Two New Cytotoxic Spirostane-Steroidal Saponins from the Roots of *Bletilla striata*

by **Jong Eel Park**[a]), **Kyeong Wan Woo**[a]), **Sang Un Choi**[b]), **Je Hyun Lee**[c]), and **Kang Ro Lee***[a])

[a]) Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea (phone: +82-31-2907710; fax: +82-31-2907730; e-mail: krlee@skku.ac.kr)

[b]) Korea Research Institute of Chemical Technology, Teajeon 305-600, Korea

[c]) Department of Korean Medicine, Dongguk University, Gyeongju-si, Gyeongbuk 780-714, Korea

Two new spirostane-steroidal saponins, bletilnoside A (**1**) and bletilnoside B (**2**), together with five known compounds, **3**–**7**, were isolated from the roots of *Bletilla striata* (THUNB.) REICHB. F. The structures of the new compounds were determined based on their 1D- and 2D-NMR spectral data. The isolated compounds **1**–**7** were tested for cytotoxicity against four human tumor cells (A549, SK-OV-3, SK-MEL-2, and HCT15) *in vitro* using a sulforhodamin B bioassay, and compounds **1**, **2**, and **5** showed significant cytotoxicities against all tested tumor cell lines with IC_{50} values ranging from 3.98 ± 0.16 to 12.10 ± 0.40 μM.

1. Introduction. – *Bletilla striata* (THUNB.) REICHB. F. (Orchidaceae) is a perennial herb that is widely distributed throughout Northeast Asia. The roots of *B. striata* have been used as a hemostatic agent in Korean traditional medicine [1]. Phenanthrenes, stilbenes, triterpenoids, and anthocyanins have been isolated from this source [2–9]. Its MeOH extract shows antimitotic [10], and antimicrobial activities [11]. In the course of our continuing search for potential lead compounds in Korean traditional medicinal plants, we studied the MeOH extract of *B. striata* roots and isolated two new spirostane steroidal saponins, bletilnoside A (**1**) and bletilnoside B (**2**), together with five known compounds, **3**–**7** (*Fig. 1*). The structures of the new compounds were elucidated by spectroscopic and chemical methods. Here, we report the isolation and structure determination of the isolated spirostane-steroidal saponins. The isolated compounds, **1**–**7**, were evaluated for their cytotoxicities against four human tumor cells *in vitro* using a sulforhodamin B bioassay.

2. Results and Discussion. – 2.1. *Structure Elucidations of New Compounds.* Compound **1** was obtained as a white amorphous powder. The molecular formula was deduced as $C_{38}H_{62}O_{12}$ from the *quasi*-molecular ion ($[M+H]^+$) peak at *m/z* 711.9083 in its positive-ion mode HR-FAB-MS spectrum. The ^{1}H-NMR spectrum (*Table 1*) of **1** showed signals of two tertiary Me groups at δ(H) 1.58 (*s*, Me(19)) and 1.03 (*s*, Me(18)), two secondary Me groups at δ(H) 1.41 (*d*, $J = 7.0$, Me(21)) and 1.35 (*d*, $J = 7.0$, Me(27)), three O-bearing CH groups at δ(H) 4.80–4.90 (*m*, H–C(16)), 4.66 (br. *s*, H–C(1)), and 4.45 (br. *s*, H–C(3)), and two O-bearing CH_2 groups at δ(H) 4.34 (*dd*, $J = 10.5$, 2.0, H_a–C(26)) and 3.64 (*dd*, $J = 10.5$, 2.0, H_b–C(26)). The ^{13}C-NMR and DEPT experiments displayed 27 C-atom signals including those of four Me groups at δ(C) 19.4

