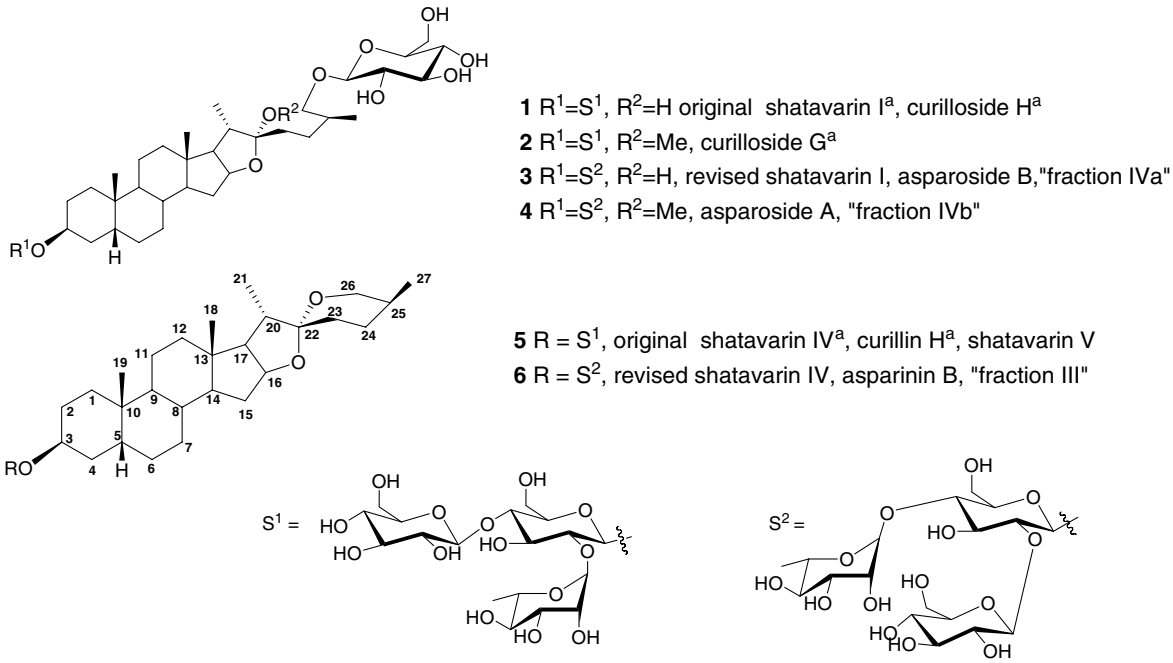


Figure 1. Structures and names of some of the previously reported furostanol (**1**–**4**) and spirostanol (**5** and **6**) saponins from *A. racemosus*, *A curillus* and *A. adscendens*. Shatavarin V, reported here, is shown to be **5**. [a]Structures originally reported and now revised in this work.

saponins present in *A. racemosus* root appeared[10] and the authors report the presence of shatavarins I and IV as the major constituents, but again with the original incorrect structures **1** and **5**.

We now report the elucidation of the structure of shatavarin V (**5**), a *minor* new saponin from the roots of *A. racemosus*, utilizing a combination of 1D (^{1}H, ^{13}C, DEPT, TOCSY) and 2D (COSY, HSQC, HMBC) NMR methods. Comparisons of chemical shifts for the sugar moieties for this saponin and those reported for other proposed structures,[5–10] clearly indicated the novelty of shatavarin V (**5**) and allowed correction/correlation of all the previously suggested structures.

Extraction and isolation: The powdered roots of *A. racemosus* were extracted (90% acetonitrile/water), assisted by sonication. The extract was 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. Under these conditions, the retention time for **5** shatavarin V, representing about 2.3% of the total saponin content of the *A. racemosus* extract, was 38.8 min (for comparison, shatavarin I **3** and shatavarin IV **6** represent 19% and 44%, respectively, of the total saponin content of the extract).

