Cytotoxic Steroidal Saponins from the Rhizomes of *Asparagus oligoclonos*

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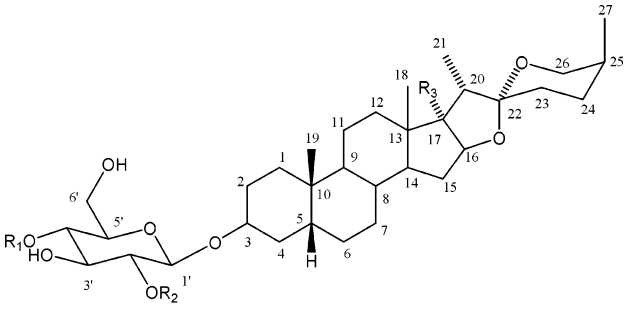
Two new steroidal saponins, aspaoligonins A (**2**) and B (**3**), were isolated from the methanolic extract of the rhizomes of *Asparagus oligoclonos* together with a known spirostanol saponin, asparanin A (**1**). Aspaoligonins A and B were characterized as (25*S**)-5*β*-spirostan-3*β*,17α-diol 3-*O*-*β*-D-glucopyranosyl (1→2)-*β*-D-glucopyranoside and (25*S**)-5*β*-spirostan-3*β*,17α-diol 3-*O*-α-L-rhamnopyanosyl (1→4)-[*β*-D-xylopyranosyl-(1→2)]-*β*-D-glucopyranoside, respectively, by spectrometric analyses including HRFABMS and 2D NMR. Compounds **1**−**3** were cytotoxic against five human tumor cell lines with IC_{50} values of 2.05−2.84 *μ*g/mL.

The genus *Asparagus*, which is widely distributed in eastern Asia including China, Korea, and Japan, has over 300 species. Steroidal saponins have been isolated from many of these species.[1−5] The rhizomes of *Asparagus oligoclonos* Maxim (Liliaceae) (Bangwoolbizaru in Korean) have been used as cough and asthma remedies in far eastern countries. In the course of searching for cytotoxic natural compounds, three steroidal saponins were isolated from the rhizomes of *A. oligoclonos*. The present paper describes the extraction, isolation, purification, structure elucidation, and cytotoxic effect of the isolated saponins (**1**−**3**) on five human tumor cell lines.

The rhizomes of *A. oligoclonos* were extracted with MeOH at room temperature. The MeOH extract was partitioned between H_2O and Et_2O. The H_2O layer was then extracted with saturated *n*-BuOH. The *n*-BuOH-soluble fraction was chromatographed on an open silica gel column, and the active fractions were purified further by reversed-phase preparative HPLC. Subsequent crystallization of the active fractions afforded **1**−**3**.

Asparanin A (**1**) was obtained as a white amorphous powder. The molecular formula was postulated as $C_{39}H_{64}O_{13}$ from the positive ion LRFABMS (*m/z* 763.2 $[M + Na]^+$) combined with ^{13}C NMR data. Acid hydrolysis of **1** gave only D-glucose as a sugar moiety. The ^1H and ^{13}C NMR data of **1** were identical to those of previously reported (25*S**)-5*β*-spirostan-3*β*-ol-3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside.[2]