© 2014 Verlag Helvetica Chimica Acta AG, Zürich

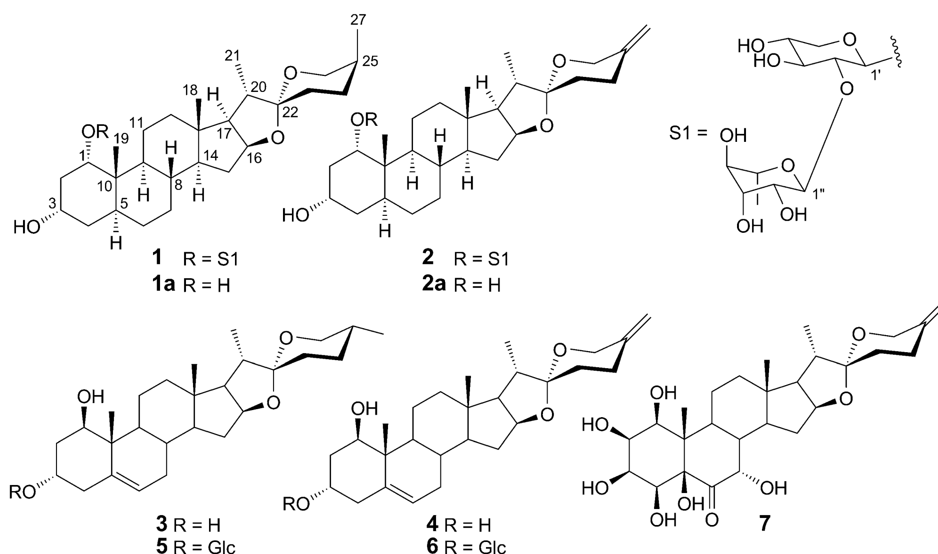

Fig. 1. *Structure of compounds* **1**–**7**, *isolated from* B. striata

(C(19)), 16.7 (C(18)), 16.3 (C(27)), and 15.0 (C(21)); three O-bearing CH groups at δ(C) 81.3 (C(16)), 76.0 (C(1)), and 67.4 (C(3)); one O-bearing CH_2 group at δ(C) 65.2 (C(26)), seven CH groups at δ(C) 63.0 (C(17)), 56.4 (C(14)), 42.0 (C(9)), 35.6 (C(8)), 31.8 (C(5)), and 27.6 (C(25)); nine CH_2 groups at δ(C) 40.3 (C(12)), 34.2 (C(15)), 32.2 (C(11)), 27.3 (C(2)), 26.4 (C(4)), 26.3 (C(24)), 26.2 (C(7) and C(23)), and 21.5 (C(6)), and three quaternary C-atoms at δ(C) 109.6 (C(22)), 40.6 (C(13)), and 39.3 (C(10)), suggesting that **1** was a spirostane-type steroid derivative [12]. The ^{1}H- and ^{13}C-NMR (including DEPT) and 2D-NMR (^{1}H,^{1}H-COSY, HMQC, HMBC, and ROESY) spectra of **1** revealed the presence of a xylose unit [13] (^{1}H-NMR: δ(H) 5.30 (d, $J = 7.5$, H–C(1′)), 4.56–4.66 (dd, $J = 12.0$, 10.5, H_a–C(5′)), 4.46–4.57 (m, H–C(2′)), 4.42–4.54 (m, H–C(3′)), 4.36–4.47 (m, H–C(4′)), and 3.94 (t, $J = 10.5$, H_b–C(5′)); ^{13}C-NMR: δ(C) 98.2 (C(1′)), 79.2 (C(3′)), 76.9 (C(2′)), 71.6 (C(4′)), and 67.4 (C(5′))) and a rhamnose unit [14] (^{1}H-NMR: δ(H) 6.38 (br. s, H–C(1″)), 5.04 (overlap, H–C(2″,3″)), 4.95–5.05 (m, H–C(5″)), 4.47–4.57 (m, H–C(4″)), and 2.01 (d, $J = 6.5$, H–C(6″)); ^{13}C-NMR: δ(C) 102.0 (C(1″)), 74.5 (C(4″)), 72.2 (C(2″)), 72.0 (C(3″)), 69.9 (C(5″)), and 18.8 (C(6″))). The coupling constant ($J = 7.5$) of the xylose anomeric H-atom suggested a β-form [13]. The configuration of rhamnose was determined to be α by comparing the ^{13}C-NMR data of C(3″) (δ(C) 72.0) and C(5″) (δ(C) 69.9) (α-form: δ(C) 72.5, 69.0; β-form: δ(C) 73.8, 73.1 [14]). The position of the sugar was confirmed by the HMBC spectrum by correlations between Xyl H–C(1′)/C(1) and Rha H–C(1″)/C(2′) (*Fig. 2*). The connectivities of the sugars were also confirmed by the ROESY correlations between xyl H–C(1′)/H–C(1) and rha H–C(1″)/H–C(2′) [14]. The broad *singlet* at δ(H) 4.66 in the ^{1}H-NMR spectrum revealed the β-orientation for H–C(1) [15], since an α-oriented H-atom at C(1) reportedly appears as a double *doublet* ($J = 12.0$, 2.0) [16]. This was further confirmed by the cross-peak between H–C(1) and

