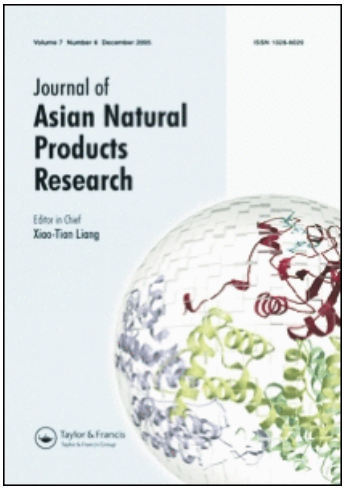

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ASPAROSIDES A AND B, TWO NEW STEROIDAL SAPONINS FROM *ASPARAGUS MEIOCLADOS*

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Asparosides A (**1**) and B (**2**), two new saponins, were isolated from the roots of *Asparagus meioclados*. On the basis of chemical and spectroscopic evidence, their structures were elucidated as 23-*O*-α-arabinopyranosyl-(5β,25s)-spirostan-3β,23α-diol-3-*O*-[β-D-xylopyranosyl(1 → 4)]-β-D-glucopyranoside and 26-*O*-β-glucopyranosyl-5β-furost-20(22)-ene-3β,26-diol-3-*O*-[β-D-xylopyranosyl(1 → 4)]-β-D-glucopyranoside, respectively.

Keywords: *Asparagus meioclados*; Liliaceae; Steroidal saponin; Asparoside A; Asparoside B

INTRODUCTION

Plants belonging to the *Asparagus* genus are widely distributed in the world and have been mainly used in diuretic preparations, laxatives and as remedies for cancer in traditional Chinese medicine. The main active constituents of *Asparagus* were the saponins that showed antifungal, cytotoxic, antiviral and molluscicide properties, etc. [1]. Joshi reported that shatavarin-I in doses of 20–500 mcg/ml produces a specific and competitive block of oxytocin-induced contraction of rats, *in vitro* and *in vivo* [2]. Steroidal saponin from *Asparagus africanus* was discovered to have antiprotozoal effect [3]. Our experiment resulted in two new saponins from the *Asparagus meioclados* grown in Yunnan province, China. In this paper, we describe the isolation, structural elucidation and chemical shift assignments of the new saponins.

RESULTS AND DISCUSSION

Compound **1** was negative to Ehrlich reagents. Its IR spectrum showed the typical signals of spirostanol saponin: 985, 915, 900, and 850 cm^{-1} suggesting that **1** was a spirostanol saponin. The intensity of the signals ($915 > 900\,cm^{-1}$) indicated that the absolute

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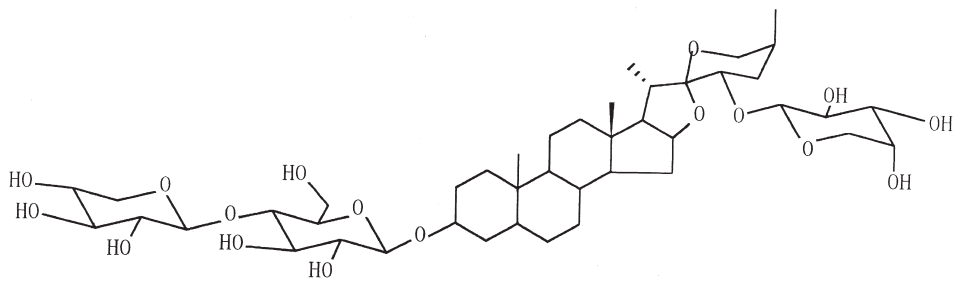
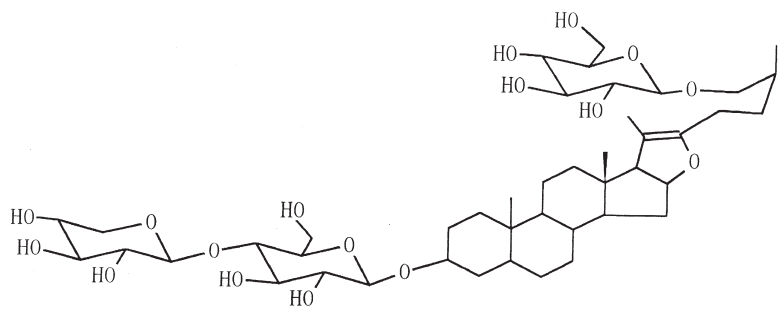
configuration of C-25 was *S*. The analysis of the ESI-MS, ^{1}H-NMR and ^{13}C-NMR spectra data gave the molecular formula of $C_{43}H_{70}O_{17}$.