The molecular formula of compound **2** ($C_{39}H_{64}O_{14}$) was determined on the basis of ^{13}C NMR and the HRFAB mass spectrum. The IR spectrum of **2** showed absorption for hydroxyl groups (3370 and 1070 cm^{-1}) and characteristic absorption bands of a (25*S**)-spirostanol steroidal type skeleton (982, 917, 896, and 850 cm^{-1}, intensity 917 > 896 cm^{-1}).[1,4,6] The 25*S**-configuration of the steroidal saponin was also confirmed by the two proton signals [*δ* 4.05 (1H, m) and 3.27 (1H, d, *J* = 10.70 Hz)], which corresponded to the H-26 in the ^1H NMR[3,7] and the higher field resonance of C-27 (*δ* 16.2) compared to the ^{13}C NMR shift of (25*R**)-spirostanes (*δ* 17.1−17.2).[8] Four methyl proton signals characteristic of the spirostane saponin appeared at *δ* 0.96 (s, H-18), 1.02 (s, H-19), 1.23 (d, *J* = 7.08 Hz, H-21), and 1.07 (d, *J* = 7.08 Hz, H-27). In the ^{13}C NMR, a quaternary carbon signal was observed at *δ* 90.0, which was not found in **1**. In HMBC analysis, this carbon showed a $^3J_{C-H}$ correlation with the signal at δ_H 1.20 (3H, d), which was assigned as H-21 (CH_3) from its splitting pattern and $^3J_{C-H}$ correlation with C-22 (*δ* 110.3). The correlation spot between δ_C 90.0 and δ_H 0.93 (3H, s, H-18) was also observed. These observations suggested the presence of a hydroxyl group at C-17. In addition, the ^{13}C signals of carbons near C-17, C-12 (*δ* 40.3), C-13 (*δ* 40.9), C-14 (*δ* 56.5), C-16 (*δ* 81.3), and C-21 (*δ* 14.9) of **1** were very different from those of **2** (*δ* 32.5, 45.5, 52.8, 90.4, and 9.5, respectively). The existence of a 17α-hydroxyl group in the structurally related spirostane steroids such as pennogenin[9] and nigruminin II[10,11] has been reported (the chemical shifts of α-hydroxyl C-17 were near *δ* 90); however, this is the first report of 17α-hydroxysarsasapogenin. Two anomeric carbon signals (*δ* 101.9 and 105.9) and two anomeric



1 R_1 = H, R_2 = Glc R_3 = H

2 R_1 = H, R_2 = Glc R_3 = OH

3 R_1 = Rha, R_2 = Xly R_3 = OH

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Table 1. NMR Data (δ) for the Aglycone Moieties of Compounds **2** and **3**

		^{13}C δ		^{1}H δ (J in Hz)	
position		**2**	**3**	**2**	**3**
1	CH_2	31.0	31.0	1.80 m	1.76 m
2	CH_2	27.0	26.9	1.17 m	2.18 m
3	CH	75.3	76.0	4.27 m	4.17 m
4	CH_2	30.7	30.8	1.80 m	1.76 m
5	CH	36.9	37.2	2.20 m	2.14 m
6	CH_2	26.8	26.8	1.93 m	2.18 m
7	CH_2	26.7	26.7	1.93 m	2.18 m
8	CH	36.1	36.2	1.59 m	1.58 m
9	CH	40.1	40.2	1.32 m	1.32 m
10	C	35.3	35.3		
11	CH_2	21.0	21.1	1.50 m	1.48 m
12	CH_2	32.5	32.5	1.47 m	1.76 m
13	C	45.5	45.5		
14	CH	52.8	52.9	2.10 m	2.14 m
15	CH_2	31.6	31.6	1.80 m	1.76 m
16	CH	90.4	90.4	4.44 m	4.41 m
17	C	90.0	90.1		
18	CH_3	17.4	17.4	0.96 s	0.93 s
19	CH_3	24.1	23.9	1.02 s	1.08 s
20	CH	45.3	45.3	2.23 m	2.18 m
21	CH_3	9.5	9.5	1.23 d (7.08)	1.20 d (7.08)
22	C	110.3	110.3		
23	CH_2	25.7	25.7	1.38 m	1.32 m
24	CH_2	26.5	26.5	1.02 m	1.32 m
25	CH	27.4	27.4	1.59 m	1.58 m
26	CH_2	64.9	64.9	4.05 m, 3.27 d (10.70)	4.02 m, 3.24 d (10.56)
27	CH_3	16.2	16.2	1.07 d (7.08)	1.04 d (7.08)