Table 1. *^{1}H- and ^{13}C-NMR* ((D$_5$)pyridine) *Data for* **1** *and* **2**. δ in ppm, *J* in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	4.66 (br. *s*)	76.0	4.35 (br. *s*)	75.9
2	2.56–2.59 (*m*), 2.51–2.53 (*m*)	27.3	2.18–2.30 (*m*), 1.99–2.08 (*m*)	27.4
3	4.45 (br. *s*)	67.4	4.15 (br. *s*)	67.3
4	1.72–1.77 (*m*), 1.63–1.67 (*m*)	26.4	1.78–1.89 (*m*), 1.27–1.36 (*m*)	26.6
5	2.74–2.82 (*m*)	31.8	1.92–2.07 (*m*)	31.7
6	1.58[a])	21.5	1.21[a])	21.5
7	2.41[a]), 2.21[a])	26.2	1.20–1.35 (*m*), 0.97–1.08 (*m*)	26.3
8	1.88–1.98 (*m*)	35.6	1.53–1.68 (*m*)	35.3
9	1.51–1.57 (*m*)	42.0	1.43[a])	41.8
10		39.3		39.4
11	2.26–2.35 (*m*), 1.64–1.75 (*m*)	32.2	2.52–2.61 (*m*), 1.89–2.03 (*m*)	32.0
12	1.92–2.21 (*m*), 1.38–1.42 (*m*)	40.3	1.59–1.72 (*m*), 1.07–1.25 (*m*)	40.1
13		40.6		40.7
14	1.36–1.45 (*m*)	56.4	1.21–1.34 (*m*)	56.3
15	2.30–2.37 (*m*), 1.90–1.99 (*m*)	34.2	1.97–2.03 (*m*), 0.99–1.08 (*m*)	33.2
16	4.80–4.90 (*m*)	81.3	4.49–4.60 (*m*)	81.4
17	2.08–2.19 (*m*)	63.0	1.76–1.86 (*m*)	63.1
18	1.03 (*s*)	16.7	0.78 (*s*)	16.6
19	1.58 (*s*)	19.4	1.05 (*s*)	19.3
20	2.12–2.23 (*m*)	42.5	1.81[a])	41.9
21	1.41 (*d*, *J* = 7.0)	15.0	1.06 (*d*, *J* = 7.0)	14.9
22		109.6		109.3
23	1.72[a]), 1.58[a])	26.2	2.24[a]), 1.53[a])	26.2
24	1.64[a]), 1.54[a])	26.3	1.05[a]), 1.03[a])	28.9
25	1.80–1.90 (*m*)	27.6		144.4
26	4.34 (*dd*, *J* = 10.5, 2.0), 3.64 (*dd*, *J* = 10.5, 2.0)	65.2	4.42 (*d*, *J* = 10.5), 3.64 (*d*, *J* = 10.5)	65.3
27	1.35 (*d*, *J* = 7.0)	16.3	4.77 (br. *s*), 4.72 (br. *s*)	108.6
Xyl				
1′	5.30 (*d*, *J* = 7.5)	98.2	4.42 (*d*, *J* = 7.5)	98.1
2′	4.46–4.57 (*m*)	76.9	4.18–4.29 (*m*)	76.8
3′	4.42–4.54 (*m*)	79.2	4.11–4.22 (*m*)	79.1
4′	4.36–4.47 (*m*)	71.6	4.16–4.28 (*m*)	71.5
5′	4.56–4.66 (*m*), 3.94 (*t*, *J* = 10.5)	67.4	4.30 (*dd*, *J* = 10.5, 2.0), 3.61 (*t*, *J* = 10.5)	67.5
Rha				
1″	6.38 (br. *s*)	102.0	6.56 (br. *s*)	101.9
2″	5.04[a])	72.2	4.68–4.79 (*m*)	72.1
3″	5.04[a])	72.0	4.66–4.74 (*m*)	71.9
4″	4.47–4.57 (*m*)	74.5	4.38–4.50 (*m*)	74.4
5″	4.95–5.05 (*m*)	69.9	4.48–4.53 (*m*)	69.8
6″	2.01 (*d*, *J* = 6.5)	18.8	1.70 (*d*, *J* = 6.5)	18.7

[a]) Overlapped with other signals.

H–C(19) in the ROESY spectrum (*Fig. 3*). The α-orientation of the OH group at C(3) was assigned based on the chemical shift of C(3) (δ(C) 67.4) in the ^{13}C-NMR spectrum