In the ^{1}H-NNR spectrum of **1** (Fig. 1), four CH_3 signals [δ 0.78(s), 1.02(s), 1.14(d, J = 6.6 Hz), 1.22(d, J = 6.7 Hz)] and the two typical protons signals of H-26 [δ 3.28(d, J = 10.2 Hz, 26-Ha), (4.05, 26-Hb)] in the spirostanol were observed. Comparison of its MS and ^{13}C-NMR spectra with that of sarsasapogenin [4] indicated that an additional hydroxyl was attached to C-23 or C-24. In the HMBC spectrum, H-20 (δ 3.05 ppm) had a cross peak with the signal (δ 72.3 ppm) suggesting that the hydroxyl group was bound to C-23. The cross signal of He-24 (δ 2.20 ppm) with C-24 (δ 34.6 ppm) in the HMQC spectrum and with He-26 (δ 3.28 ppm) in the ^{1}H–^{1}H COSY spectrum excluded the possibility of the hydroxyl group at C-24. The aglycone proposed to be 5β-spirostan-3β,23α-diol was determined by comparing its ^{13}C-NMR data with those of timosaponin G [5] from the rhizome of *Anemarrhena asphodeloides* Bge. The 9 ppm downfield shift of C-23 and the 1.6 ppm upfield shift of C-24 displayed that a sugar moiety was at C-23, which was further confirmed by correlation between C-23 (δ 72.3 ppm) and an anomeric proton (δ 4.95 ppm) in the HMBC spectrum. The absolute configuration of C-23 was assigned as *S* by the correlation between H-20 (δ 3.00 ppm) and H-23 (δ 4.24 ppm) in the NOESY spectrum (Fig. 2).

Positive ion mode ESI-MS gave *m/z* 876 $[M + H_2O]^+$, 859 $[M + H]^+$, 727 $[M - 132 + H]^+$, 577 $[M - 132 - 132 - H_2O + H]^+$, 565 $[M - 132 - 162 + H]^+$, 432 $[M - 132 - 132 - 162]^+$, 415 $[M - 132 - 132 - 162 - H_2O + H]^+$ peaks. On acid hydrolysis, it gave glucosyl, xylosyl and arabinosyl units, identified by comparing with authentic samples on TLC (solvent system d, glucose: R_f = 0.23; arabinose: R_f = 0.52; xylose: R_f = 0.58). The coupling constants and ^{13}C-NMR data of the anomeric signals revealed the β configuration for glucose (J = 7.7 Hz, δ 102.8 ppm) and xylose (J = 7.6 Hz, δ 105.5 ppm) while α configuration for the arabinose (J = 7.7 Hz, δ 106.2 ppm). The linkage of the sugars was deduced from the ^{3}J-correlations between the anomeric



1



2

FIGURE 1 The structures of **1** and **2**.

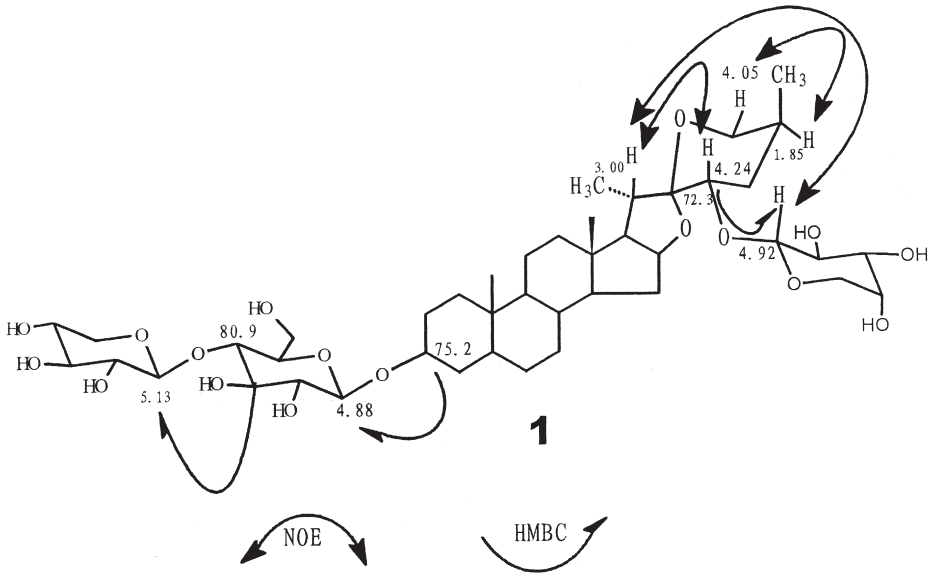


FIGURE 2 The key HMBC and NOE correlations of **1**.