Structure elucidation: Shatavarin V **5** was isolated as a white solid (mp: 285–288 °C (decomp.), $[\alpha]_D$ −56.7 (*c*, 0.12, pyridine)). Positive-ion ESI-HRMS provided an ion at 909.4813 ([M+Na$^+$]) indicating a molecular formula of $C_{45}H_{74}O_{17}$. The aglycon of **5** was identified as sarsasapogenin by the comparison of NMR data with those reported in literature.[11] Acid hydrolysis[12] and GCMS analysis via comparison with authentic standards revealed the presence of 2 equiv of glucose and one of rhamnose. The ^{1}H NMR spectrum (in d_5-pyridine) revealed the presence of two methyl groups (δ 0.80 and 1.06 ppm) attached to quaternary carbons, corresponding to the angular methyl groups of a steroidal sapogenin, as well as two methyl groups [δ 1.14 (*J* 7.0 Hz) and δ 1.06 (*J* 6.8 Hz)] on secondary carbons. The presence of another methyl group at δ 1.72 (d, *J* 6.2 Hz) attached to a secondary centre and three anomeric proton signals [4.80 (d, *J* 7.8 Hz), δ 5.12 (d, *J* 7.6 Hz), δ 6.36 (d, *J* 1.4 Hz)] suggested the presence of three monosaccharides including one deoxyhexose. The vicinal coupling constants for the sugars were determined through 1D-TOCSY experiments, which indicated the presence of two β-D-glucopyranoses and one α-rhamnopyranose (see Table 1), in agreement with the sugars indicated by GCMS analysis after acid hydrolysis of the saponin.

In the HMBC spectrum (see Fig. 2), a cross-peak between the ^{1}H NMR signal at δ 4.80 (H1′, 2,4-disubstituted glucose) and the carbon signal at δ 76.1 (C-3, aglycon) indicated glycosylation of the aglycon at C-3. Similarly, anomeric protons at δ 5.12 (H1″, terminal glucose) and δ 6.36 (H1‴, rhamnose) showed cross-peaks with the carbon signals at δ 82.3 (C-4′ of the 2,4-disubstituted glucose), and at δ 76.6 (C-2′ of the 2,4-disubstituted glucose), respectively. Shatavarin V was therefore identified as 3-*O*-{[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→4)]-β-D-glucopyranosyl}-(25*S*)-5β-spirostan-3β-ol (**5**).

Table 1. ^{1}H and ^{13}C spectral data (δ in ppm) for the sugar moieties of shatavarin V **5** (500/125 MHz, pyridine-d_5, J in Hz)

Aglycone shatavarin V 5			Sugar units shatavarin V 5		
Nucleus	^{1}H, δ (J)	^{13}C, δ	Nucleus	^{1}H, δ (J)	^{13}C, δ
1	1.82 m	31.0	3-O-β-D-Glucose		
	1.48 m		1′	4.80 d (7.8)	102.0
2	1.48 m	26.9	2′	4.25 dd (7.8, 9.3)	76.6
	1.90 m		3′	4.20 m	78.2
3	4.24 m	76.1	4′	4.20 m	82.3
4	1.83 m	30.9	5′	3.81 ddd (3.1, 3.8, 12.0)	76.2
	1.83 m		6′	4.44 dd (3.1, 12.0)	62.1
5	2.14 m	37.2		4.50 dd (3.9, 12.0)	
6	1.53 m	26.8	2′-O-L-Rhamnose		
	1.91 m		1″	6.36 d (1.4)	101.4
7	0.97 m	26.9	2″	4.71 dd (1.4, 3.3)	72.4
	1.28 m		3″	4.53 dd (3.3, 9.3)	72.7
8	1.50–1.56 m	35.6	4″	4.31 dd (9.4, 9.4)	74.1
9	1.21–1.31 m	40.4	5″	4.79 dq (6.2, 9.4)	69.4
10		35.3	6″	1.72 d (6.2)	18.8
11	1.21 m	21.2	4′-O-β-D-Glucose		
	1.32 m		1‴	5.12 d (7.6)	105.3
12	1.08 m	40.4	2‴	4.05 dd (7.6, 9.1)	75.0
	1.68 m		3‴	4.20 dd (9.3, 9.3)	78.3
13		40.9	4‴	4.27 dd (9.2, 9.2)	71.3
14	1.06 m	56.6	5‴	3.95 ddd (2.5, 4.9, 9.2)	78.5
15	1.35–1.41 m	32.2	6‴	4.31 dd (5.0, 12.2)	62.1
	2.01 m			4.43 dd (2.5, 12.2)	
16	4.56 m	81.4			
17	1.81 m	63.0			
18	0.80 s	16.6			
19	1.06 s	23.4			
20	1.90 m	42.5			
21	1.14 d (7.0)	14.9			
22		109.7			
23	1.88 m	26.4			
	1.42 m				
24	1.33 m	26.3			
	2.12 m				
25	1.57 m	27.5			
26	3.35 br d (12.0)	65.1			
	4.06 m				
27	1.06 d (6.8)	16.3			

