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A New Triterpene Saponin from the Tubers of *Stachys sieboldii*

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Stachys sieboldii MIQ. (Labiatae) is native to Northern China and widely distributed in North America, Asia, and Europe.[1] *S. sieboldii* has been used in Chinese folk medicine for the treatment of ischemic stroke, senile dementia, and various gastrointestinal problems.[2] Previous phytochemical and pharmacological studies on this plant reported the isolation of terpenes,[3] flavonoids,[4] and phenolic compounds,[5] and it having antimicrobial,[6] antioxidant,[7] and antitumor activities.[8] In our continuing efforts to study the secondary metabolites of natural plant sources, the MeOH extract of *S. sieboldii* was investigated, and a new triterpene saponin, Sieboldii saponin A (**1**), was isolated, together with five known compounds (**2-6**). The structures of the isolates (**1-6**) were elucidated by means of spectroscopic methods and chemical evidences.

Sieboldii saponin A (**1**) was isolated as a colorless gum. The molecular formula was determined to be $C_{42}H_{66}O_{15}$ from the molecular ion peak $[M + H]^+$ at *m/z* 811.4480 (calcd. for $C_{42}H_{67}O_{15}$: 811.4480) in the positive-ion HR-FABMS. The IR spectrum at 3421 cm^{-1} indicated that **1** possessed hydroxyl group. The ^{1}H-NMR spectrum of **1** (Table 1) displayed the signals of an olefinic proton at δ_H 5.30 (1H, br t, J = 3.5 Hz, H-12), a couple of exomethylene protons at δ_H 4.72 and 4.67 (each 1H, br s, H-30), two oxygenated methine protons at δ_H 3.92 (1H, m, H-2) and 3.82 (1H, m, H-3), a couple of oxymethylene protons at δ_H 3.55 and 3.28 (each 1H, d, J = 11.2 Hz, H-23), one methine proton at δ_H 2.30 (1H, d, J = 11.9 Hz, H-18), four tertiary methyl protons at δ_H 1.21 (3H, s, H-27), 1.07 (3H, s, H-25), 0.86 (3H, s, H-26), and 0.82 (3H, s, H-24), one secondary methyl proton at δ_H 1.05 (3H, d, J = 6.3 Hz, H-29), and two sugar anomeric protons at δ_H 5.45 (1H, d, J = 7.7 Hz, H-1′) and 4.77 (1H, d, J = 7.7 Hz, H-1″). The ^{13}C-NMR spectrum showed a total of 42 carbon signals, of which 30 carbons were to be assigned to the aglycone and the remaining 12 carbons to the sugar moieties. The ^{13}C-NMR and DEPT spectra included one carboxylic carbon at δ_C 175.9, five methyl carbons at δ_C 22.7, 16.5, 16.2, 16.0, and 15.3, four olefinic carbons at δ_C 152.9, 138.9, 125.8, and 104.1, two oxygenated methine carbons at δ_C 77.3 and 65.8, one oxygenated methylene carbon at δ_C 69.9, four methine carbons at δ_C 54.9, 47.3, 42.9, and 37.1, eight methylene carbons at δ_C 41.0, 38.4, 32.4, 31.8, 28.6, 23.5, 23.1, and 17.5, five quaternary carbons at δ_C 48.1, 42.0, 41.2, 39.7, and 37.3, and 12 remaining signals at δ_C 102.6, 77.4, 76.6, 74.5, 71.0, and 62.2, and at δ_C 92.6, 77.3, 77.2, 76.8, 69.4, and 61.0 assignable to two glucose moieties. From these data, compound **1** was presumed to be of urs-12-en-28-oic acid skeleton[9,10] with terminal double bond. The HMBC correlations of H-29/C-20 and H-30/C-19 and C-21 confirmed a secondary