protons and respective carbons, Hg-1 (δ 4.88 ppm) with C-3 (δ 75.2 ppm), Hx-1 (δ 5.13 ppm) with Cg-4 (δ 80.9 ppm) and Ha-1 (δ 4.92 ppm) with C-23 (δ 72.3 ppm) in the HMBC spectrum. All the proton and carbon signals were fully assigned by $^1H-^1H$ COSY, TOCSY, HMQC and HMBC experiments (see Tables I and II). In conclusion, the structure of **1**

TABLE I ^{13}C-NMR chemical shifts of compounds **1** and **2** (125 MHz in C_5D_5N, δ in ppm)

Carbon	**1**	**2**	*Carbon*	**1**	**2**
1	30.8(t)	30.9(t)	15	31.8(d)	31.4(d)
2	26.8(t)	26.8(t)	16	81.4(d)	84.6(d)
3	75.2(d)	75.2(d)	17	62.8(d)	64.7(d)
4	30.6(t)	30.6(t)	18	17.2(q)	14.4(q)
5	36.9(d)	37.0(d)	19	23.8(q)	23.9(q)
6	26.9(t)	27.0(t)	20	36.2(d)	103.5(s)
7	26.7(t)	27.0(t)	21	14.5(q)	11.8(q)
8	35.3(d)	35.2(d)	22	111.2(s)	152.4(s)
9	40.1(d)	40.1(d)	23	72.3(d)	34.4(d)
10	35.2(s)	35.2(s)	24	34.6(t)	23.6(t)
11	21.1(t)	21.3(t)	25	30.4(d)	33.7(d)
12	40.7(t)	40.2(t)	26	64.0(t)	75.0(t)
13	41.3(s)	43.8(s)	27	17.7(q)	17.2(q)
14	56.2(d)	54.8(d)			

	1			**2**		
	3-Glu	Xyl	23-Ara	3-Glu	Xyl	26-Glu
1	102.8(d)	105.5(d)	106.2(d)	103.0(d)	102.5(d)	105.2(d)
2	74.5(d)	74.4(d)	72.1(d)	75.0(d)	74.7(d)	75.0(d)
3	76.8(d)	78.5(d)	74.3(d)	76.5(d)	78.4(d)	78.5(d)
4	80.9(d)	70.7(d)	68.6(d)	81.0(d)	70.8(d)	71.8(d)
5	76.5(d)	67.3(t)	65.8(t)	76.6(d)	67.4(t)	78.6(d)
6	61.8(t)			61.9(t)		62.9(t)

was elucidated as 23-*O*-α-arabinopyranosyl-(5β,25s)-spirostan-3β,23α-diol-3-*O*-[β-D-xylopyranosyl(1 → 4)]-β-D-glucopyranoside, named asparoside A.

Compound **2** was positive to Ehrlich reagent and there was no typical spiranol signal in its IR spectrum. Therefore, it was deduced to be a furostanol saponin. The ESI-MS, ^{1}H-NMR and ^{13}C-NMR spectra suggested that the molecular formula should be $C_{43}H_{70}O_{16}$.

In the ^{1}H-NMR, **2** gave four CH_3 signals [δ 0.68(s), 0.85(s), 1.02(d, $J = 6.4$ Hz), 1.61(s)] clarifying that CH_3 (δ 1.61, s) combined with an electron acceptor group. EI-MS *m/z*: 181, 163 peaks showed that there was a C = C bond between the C-20 and C-22 [6]. The signals at δ 103.5 and 152.4 ppm in ^{13}C-NMR spectrum further verified the presence of the C = C–O bond, consequently, the aglycone was deduced to be 20(22)-ene-furostanol. The comparison of the ^{13}C-NMR spectral data with the known anemarsaponin B [6] led to the result that **2** had the same aglycone as that of anemarsaponin B, that is 5β-furost-20(22)-ene-3β,26-diol. The downfield shifts of the C-3 and C-26 demonstrated that they were glycosided.