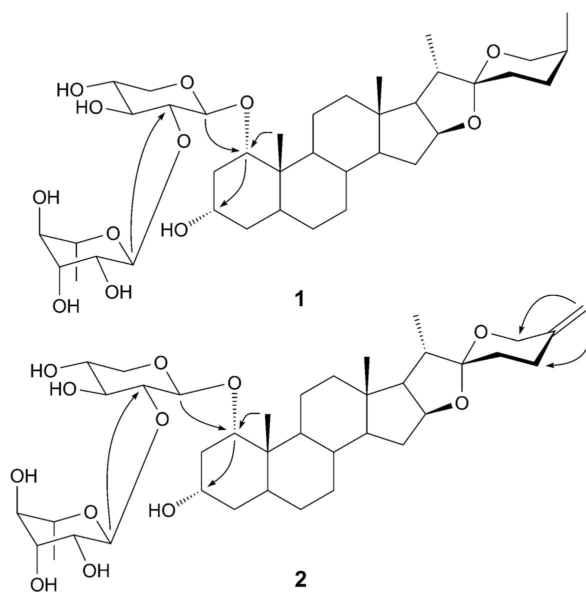

Fig. 2. *Key HMBCs* (H → C) *of compounds* **1** *and* **2**

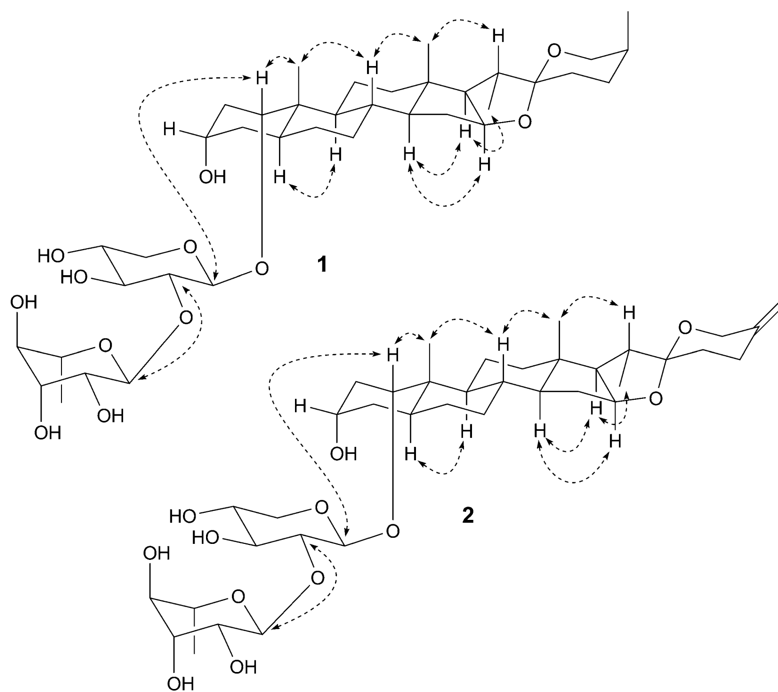

Fig. 3. *Key ROESY correlations* (H ↔ H) *of compounds* **1** *and* **2**

(δ(C) 66.0 for the α-orientation of HO–C(3); δ(C) 71.1 for the β-orientation of HO–C(3) [16][17]). The configurations of all other ring junctions were identified as *trans* by ROESY correlations (*Fig. 3*) [16]. The configuration at C(25) was confirmed to be (*S*), based on the comparison of the ^{1}H-NMR data of the Me group at C(25) (δ(H) 1.35 (δ(H) 1.10 for the (*S*)-form [18]) and the ^{13}C-NMR spectral data of C(23), C(24), C(25), C(26), and C(27) [19][20]. Acid hydrolysis of **1** with 1N HCl yielded an aglycone **1a** and two sugars. The aglycone (25*S*)-5α-spirostan-1α,3α-diol (**1a**) was identified by comprehensive ^{1}H- and ^{13}C-NMR, 2D-NMR, and HR-EI-MS analyses. Two sugars were confirmed by GC analysis after derivatization (D-xylose, t_R: 6.16 min, L-rhamnose, t_R: 6.11 min) [21]. Thus, compound **1** was identified as (1α,3α,25*S*)-1-[(β-D-xylopyranosyl-(2 → 1)-α-L-rhamnopyranosyl)oxy]-5α-spirostan and named bletilnoside A.

Compound **2** was obtained as a white amorphous powder. The molecular formula was deduced as $C_{38}H_{60}O_{12}$ from the $[M+Na]^+$ peak at *m/z* 731.8744 (calc. for $C_{38}H_{60}NaO_{12}^+$: 731.8746) in the HR-FAB-MS spectrum. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1**. The major difference was the presence of two olefinic H-atom signals (δ(H) 4.77 (br. *s*, H_a–C(27)) and 4.72 (br. *s*, H_b–C(27)); δ(C) 144.4 (C(25)) and 108.6 (C(27))) in **2**. The position of the exocyclic CH_2 group was confirmed by the HMBC spectrum, in which a correlation was observed between the CH_2(27) (δ(H) 4.77 and 4.72), and C(26) and C(24) (δ(C) 65.3, 28.9, resp.; *Fig. 2*). The configuration of **2** was assumed to be same as **1** by comparson of their NMR data, which were confirmed by ROESY correlations (*Fig. 3*). Acid hydrolysis of **2** yielded aglycone (**2a**) and two sugars. The aglycone (5α)-spirost-25(27)-ene-1α,3α-diol (**2a**) was identified by ^{1}H-NMR and HR-EI-MS analyses, and two sugars were identified as D-xylose and L-rhamnose by GC analysis [21]. Thus, compound **2** was identified as (1α,3α)-1-[(β-D-xylopyranosyl-(2 → 1)-α-L-rhamnopyranosyl)oxy]-25(27)-ene-5α-spirostan and named bletilnoside B.