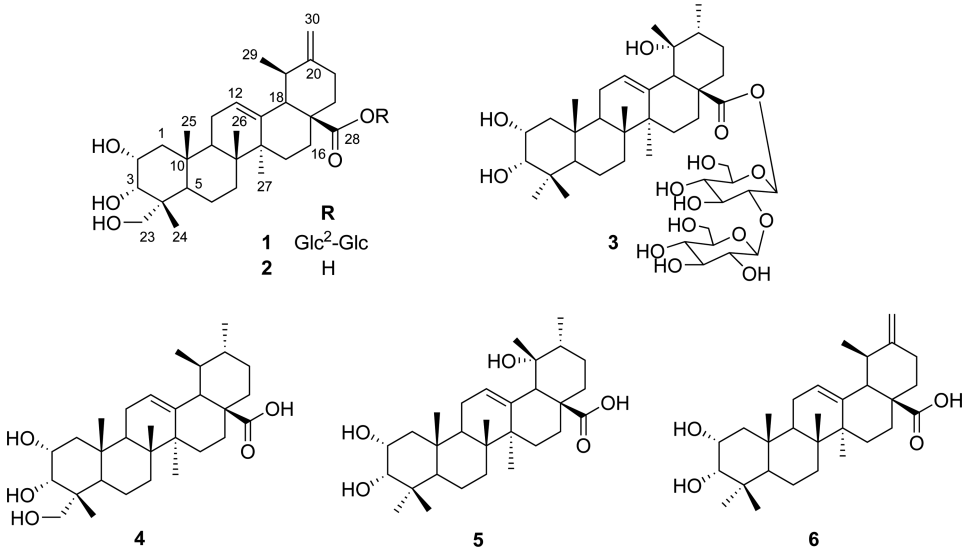

Figure 1. Structures of compounds **1-6**.

Table 1. ^{1}H- and ^{13}C-NMR data of **1**[a] in CD_3OD

Position	Aglycone		Position	Sugar	
	δ_H	δ_C		δ_H	δ_C
1	1.63 (m), 1.32 (m)	41.0	1′	5.45 (d, 7.7)	92.6
2	3.92 (m)	65.8	2′	3.63 (m)	77.2
3	3.82 (d, 1.4)	77.3	3′	3.36 (m)	76.8
4	-	41.2	4′	3.43 (m)	69.4
5	1.54 (m)	42.9	5′	3.36 (m)	77.3
6	1.60 (m), 1.42 (m)	17.5	6′	3.80 (dd, 11.2, 2.1), 3.68 (m)	61.0
7	2.37 (m), 2.21 (m)	32.4	1″	4.77 (d, 7.7)	102.6
8	-	39.7	2″	3.23 (m)	74.5
9	1.77 (m)	47.3	3″	3.30 (m)	76.6
10	-	37.3	4″	3.43 (m)	71.0
11	2.03 (m), 1.12 (m)	23.1	5″	3.63 (m)	77.4
12	5.30 (br t, 3.5)	125.8	6″	3.91 (dd, 11.2, 2.1), 3.66 (m)	62.2
13	-	138.9			
14	-	42.0			
15	1.97 (m), 1.12 (m)	28.6			
16	2.27 (m), 2.00 (m)	23.5			
17	-	48.1			
18	2.30 (d, 11.9)	54.9			
19	2.24 (m)	37.1			
20	-	152.9			
21	2.37 (m), 2.24 (m)	31.8			
22	1.94 (m), 1.65 (m)	38.4			
23	3.55 (d, 11.2), 3.28 (d, 11.2)	69.9			
24	0.82 (s)	16.2			
25	1.07 (s)	16.0			
26	0.86 (s)	16.5			
27	1.21 (s)	22.7			
28	-	175.9			
29	1.05 (d, 6.3)	15.3			
30	4.72 (br s), 4.67 (br s)	104.1			

[a] ^{1}H- and ^{13}C-NMR run at 700 MHz and 175 MHz, respectively, proton coupling constants (*J*) in Hz are given in parentheses.

methyl and exomethylene group to be located at C-29 and C-30, respectively (Fig. 2(a)). The relative stereochemistry of the aglycone was assumed to be similar with that of 2α,3α,23-trihydroxyursa-12,20(30)-dien-28-oic acid (**2**) by comparing ^{13}C-NMR of **1**,[11] and corroborated by NOESY cross-peaks of H-2/H-3, H-24, and H-25, H-3/H-24, H-5/H-9 and H-23, and H-9/H-27 (Fig. 2(b)). The full NMR assignments and connectivities were determined by ^{1}H-^{1}H COSY, HMQC and HMBC. The anomeric configurations for two glucoses were to be a *β*-form from the coupling constant of 7.7 Hz.[12] Acid hydrolysis of **1** with 1 N HCl yielded **2**, whose ^{1}H-NMR and MS data were in good agreement with values reported previously,[11] and D-glucose ($[\alpha]_D^{25}$ +49.4° *c* = 0.04 in H_2O), which was identified by GC and co-TLC (EtOAc-MeOH-H_2O = 9:3:1, R_f value: 0.2)[13] with glucose standard (Aldrich Co., U.S.A.). The positions of the glucoses