protons [δ 4.96 (d, J = 7.32 Hz) and 5.40 (d, J = 7.80 Hz)] were observed. Acid hydrolysis of **2** with 1 M hydrochloric acid in dioxane−H_2O (1:1) gave D-glucose as the only sugar moiety. The ^{1}H and ^{13}C NMR data of **1** and **2** suggested that both compounds had the same sugar moieties and configurations (β-anomers).[1,2] It was evident that the sugar moiety of **2** was linked at C-3 from the correlation between δ 4.96 (H-1′ for inner glucose) and δ 75.3 (C-3) in the HMBC spectrum. The upfield shift of C-1′ (δ 101.9) of **2** due to the β -effect[12] indicated that the terminal glucose was linked at C-2′ (δ 83.1) of the inner glucose. The linkage pattern of the sugar units was also confirmed by the correlation between δ 5.40 (H-1″) and 83.13 (C-2′) in the HMBC spectrum. All carbon signals of **2** were assigned on the basis of 2D NMR data including ^{1}H−^{1}H COSY, DEPT, HMQC, and HMBC, as well as the comparison of NMR data with that of compounds reported previously.[1,7−11,13,14] From these results, **2** was characterized as (25*S**)-5β-spirostan-3β,17α-diol 3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside and named aspaoligonin A.

Compound **3** was obtained as a white amorphous powder. The molecular formula ($C_{44}H_{72}O_{17}$) was deduced from ^{13}C NMR data and the HRFAB mass spectrum. The IR spectrum of **3** also showed absorption bands characteristic of a 25(*S**)-spirostanol moiety (982, 914, 897, and 848 cm^{-1}, intensity 914 > 897 cm^{-1}).[1,4,6] In the ^{13}C NMR spectrum, ^{13}C signals for the aglycone moiety were very similar to those of **2**, suggesting that they have the same aglycone. Acid hydrolysis of **3** gave D-glucose, L-rhamnose, and D-xylose (1:1:1) with several degraded aglycones. Three anomeric carbon signals of **3** were observed at δ 101.9 (C-1′), 101.6 (C-1″), and 105.7 (C-1‴), which corresponded to three anomeric proton signals at δ 4.79 (d, J = 6.84 Hz, H-1′), 6.33 (d, J = 1.20 Hz, H-1″), and 4.99 (d, J = 7.80 Hz, H-1‴), respectively. The ^{13}C chemical shifts of the sugar moiety were very similar to those of monodesmosides or bisdesmosides having terminal rhamnose and xylose.[15−18] The ^{13}C NMR assignments of the sugar moiety of **3** were

Table 2. NMR Data for the Sugar Moieties of Compounds **2** and **3**

		^{13}C δ		^{1}H δ (J in Hz)	
position		**2**	**3**	**2**	**3**
3-*O*-Glc					
1′	CH	101.9	101.9	4.96 d (7.32)	4.79 d (6.84)
2′	CH	83.1	81.7	4.26 m	4.17 m
3′	CH	78.2[a]	77.6	3.97 m	4.11 m
4′	CH	71.8	76.9	4.34 m	4.17 m
5′	CH	78.5	76.2	3.87 m	3.75 m
6′	CH_2	62.9[b]	61.8	4.58 m	4.41 m
Glc					
1″	CH	105.9		5.40 d (7.80)	
2″	CH	77.0		4.07 m	
3″	CH	78.1[a]		4.28 m	
4″	CH	71.6		4.19 m	
5″	CH	77.9		3.87 m	
6″	CH_2	62.7[b]		4.30 m	
Rha					
1‴	CH		101.6		6.33 d (1.20)
2‴	CH		72.3		4.75 m
3‴	CH		72.7		4.52 m
4‴	CH		74.0		4.30 m
5‴	CH		69.5		4.75 m
3‴	CH_2				4.11 m
4‴					
5‴					4.23 m, 3.64 m

[a,b] Assignments with the same superscripts may be exchangeable with each other in each column.