The known compounds were identified as 3-epiruscogenin (**3**), 3-epineoruscogenin (**4**) [22], 3-*O*-β-D-glucopyranosyl-3-epiruscogenin (**5**), 3-*O*-β-D-glucopyranosyl-3-epineoruscogenin (**6**) [23], (20*S*,22*R*)-1β,2β,3β,4β,5β,7α-hexahydroxyspirost-25(27)-en-6-one (**7**) [24], by comparing their spectroscopic data with those in previous reports. To the best of our knowledge, all compounds were isolated for the first time from this plant source and the genus *Bletilla*. In addition, this is the first report on the occurrence of steroidal saponins in the genus *Bletilla*.

2.2. *Biological Evaluation of Compounds.* The cytotoxic activities of the isolated compounds **1**–**7** were evaluated by determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) *in vitro* using the sulforhodamine B (SRB) assay [25]. The compounds **1**, **2**, and **5** showed significant cytotoxicities against all tested tumor cell lines with IC_{50} values ranging from 3.98 ± 0.16 to 12.10 ± 0.40 μM (*Table 2*). Especially, the new compounds **1** and **2** exhibited potent cytotoxicities against all of the cell lines tested with IC_{50} values in the range of 3.98 ± 0.16–9.29 ± 1.23 μM. Compounds **3**, **4**, and **6** showed low cytotoxicities against tested cell lines ($IC_{50} > 30$ μM).

This work was supported by the *Basic Science Research Program* through the *National Research Foundation of Korea* (NRF) funded by the *Ministry of Education, Science and Technology*

Table 2. *Cytotoxic Activities of Compounds* **1**, **2**, *and* **5** *against Four Cultured Human Tumor Cell Lines in the SRB Bioassay*

Compound	IC_{50} [μM][a])			
	A549	SK-OV-3	SK-MEL-2	HCT-15
1	4.56 ± 0.29	4.00 ± 0.06	3.98 ± 0.16	5.08 ± 0.51
2	8.79 ± 1.01	8.08 ± 0.83	5.29 ± 0.34	9.29 ± 1.23
5	12.10 ± 0.40	11.80 ± 0.28	11.55 ± 0.27	11.00 ± 0.23
Doxorubicin[b])	0.0035 ± 0.0025	0.0037 ± 0.0022	0.0009 ± 0.0001	0.1574 ± 0.0569

[a]) The concentration of the compound that caused a 50% inhibition of cell growth. The data are presented as the mean ± SEM of at least three distinct experiments. [b]) Positive control.

(20110028285). We thank Drs. *E. J. Bang*, *S. G. Kim*, and *J. J. Seo* at the Korea Basic Science Institute for their aid in obtaining the NMR and mass spectra.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 230–400 mesh; *Merck*, Germany) and *Lichroprep RP$_{18}$* gel (40–60 μM, *Merck*, DE-Darmstadt). TLC: Silica gel *60 F$_{254}$* and *RP-18 F$_{254s}$* silica gel plates (*Merck*); detection under UV light and by spraying with 10% aq. H_2SO_4 soln., followed by heating at 120° for 1 min. HPLC: Prep. HPLC *Gilson 306 pump*, *Gilson-101 RI* detector, *Phenomenex-Luna-C$_{18}$-(2)* column (250 mm × 10.00 mm (i.d.), 5 μM); 85, 90% MeOH as mobile phase (2 ml/min); t_R in min. M.p.: *Gallenkamp* apparatus; uncorrected. IR Spectra: *Bruker IFS-66/S* FT-IR spectrometer; KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Varian UNITY INOVA 500* FT-NMR instrument; δ in ppm rel. to Me_4Si as internal standard, J in Hz. FAB-, HR-FAB-, and EI-MS: *JEOL JMS-700* (*Jeol*, Japan); in m/z.