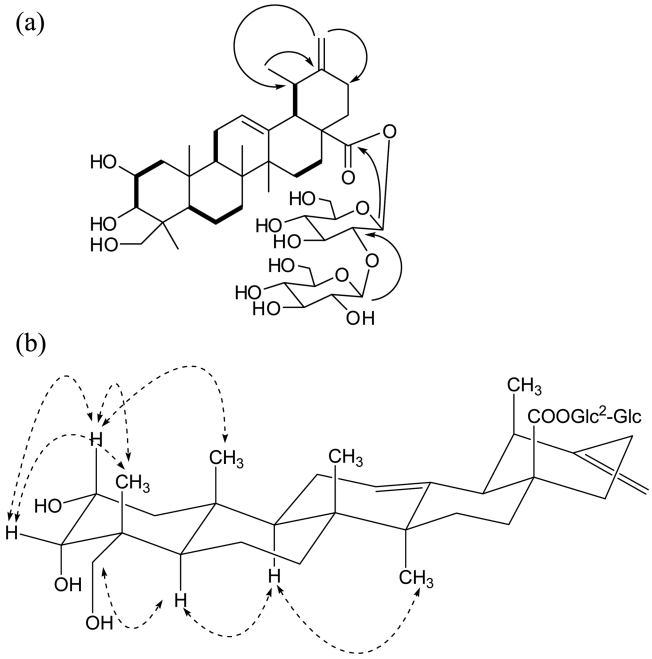

Figure 2. Key HMBC (H→C), ^{1}H-^{1}H COSY (—) correlations (a) and NOESY (◂⋯▸) correlations of **1** (b).

were confirmed by the HMBC correlations of H-1′/C-28 and H-1″/C-2′ (Fig. 2(a)). Thus, the structure of **1** was established as 28-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]-2α,3α,23-trihydroxyursa-12,20(30)-dien-28-oic acid, named sieboldii saponin A.

The known compounds, 2α,3α,23-trihydroxyursa-12,20(30)-dien-28-oic acid (**2**),[11] pruvuloside A (**3**),[14] esculentic acid (**4**),[15] euscaphic acid (**5**),[16] and 2α,3α-dihydroxyursa-12,20(30)-dien-28-oic acid (**6**)[17] were identified by comparing their spectroscopic data with those in the literatures. The compounds **2-6** were reported from this source for the first time.

Experimental Section

General Procedures. Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. HR-FAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra, including ^{1}H-^{1}H COSY, DEPT, HMQC, HMBC and NOESY experiments, were recorded on a Varian UNITY INOVA 700 NMR spectrometer operating at 700 MHz (^{1}H) and 175 MHz (^{13}C) with chemical shifts given in ppm (δ). Preparative HPLC was conducted using a Gilson 306 pump with Shodex refractive index detector and Econosil RP-C_{18} 10 μm column (250 × 10 mm). Silica gel 60 (Merck, 70-230 mesh and 230-400 mesh) and RP-C_{18} silica gel (YMG GEL ODS-A, 12 nm, S-75 μm) were used for column chromatography. Spots were detected on TLC under UV light or by heating after spraying with 10% H_2SO_4 in C_2H_5OH (v/v). A Hewlett-Packard (HP) GC system 6890 Series equipped with a 5973 Mass Selective Detector (MSD) system. The system was controlled by the Enhanced ChemStation Version B.01.00 program. The capillary column used

for GC was an Agilent J&W HP-5MS UI (30.0 m × 0.25 mm i.d., 0.25 μm film thickness coated 5% diphenyl 95% dimethylpolysiloxane).