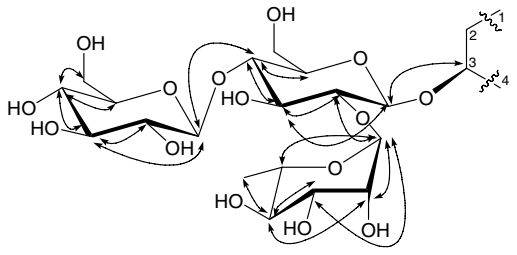
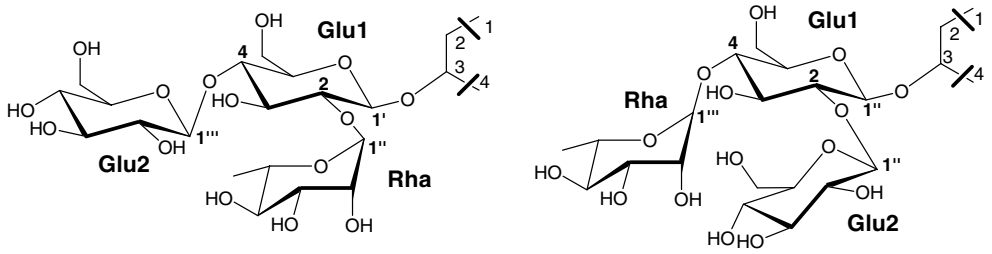


Figure 2. HMBC correlations for shatavarin V **5**.

Previously, this structure was reported for curillin H,[9] and wrongly assigned to shatavarin IV.[5,10] However, no spectroscopic data for curillin H was presented in the original report.[9] Thus, this structural assignment must be regarded as tentative at best, and from comparison of the spectroscopic data for compounds correlated in the original report,[9] it now appears erroneous (vide infra). It should also be noted that although shatavarin V (**5**) possesses the structure originally reported for shatavarin IV, they are distinct: shatavarin IV is the major saponin (44%) in *A. racemosus* roots, whilst shatavarin V is a minor constituent (2%). Additionally, the physical data originally presented for shatavarin IV (mp, $[\alpha]_D$) exactly match with that obtained for shatavarin IV isolated by us previously[4] and are different to that of shatavarin V reported here. The recent report by Jadhav and Bhutani[10] assigning shatavarins I and IV as **1** and **5**, respectively, is difficult to understand. It is clear, however, from comparison of spectral data[13] that the compounds they had isolated are identical to shatavarins I (**3**) and IV (**6**) we had previously characterised and distinct from shatavarin V **5**.[13]

The availability of NMR data for shatavarins I, IV and V allows some general observations to be made and conclusions drawn as to the validity of several literature structures. Comparisons of the chemical shifts for the anomeric proton (see Table 2) within the sugar moieties of the steroidal saponins with glycosidic linkages of Type I (i.e., shatavarin V **5**) and Type II (e.g., shatavarins I (**3**) and IV (**6**)), revealed upfield shifts of

Table 2. Comparison of chemical shift of the anomeric protons in pyridine-d_5 of the different sugar moieties of shatavarin V **5** and published structures



Type I **Type II**

Plant		Saponins		δ_H Anomeric proton (ppm)				Ref.
Source	Part	Name	Type	Glu1	Glu2	Rha	Solvent[a]	
Spirostanol glycosides from Asparagus sp.								
A. racemosus	Root	Shatavarin V	I	4.80	5.12	6.36	P	This work
		Shatavarin IV	II	4.87	5.48	5.78	P	4
A. adscendens	Fruits	Asparanin B	II	4.85	5.43	5.88	P	7
A. curillus	Fruits	Fraction III	II	4.85	5.43	5.88	P	8
Furostanol glycosides from Asparagus sp.								
A. racemosus	Root	Shatavarin I	II	4.85	5.46	5.92	P	4
A. curillus	Leaves	Curilloside G	I/II[b]	4.85	5.43	5.88	P	9
A. adscendens	Fruits	Asparoside A	II	4.82	5.41	5.90	P	7

[a] P: pyridine-d_5.
[b] Reported as Type I but NMR comparison indicates Type II.