ESI-MS showed 873 $[M + H]^+$, 741 $[M - 132 + H]^+$, 579 $[M - 132 - 162 + H]^+$ peaks and EI-MS gave 578 $[M - 132 - 162]^+$, 416 $[M - 132 - 162 - 162]^+$ peaks. The NMR spectra indicated three anomeric protons [δ 4.82(d, $J = 7.7$ Hz], 4.90(d, $J = 8.1$ Hz), 5.14(d, $J = 7.7$ Hz)] and ^{13}C-NMR had three anomeric carbons (δ 103.0, 105.2 and 105.6 ppm, respectively). On acid hydrolysis, **2** produced glucose ($R_f = 0.23$, solvent system d) and xylose ($R_f = 0.58$, solvent system d). From the spectral and chemical data, two glucoses and one xylose in **2** were concluded. A detailed comparison of the ^{1}H- and ^{13}C-NMR

TABLE II HMQC/HMBC correlations of asparoside A

Position	*Proton*	*HMQC (δ C in ppm)*	*HMBC (δ H in ppm)*
16	4.60	81.4	1.40(H-15), 1.90(H-17)
17	1.90	62.8	2.00(H-15), 1.02(H-18), 1.22(H-21), 3.00(H-20)
20	3.00	36.2	1.22(H-21), 1.90(H-17)
21	1.22	14.5	1.90(H-17), 3.00(H-20), 1.40(H-15)
22	–	111.2	1.22(H-21), 3.28(H-26), 3.00(H-20), 1.22(H-21)
23	4.24	72.3	2.40(H-24), 4.92(Ha-1)
24	2.20, 2.40	34.6	1.14(H-27), 3.28(H-26)
25	1.85	30.4	1.14(H-27), 2.40(H-24)
26	3.28,4.05	64.0	1.14(H-27)
27	1.14	17.7	2.40(H-24), 4.05(H-26), 3.28(H-26), 1.85(H-25),
		Glu	
1	4.88(d, $J = 7.7$ Hz)	102.8	4.04(Hg-2)
2	4.04	74.4	4.32(Hg-4)
3	4.26	76.8	4.04(Hg-2), 4.32(Hg-4)
4	4.32	80.9	5.13(Hx-1), 4.26(Hg-3)
5	3.84	76.5	4.32(Hg-4)
6	4.44,4.54	61.8	4.32(Hg-4)
		Xyl	
1	5.13(d, $J = 7.6$ Hz)	105.5	3.64(Hx-5), 4.00(Hx-2)
2	4.00	74.7	4.10(Hx-4), 4.22(Hx-5)
3	4.24	78.5	4.00(Hx-2), 3.64(Hx-5)
4	4.10	70.7	3.64(Hx-5), 4.10(Hx-5), 4.24(Hx-3)
5	3.64,4.22	67.3	
		Ara	
1	4.92(d, $J = 7.7$ Hz)	106.2	3.82(Ha-5), 4.40(Ha-2), 4.22(Ha-3), 4.25(H-23)
2	4.40	72.1	4.92(Ha-1)
3	4.22	74.3	4.34(Ha-4)
4	4.34	68.6	4.28(Ha-5)
5	3.82, 4.28	65.8	4.92(Ha-1)

Hg-1 means the 1st proton of glucose; Hx-1, the 1st proton of xylose; Ha-1, the 1st proton of arabinose, etc.

data for compound **2** and aspafilioside C [7] showed that the saccharide chain was identical in both compound. The coupling constants determined the β configuration for two glucoses ($J = 7.7$ Hz; $J = 8.1$ Hz) and the xylose ($J = 7.7$ Hz). Partial acid hydrolysis resulted in compound **2a**, proved to be aspafilioside A [7] by TLC and IR spectrum. The obvious IR absorption of **2a** at 918 and 900 cm^{-1} ($918 > 900\,cm^{-1}$) suggested the *S* absolute configuration on C-25 of **2** and **2a**. Thus **2** was characterized as 26-*O*-β-glucopyranosyl-5β-furost-20(22)-ene-3β,26-diol-3-*O*-[β-D-xylopyranosyl(1 → 4)]-β-D-glucopyranoside, named asparoside B.

EXPERIMENTAL SECTION

General Experimental Procedures

Mps were determined on a Kofler micromelting point apparatus and are uncorrected. $[\alpha]_D$ was carried out on Perkin-Elmer polarimeter model 241. IR were recorded on a JASCO FT-IR-230 in KBr pellet. EI-MS were performed on HP5989 and ESI-MS were carried on PE MARINER. ^{1}H- and ^{13}C-NMR were measured on Varian UNITY 500 with TMS as internal standard. CC: silical gel H (200–300 mesh. Qingdao Plant of Oceanic Chemistry). TLC: silica gel 254 (Yantai), preparative-TLC: silica gel (Yantai). Spots on the plate were observed under UV light and visualized by spraying with 10% H_2SO_4 followed by heating. Sugars were ordered from the Shanghai Company of Chemical Agents. Solvents for CC: $CHCl_3$–MeOH (8:2) (solvent a), $CHCl_3$–MeOH–H_2O (58:28:4) (solvent b), $CHCl_3$–MeOH–H_2O (58:28:1) (solvent c). EtOAc–MeOH–H_2O–HOAc (6:2:1:1) (solvent d).