Plant Materials. The tubers of *S. sieboldii* were collected at Yecheon, Gyeongsangbuk-Do, Korea, in June 2012, and identified by one of the authors (K. R. Lee). A voucher specimen (SKKU-NPL 1211) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The dried tubers of *S. sieboldii* (5 kg) were extracted with 80% MeOH three times at 60 °C. The resulting MeOH extract (1 kg) was suspended in distilled water (1.8 L) and partitioned with solvent to give *n*-hexane (7 g), $CHCl_3$ (20 g), EtOAc (12 g), and *n*-BuOH (24 g) layers. The EtOAc-soluble layer (12 g) was separated on a RP-C_{18} silica gel column (230-400 mesh, 400 g), and eluted gradiently with MeOH-H_2O (1:1, 1.5:1, 4:1, and 1:0) to afford eight fractions (fr. E1-E8) based on a TLC analysis. Fr. E5 (85 mg) was separated by Lobar-A column eluted with $CHCl_3$-MeOH (20:1) and then purified by reversed-phase preparative HPLC with MeOH-H_2O (12:7) at a flow rate of 2.0 mL/min (Econosil RP-C_{18} 10 μm column; 250 × 10 mm; 10 μm particle size; Shodex refractive index detector) to obtain **1** (6 mg, t_R = 16.0 min) and **3** (6 mg, t_R = 11.5 min). The $CHCl_3$-soluble layer (20 g) was chromatographed on a RP-C_{18} silica gel open column (230-400 mesh, 550 g) eluting with a gradient solvent system of MeOH-H_2O (1:1 and 1:0), yielding nine subfractions (fr. C1-C9). Fr. C4 (8 g) was separated on a RP-C_{18} silica gel column (230-400 mesh, 350 g) with 80% MeOH and further separated by silica gel column using *n*-hexane-EtOAc (1:1) to give six subfractions (fr. C41-C46). Fr. C44 (17 mg) was purified by reversed-phase preparative HPLC with 65% CH_3CN to yield **2** (5 mg, t_R = 15.1 min) and **4** (3 mg, t_R = 19.0 min). Fr. C46 (25 mg) was purified by reversed-phase preparative HPLC using 75% CH_3CN to yield **5** (10 mg, t_R = 13.6 min) and **6** (5 mg, t_R = 16.6 min).

Sieboldii Saponin A (1): Colorless gum. $[\alpha]_D^{25}$ +121.3 (MeOH); IR (KBr) ν_{max}: 3421, 2936, 1739, 1368, 1216, 1055 cm^{-1}; ^1H (CD_3OD, 700 MHz) and ^{13}C-NMR (CD_3OD, 175 MHz) see Table 1; HR-FABMS *m/z* 811.4480 $[M + H]^+$ (calcd. for $C_{42}H_{67}O_{15}$: 811.4480).

Acid Hydrolysis of 1: Compound **1** (1 mg) was treated with 1 N HCl (2 mL) at 80 °C for 1.5 h. After cooling, the hydrolysate was extracted with $CHCl_3$ and the extract was evaporated in vacuo to yield **2** as a colorless gum. The sugar in water layer was identified as D-glucose by co-TLC (EtOAc-MeOH-H_2O = 9:3:1, R_f value: 0.2) with D-glucose standard (Aldrich Co., U.S.A.).

2: $[\alpha]_D^{25}$ +42.8 (MeOH); ^1H-NMR (C_5D_5N, 700 MHz) δ_H 5.44 (1H, br t, *J* = 3.0 Hz, H-12), 5.00 (1H, br s, H-30$_a$), 4.78 (1H, br s, H-30$_b$), 4.27 (1H, m, H-2), 4.15 (1H, d, *J* = 2.4 Hz, H-3), 3.91(1H, d, *J* = 10.7 Hz, H-23$_a$), 3.76 (1H, d, *J* = 10.7 Hz, H-23$_b$), 2.73 (1H, d, *J* = 11.2 Hz, H-18), 1.09 (3H, s, H-27), 1.06 (3H, d, *J* = 6.4 Hz, H-29), 1.01 (3H, s, H-25), 0.98 (3H, s, H-26), 0.85 (3H, s, H-24); HR-FABMS *m/z* 487.3422 $[M + H]^+$ (calcd. for $C_{30}H_{47}O_5$: 487.3423).

Determination of the Sugar of 1. The sugar obtained from the hydrolysis of **1** was dissolved in anhydrous pyridine (0.1 mL) and L-cysteine methyl ester hydrochloride (2 mg) was added. The mixture was stirred at 60 °C for 1.5 h. After the reaction mixture was dried in *vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 mL each), and the organic layer (1 μL) was analyzed by GC-MS.[13] The identification of D-glucose for **1** was detected by co-injection of the hydrolysate with standard silylated samples, giving single peaks at 16.429 min. Retention time of authentic sample treated in the same way with 1-trimethylsilylimidazole in pyridine was 16.396 min.

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