~0.35 ppm for H-1 of the terminal glucose (Glu2) when linked at the C-2 position (Type II), rather than linkage through C-4 (Type I) of the glucose bonded directly to the sarsasapogenin skeleton. Similarly, a downfield shift of ~0.50 ppm for the anomeric proton of the rhamnose moiety occurs when linked at the C-2 position (Type I) rather than at the C-4 (Type II) position of the 2,4-disubstituted glucose unit.

From inspection of the values for the corresponding protons reported in the literature for asparinin B and 'fraction III' for *A. curillus*, it appears that these have the arrangement of sugars as seen in shatavarin I and indeed are all identical with structure **6** as reported here. Equally, however, curilloside G from *A. curillus* possesses identical anomeric proton shifts to those found in the Type II systems, such as shatavarin I **3** and asparoside A **4**. It is likely therefore that curilloside G is the methyl acetal of shatavarin I. As the original report[9] chemically correlated curilloside G with curilloside H and curillin H, it also appears that these latter compounds are in fact shatavarins I (**6**) and IV (**3**).[14]

In summary, a new steroidal saponin, shatavarin V (**5**), has been isolated from the roots of *A. racemosus*. Although this same structure has been attributed previously to a number of other saponins, mainly on the basis of mass spectroscopy, careful analyses and comparisons of the NMR data for the suite of different candidates confirm the novelty of this structure, and that all the previously reported saponins, curillin H and curilloside H and G, are in fact (revised[4]) shatavarins IV (**3**) and I (**6**).

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12. The saponins (0.5–1 mg) were heated with 90% formic acid (0.4 mL) for 1 h at 100 °C then cooled and concentrated.

The residue was then heated with 2 M TFA (0.3 mL) for 2 h at 120 °C, and after cooling the solution was evaporated to dryness and rinsed twice with methanol (2×0.5 mL). The resulting product was reduced at room temperature for 30 min using a solution of 0.25 M $NaBH_4$ in NH_4OH (0.3 mL) and then quenched by a solution of 10% of acetic acid in MeOH (4×0.5 mL). The reduced product was then acetylated with acetic anhydride–pyridine (1:1) at 100 °C for 1 h. This mixture was diluted with water and extracted with ethyl acetate and the extract analysed by GCMS and compared with authentic standards.

13. The shift of the anomeric protons of the three sugar moieties can be compared for example. Shatavarin I (in CD_3OD):[10] Glucose1: δ_H 4.45; Glucose2 4.69; Rhamnose 4.83. Shatavarin I (in CD_3OD this work): Glucose1: δ_H 4.39; Glucose2 4.62; Rhamnose 4.81. Shatavarin IV (in CD_3OD:$CDCl_3$ 1:1)[10] Glucose1: δ_H 4.42; Glucose2 4.64; Rhamnose 4.86. Shatavarin IV (in CD_3OD:$CDCl_3$ 1:1 this work) Glucose1: δ_H 4.39; Glucose2 4.62; Rhamnose 4.81. Shatavarin V: (in CD_3OD:$CDCl_3$ 1:1 this work) Glucose1: δ_H 4.67; Glucose2 4.36; Rhamnose 5.23.
14. No spectral data is available for asparoside A,[7] 'fraction IVa' or 'fraction IVb'.[8] However, as these compounds appear to have been correlated with the correctly assigned asparoside B (**3**)[7] in the case of asparoside A and 'fraction III' (**6**)[8] in the cases of 'fractions IVa and IVb' we presume these are correctly assigned. Thus, their structures are: asparoside A **4**; 'fraction IVa' **3**; and 'fraction IVb' **4**.