Table 3. Cytotoxicity of Compounds **1**−**3**

	IC_{50} (μg/mL)				
compound	A549[a]	SK-OV-3[b]	SK-MEL-2[c]	XF498[d]	HCT15[e]
1	2.05	2.43	2.41	2.56	2.47
2	2.25	2.66	2.53	2.84	2.59
3	2.55	2.73	2.59	2.51	2.57
adriamycin[f]	0.04	0.18	0.03	0.12	0.08

[a] Human lung carcinoma. [b] Human adenocarcinoma, ovary malignant ascites. [c] Human malignant melanoma, metastasis to skin of thigh. [d] Human central nerve system tumor. [e] Human colon adenocarcinoma. [f] Positive antitumor control.

achieved by referring to those of authentic methyl glycosides and taking into account the known effects of *O*-glycosylation, and indicated the presence of a terminal α-rhamnopyranosyl unit, a terminal β-xylosyl, and a 2,4-disubstituted β-glucopyranosyl unit.[12] In the HMBC spectrum, the correlations between H-1‴/C-4′ and H-1‴/C-2′ indicated that rhamnose and xylose were linked at C-4′ and C-2′ of the inner glucose, respectively. The downfield-shifted carbon signals at C-4′ (δ 76.9) and C-2′ (δ 81.7) of glucose also supported the above observations. Thus, **3** was determined to be (25*S**)-5β-spirostan-3β,17α-diol 3-*O*-α-L-rhamnopyanosyl (1→4)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranoside and was named aspaoligonin B.

Steroidal saponins **1**−**3** were evaluated for cytotoxicity in vitro against human tumor cell lines A549, SK-OV-3, SK-MEL-2, XF498, and HCT15. The IC_{50} values are listed in Table 3. Compounds **1**−**3** showed significant levels of cytotoxicity (IC_{50} 2.05−2.84 μg/mL), which were similar to those of carboplatin (IC_{50} 2.28−2.95 μg/mL); however, they were less potent than adriamycin, which was used as a positive control. The cytotoxic saponins from *A. officinalis*[17,18] and *A. cochinchinensis*[20] have been reported, but this is the first report of the cytotoxic steroidal saponins from *A. oligoclonos*.

Experimental Section

General Experimental Procedures. Optical rotation was recorded on a JASCO P-1020 polarimeter. IR spectra were carried out on a Perkin-Elmer FT-IR spectrophotometer. ^{1}H,

^{13}C, DEPT, COSY, HMQC, and HMBC NMR spectra were recorded on a JEOL JNM-LA400 FT-NMR spectrometer in pyridine-d_5. FABMS was measured with a JEOL HX-110/110A tandem mass spectrometer. GC was performed on a Varian CP-3800 with a flame ionization detector. Silica gel (silica gel 60, 70−230 mesh, Merck) was used for open column chromatography. TLC was performed on Merck TLC plates precoated silica gel 60 F_{254} with visualization by spraying with 10% H_2SO_4. Preparative HPLC was carried out on a SpectraSYSTEM P2000 pump connected with a Alltech 500 ELSD detector, using a GL Sciences Inertsil PREP ODS column (20 i.d. × 250 mm, 10 μm).

Plant Material. The rhizomes of *A. oligoclonos* were collected at the Yeongnam Agricultural Research Institute, NICS, RDA, Miryang, Korea, and identified by Dr. Jung-Sook Sung of NICS, RDA, Suwon, Korea. A voucher specimen (No. MPS 000715) has been deposited in the Herbarium of Ginseng & Medicinal Crops Division, NICS.

Extraction and Isolation. The rhizomes of *A. oligoclonos* (3 kg) were dried in an oven at 60 °C, then the dried material was powdered and extracted with MeOH (5 × 10 L) at room temperature. After filtration, the MeOH extract was concentrated in vacuo. The viscous concentrate (1 kg) was partitioned between H_2O−Et_2O (1:1, 4 L) three times, and the H_2O layer was extracted consecutively with *n*-BuOH saturated with H_2O (5 × 4 L). A portion (30 g) of the *n*-BuOH extract (125 g) was applied to a silica gel column (5 × 40 cm, 70−230 mesh, 350 g) and eluted with a $CHCl_3$−MeOH−H_2O gradient solvent system (9:3:1 → 7:3:1 → 65:35:10 → 6:4:1) to yield 58 fractions (each 250 mL). Compound **1** (36 mg) and compound **3** (94 mg) were obtained from fractions 25/26 and fractions 23/24, respectively. Fraction 33 was further purified by means of preparative HPLC eluted with an acetonitrile−H_2O gradient (0 to 30% acetonitrile for 20 min, 30 to 55% acetonitrile for 10 min, and 100% MeOH for 20 min) at a flow rate of 7 mL/min to yield **2** (60 mg).