Plant Material

The plant material was purchased from Yunnan Company of Chinese Material Medica, Kunming, P.R. China, in July, 1995. The crude drug was identified as the root of *A. meioclados* (Liliaceae) by Dr Dao-Feng Chen. A voucher specimen (TMD9501) is deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, P.R. China.

Extraction and Isolation

The plant material of *A. meioclados* (10 kg) was grounded and exhaustively extracted with 95% alcohol, the extract was concentrated under vacuum after centrifugation, dissolved in water, defatted with EtOAc, and extracted with *n*-Butanol saturated with water. From the combined *n*-Butanol phase, solvent was removed to get saponin mixture. The residue (300*g*) was chromatographed on 2 kg silica gel with gradient $CHCl_3$–MeOH solvents ($90:10 \sim 0:100$) as eluents to give fractions 1–5. Part of fraction 2 (3.5 g) and 4 (513 mg) were submitted to repeated column chromatography on silica gel (200–300 mesh) to afford compound **1** (446 mg, solvent system a) and **2** (15 mg, solvent system b).

Asparoside A: White, amorphous powder, $C_{43}H_{70}O_{17}$. Mp 196–198°C. $[\alpha]_D = -50.4$ ($c = 2.13$ MeOH–$CHCl_3 = 1:1$). Negative to Ehrlich reagents. IR(KBr) ν_{max} =980, 918, 900 and 850 cm^{-1}. (intensity $918 > 900\,cm^{-1}$) (+)ESI-MS *m/z*: 876 $[M + H_2O]^+$, 859 $[M + H]^+$, 841 $[M - H_2O + H]^+$, 727 $[M - 132 + H]^+$, 577 $[M - 132 - 132 - H_2O + H]^+$, 565 $[M - 132 - 162]^+$, 432 $[M - 132 - 132 - 162]^+$, 415 $[M - 132 - 132 - 162 - H_2O + H]^+$. ^{1}H-NMR($C_5D_5N$, 500 MHz): δ 0.78(3H, s), 1.02(3H, s), 1.14(3H, d, $J = 6.6$ Hz), 1.22(3H, d, $J = 6.7$ Hz), 3.28(1H, d, $J = 10.2$ Hz), 4.05(1H, m),

4.82(1H, d, $J = 7.7$ Hz), 5.02(1H, d, $J = 7.7$ Hz), 5.48(1H, d, $J = 7.7$ Hz). ^{13}C-NMR data are given in Table I.

Asparoside B: White, amorphous powder, $C_{43}H_{70}O_{16}$. Mp 278–280°C $[\alpha]_D = -24.0$ ($c = 2.37$ MeOH–$CHCl_3 = 1:1$). Positive to both Libermann–Burchard and Ehrlich reagents. IR(KBr) $\nu_{max} = 3440$, 2940, 1450 and 1030 cm^{-1}, respectively. (+)ESI-MS *m/z*: 873 $[M + H]^+$, 741 $[M - 132 + H]^+$, 579 $[M - 132 - 162 + H]^+$, EI-MS *m/z*: 578(11), 415(50), 397(27), 181(100), 163(35). ^{1}H-NMR(C_5D_5N, 500 MHz): δ0.68(3H, s), 0.85(3H, s), 1.02(3H, d, $J = 6.4$ Hz), 1.61(3H, s), 3.75(2H, m, 26-2H), 3.90(1H,m), 4.82(1H,d, $J = 7.7$ Hz), 4.90(1H, d, $J = 8.1$ Hz), 5.14(1H, d, $J = 7.7$ Hz). ^{13}C-NMR data are given in Table II.

Partially Acid Hydrolysis of 2

Fourteen milligrams of compound **2** were partially hydrolysed with 8 ml, 2 mol/l HCl/EtOH(85%) at 90°C under reflux for 30 min. The reaction mixture was evaporated to dryness, dissolved in MeOH. TLC was carried on with solvent system d, glucose and xylose were identified by comparing with authentic samples (glucose: $R_f = 0.23$; xylose: $R_f = 0.58$). The residue was eluted on preparative TLC to give **2a** with solvent system a, identified as aspafilioside A ($R_f = 0.45$, $CHCl_3$–$CH_3OH = 85:15$) by TLC and IR spectrum, and it has 25-*S* configuration on IR spectrum (918 > 900 cm^{-1}).

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