Plant Material. The roots of *B. striata* (3.6 kg) were purchased from Kyungdong herbal market, Seoul, Korea, in June 2011, and were identified by one of the authors (*K. R. L.*). A voucher specimen (SKKU-NPL 1106) was deposited with the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. Dried roots of *B. striata* (Orchidaceae) (3.6 kg) were extracted three times with 80% MeOH under reflux. The resulting MeOH extracts (568 g) were suspended in dist. H_2O (4 × 800 ml), and then successively partitioned with hexane, $CHCl_3$, AcOEt, and BuOH, yielding residues of 3, 42, 51, and 159 g, resp. The $CHCl_3$-soluble extract (40 g) was fractionated by CC (*RP-C$_{18}$* (1 kg); $CHCl_3$/MeOH 50:1 → 1:1) to give seven fractions, *Frs. A–G*. *Fr. D* (4.0 g) was subjected to CC (*RP-C$_{18}$* (200 g); MeOH/H_2O 3:2 → 1:0): *Frs. D$_1$–D$_{10}$*. *Fr. D$_{10}$* (119 mg) was further separated by CC (SiO_2; $CHCl_3$/MeOH 20:1): *Frs. D$_{10\text{-}1}$–D$_{10\text{-}4}$*. *Frs. D$_{10\text{-}2}$* (10 mg) and *D$_{10\text{-}4}$* (15 mg) were purified by prep. HPLC (*RP-C$_{18}$*; 85% MeOH) to yield **3** (t_R 12.5; 5 mg) and **4** (t_R 14.3; 7 mg). *Fr. F* (7.2 g) was subjected to CC (*RP-C$_{18}$* (400 g); MeOH/H_2O 7:3 → 1:0) to furnish six fractions, *Frs. F$_1$–F$_6$*. *Fr. F$_4$* (88 mg) in MeOH was filtered using filter paper. Then, the precipitate was washed with MeOH and subsequently dried to yield compound **7** (26 mg). *Fr. F$_5$* (2.9 g) was separated by CC (SiO_2 (150 g); $CHCl_3$/MeOH 30:1 → 1:1): *Frs. F$_{5\text{-}1}$–F$_{5\text{-}2}$*. *Fr. F$_{5\text{-}1}$* (1.5 g) was purified by prep. HPLC (*RP-C$_{18}$*; 85% MeOH) to give **5** (t_R 15.7; 76 mg) and **6** (t_R 19.2; 53 mg). *Fr. F$_6$* (378 mg) was separated by CC (SiO_2 (50 g); $CHCl_3$/MeOH 20:1) and further purified by prep. HPLC (*RP-C$_{18}$*; 90% MeOH) to afford **1** (t_R 11.5; 30 mg) and **2** (t_R 13.8; 5 mg).

Bletilnoside A (= *(1α,3α,25S)-1-[(β-*D*-Xylopyranosyl-(2 → 1)-α-*L*-rhamnopyranosyl)oxy]-5α-spirostan* = *(2*S*,3*R*,4*R*,5*R*,6*S*)-2-{[(2*S*,4*S*,5*R*)-Tetrahydro-4,5-dihydroxy-2-{[(1α,3α,5α,25*S*)-3-hydroxyspirostan-1-yl]oxy}-2*H*-pyran-3-yl]oxy}-6-methyltetrahydro-2*H*-pyran-3,4,5-triol*; **1**). White amorphous powder. M.p. 210–215°. $[\alpha]_D^{25} = -4.7$ ($c = 0.235$, MeOH). IR (KBr) 3407, 2951, 1054, 1032, 1017, 698. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-FAB-MS: 711.9083 ([M + H]$^+$, $C_{38}H_{63}O_{12}^+$; calc. 711.4320).

Bletilnoside B (=*(1α,3α)-1-[(β-D-Xylopyranosyl-(2→1)-α-L-rhamnopyranosyl)oxy]-25(27)-ene-5α-spirostan*=*(2S,3R,4R,5R,6S)-2-{[(2S,4S,5R)-Tetrahydro-4,5-dihydroxy-2-{[(1α,3α,5α)-3-hydroxy-spirost-25(27)-en-1-yl]oxy}-2H-pyran-3-yl]oxy}-6-methyltetrahydro-2H-pyran-3,4,5-triol*; **1**). White amorphous powder. M.p. 209–213°. $[\alpha]_D^{25} = -3.5$ ($c = 0.175$, MeOH). IR (KBr): 3423, 2949, 2843, 1656, 1450, 1032, 1018, 694. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-FAB-MS: 731.8744 ($[M+\mathrm{Na}]^+$, $C_{38}H_{60}NaO_{12}^+$; calc. 731.3982).

Acid Hydrolysis of **1** *and* **2**. A soln. of **1** (5.1 mg) in 1N HCl (dioxane/H_2O 1:1, 3 ml) was heated for 4 h at 100°. The hydrolysate was extracted with $CHCl_3$ and evaporated under reduced pressure to yield aglycone **1a** (0.9 mg). In the same way, **2** (4.7 mg) was treated with 1N HCl soln. to furnish aglycone **2a** (0.7 mg).