Asparanin A (1): identified by comparing its ^1H and ^{13}C NMR data with those in refs 1 and 2.

Aspaoligonin A (2): white amorphous powder; $[\alpha]^{25}_D$ −14.29° (*c* 0.05, pyridine); IR (KBr) ν_{max} 3370 (OH), 2931 (CH), 1070 (OH), 982, 917, 896, 850 cm^{-1} (intensity 917 > 896, 25*S* spiroketal); ^1H (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectral data, see Tables 1 and 2; HRFABMS (positive ion mode) *m/z* 779.4223 $[M + Na]^+$ (calcd for $C_{39}H_{64}O_{14}Na$, 779.4194).

Aspaoligonin B (3): white amorphous powder; $[\alpha]^{25}_D$ −62.07° (*c* 0.03, pyridine); IR (KBr) ν_{max} 3397 (OH), 2930 (CH), 1047 (OH), 982, 914, 897, 848 cm^{-1} (intensity 914 > 897, 25*S* spiroketal); ^1H (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectral data, see Tables 1 and 2; HRFABMS (positive ion mode) *m/z* 895.4667 $[M + Na]^+$ (calcd for $C_{44}H_{72}O_{17}Na$, 895.4667).

Acid Hydrolysis of 1, 2, and 3. A solution of each of **1** (5.3 mg), **2** (5.0 mg), and **3** (5.6 mg) in 5 mL of 1 M HCl (dioxane−H_2O, 1:1) was refluxed at 100 °C for 3.5 h under N_2 atmosphere. The reaction mixture was neutralized with 2 M NaOH, diluted with water, and extracted with Et_2O. Each aqueous layer was concentrated in vacuo to appropriate volume, and the solution was examined by TLC with two solvent systems for sugar analysis (A: BuOH−Me_2CO−H_2O−HOAc = 4:5:1:1, B: EtOAc−BuOH−H_2O−HOAc = 4:4:1:1). R_f values of rhamnose, xylose, and glucose were 0.72, 0.64, and 0.48 in solvent A and 0.56, 0.46, and 0.25 in solvent B, respectively. The remaining aqueous layer was concentrated to dryness to give a residue, which was dissolved in dry pyridine, to which was added L-cysteine methyl ester hydrochloride.[22] The reaction mixtures were heated for 2 h at 60 °C and concentrated to dryness with N_2 gas. Trimethylsilylimidazole was added to the residue, and the mixture was heated for 1 h at 60 °C, followed by extraction with *n*-hexane and water. The organic layers were analyzed by GC [column: OV-17 (0.32 mm × 30 m), detector: FID, detector temp: 270 °C, injector temp: 270 °C, column temp: 230 °C, carrier gas: He]. Peaks corresponding to D-glucose, L-rhamnose, and D-xylose appeared at 11.65, 9.96, and 9.07 min, respectively. In this procedure, the aglycones of **2** and **3** decomposed under acidic conditions.

Cytotoxicity Assay. The cytotoxicity on human tumor cell lines was evaluated by the Pharmacology Research Group, Medicinal Science Division, Korea Research Institute of Chemical Technology, Korea, using the standard SRB assay[21] on A549 (human lung carcinoma), SK-OV-3 (human adenocarcinoma, ovary malignant ascites), SK-MEL-2 (human malignant melanoma, metastasis to skin of thigh), XF-498 (human central nerve system tumor), and HCT 15 (human colon adenocarcinoma). Adriamycin was used as a positive control.

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