Data of **1a**. White amorphous powder. ^{1}H-NMR (500 MHz, (D_5)pyridine): 4.39 (q, $J = 7.0$, H–C(16)); 4.17 (br. s, H–C(4)); 3.95 (dd, $J = 10.5$, 2.0, H_a–C(26)); 3.84 (s, H–C(1)); 3.29 (dd, $J = 10.5$, 2.0, H_b–C(26)); 2.72–2.81 (m, H_a–C(2)); 2.49–2.55 (m, H–C(8)); 2.20–2.29 (m, H_b–C(2)); 2.19–2.28 (m, H–C(6)); 2.15–2.26 (m, H_a–C(11)); 2.13–2.23 (m, H_a–C(15)); 2.12–2.20 (m, H_a–C(7)); 2.10–2.19 (m, H_a–C(24)); 2.09–2.17 (m, H_b–C(7)); 2.01–2.08 (m, H–C(5)); 1.95–2.08 (m, H–C(20)); 1.88–1.90 (m, H_a–C(23)); 1.85–1.96 (m, H_a–C(4)); 1.79–1.84 (m, H_b–C(11)); 1.78–1.88 (m, H–C(17)); 1.75–1.85 (m, H_b–C(15)); 1.70–1.76 (m, H–C(9)); 1.63–1.73 (m, H_a–C(12)); 1.60–1.69 (m, H_b–C(4)); 1.39–1.48 (m, H_b–C(23)); 1.30–1.40 (m, H_b–C(24)); 1.20–1.29 (m, H–C(25)); 1.13 (s, Me(19)); 1.08–1.19 (m, H–C(14)); 1.08 (d, $J = 7.0$, H–C(21)); 1.05–1.14 (m, H_b–C(12)); 0.98 (d, $J = 7.0$, H–C(27)); 0.77 (s, Me(18)). ^{13}C-NMR (125 MHz, (D_5)pyridine): 109.7 (C(22)); 81.2 (C(16)); 73.3 (C(1)); 68.1 (C(3)); 65.1 (C(26)); 63.1 (C(17)); 56.4 (C(14)); 42.5 (C(20)); 42.1 (C(9)); 40.7 (C(13)); 40.6 (C(10)); 40.3 (C(12)); 35.8 (C(8)); 34.2 (C(15)); 32.9 (C(5)); 32.2 (C(11)); 29.9 (C(2)); 27.5 (C(25)); 26.7 (C(4)); 26.4 (C(23)); 26.2 (C(7,24)); 21.0 (C(6)); 19.3 (C(19)); 16.6 (C(18)); 16.2 (C(27)); 14.8 (C(21)). HR-EI-MS: 432.3239 (M^+, $C_{27}H_{44}O_4^+$; calc. 432.3240).

Data of **2a**. White amorphous powder. ^{1}H-NMR (500 MHz, ((D_5)pyridine): 4.78 (br. s, H_a–C(27)); 4.70 (br. s, H_b–C(27)); 4.41 (d, $J = 10.5$, H_a–C(26)); 4.36 (q, $J = 7.0$, H–C(16)); 4.15 (br. s, H–C(3)); 3.80 (br. s, H–C(1)); 3.62 (d, $J = 10.5$, H_b–C(26)); 2.70–2.79 (m, H_a–C(2)); 2.50–2.59 (m, H_a–C(11)); 2.21–2.30 (m, H_b–C(2)); 2.21–2.24 (m, H_a–C(23)); 2.20–2.27 (m, H–C(6)); 1.96–2.07 (m, H–C(5)); 1.93–2.00 (m, H_a–C(15)); 1.90–1.97 (m, H_b–C(11)); 1.89–1.94 (m, H_a–C(4)); 1.80–1.84 (m, H–C(17)); 1.79–1.87 (m, H–C(20)); 1.62–1.71 (m, H_a–C(12)); 1.60–1.67 (m, H_b–C(4)); 1.50–1.55 (m, H_b–C(23)); 1.49–1.53 (m, H–C(8)); 1.38–1.47 (m, H–C(9)); 1.30–1.40 (m, H_a–C(7)); 1.20–1.30 (m, H–C(14)); 1.10 (s, Me(19)); 1.08–1.20 (m, H_b–C(12)); 1.06 (d, $J = 7.0$, H–C(21)); 1.00–1.07 (m, H_a–C(24)); 0.99–1.05 (m, H_b–C(15)); 0.98–1.07 (m, H_b–C(7)); 0.98–1.04 (m, H_b–C(24)); 0.78 (s, Me(18)). HR-EI-MS: 430.3287 (M^+, $C_{27}H_{42}O_4^+$; calc. 430.3083).

Determination of Absolute Configuration of the Monosaccharide Units. The sugars obtained from the hydrolysis were dissolved in anh. pyridine (0.5 ml), and L-cysteine methyl ester hydrochloride (2 mg) was added. The mixture was stirred at 60° for 1.5 h and trimethylsilylated with 1-(trimethylsilyl)-1*H*-imidazole (0.1 ml) for 2 h. The mixture was partitioned between hexane and H_2O (1 ml each), and org. layer (1 µl) was analyzed by GC-MS [21]. D-Xylose and L-rhamnose were identified in the hydrolysates of **1** and **2** by co-injection with derivatized authentic samples, giving single peaks at 6.16 (D-xylose) and 6.11 min (L-rhamnose). Retention times of authentic samples treated in the same way were 6.16 (D-xylose) and 6.11 min (L-rhamnose).

Cytotoxicity Assay. A sulforhodamine B (SRB) bioassay was used to evaluate the cytotoxicity of each compound isolated against four cultured human tumor cell lines [25]. The assays were performed at the *Korea Research Institute of Chemical Technology.* The cell lines used were A549 (non-small-cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). Doxorubicin was used as a positive control.

REFERENCES

[1] C. A. Toh, *Kor. J. Pharmacogn.* **1994**, *25*, 293.
[2] J.-Q. Feng, R.-J. Zhang, W.-M. Zhao, *Helv. Chim. Acta* **2008**, *91*, 520.

[3] F. Figueiredo, F. George, F. Tatsuzawa, K. Toki, N. Saito, R. Brouillard, *Phytochemistry* **1999**, *51*, 125.
[4] M. Yamaki, C. Honda, T. Kato, L. Bai, S. Takagi, *Nat. Med.* **1997**, *51*, 493.
[5] N. Saito, M. Ku, F. Tatsuzawa, T. S. Lu, M. Yokoi, A. Shigihara, T. Honda, *Phytochemistry* **1995**, *40*, 1523.
[6] L. Bai, T. Kato, K. Inoue, M. Yamaki, S. Takagi, *Phytochemistry* **1993**, *33*, 1481.
[7] M. Yamaki, T. Kato, L. Bai, K. Inoue, S. Takagi, *Phytochemistry* **1993**, *34*, 535.
[8] M. Yamaki, L. Bai, T. Kato, K. Inoue, S. Takagi, Y. Yamagata, K. Tomita, *Phytochemistry* **1993**, *33*, 1497.
[9] M. Yamaki, L. Bai, T. Tomoko, L. Inoue, S. Takagi, *Phytochemistry* **1993**, *32*, 427.
[10] H. Morita, K. Koyama, Y. Sugimoto, J. Kobayashi, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1051.
[11] S. Takagi, M. Yamaki, K. Inoue, *Phytochemistry* **1983**, *22*, 1011.
[12] P. K. Agrawal, D. C. Jain, R. K. Gupta, R. S. Thakur, *Phytochemistry* **1985**, *24*, 2479.
[13] S.-Y. Lee, S.-U. Choi, K.-R. Lee, *Bull. Korean Chem. Soc.* **2011**, *32*, 3813.
[14] R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, O. Tanaka, *Tetrahedron* **1979**, *35*, 1427.
[15] A. H. Banskota, Y. Tezuka, K. Q. Tran, K. Tanaka, I. Saiki, S. Kadota, *Chem. Pharm. Bull.* **2000**, *48*, 496.
[16] Y. Mimaki, Y. Takaashi, M. Kuroda, Y. Sashida, *Phytochemistry* **1997**, *45*, 1229.
[17] Y. Mimaki, M. Kuroda, Y. Takaashi, Y. Sashida, *Phytochemistry* **1998**, *47*, 79.
[18] P. K. Agrawal, *Magn. Reson. Chem.* **2003**, *41*, 965.
[19] P. K. Agrawal, D. C. Jain, A. K. Pathak, *Magn. Reson. Chem.* **1995**, *33*, 923.
[20] K. Miyahara, K. Kudo, T. Kawasaki, *Chem. Pharm. Bull.* **1983**, *31*, 348.
[21] W. Jiang, W. Li, L. Han, L. Liu, Q. Zhang, S. Zhang, T. Nikaido, K. Koike, *J. Nat. Prod.* **2006**, *69*, 1577.
[22] W.-B. Pan, F.-R. Chang, L.-M. Wei, Y.-C. Wu, *J. Nat. Prod.* **2003**, *66*, 161.
[23] T. Masayuki, K. Yoshikazu, K. Genjiro, N. Shigeo, *Yakagaku Zasshi* **1979**, *99*, 528.
[24] W.-B. Pan, F.-R. Chang,Y.-C. Wu, *J. Nat. Prod.* **2000**, *63*, 861.
[25] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Nat. Cancer Inst.* **1990**, *82*, 1107.

Received February 26, 2013