

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

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Received December 10, 2013, Accepted January 6, 2014

Key Words : *Stachys sieboldii* MIQ., Labiatae, Triterpene, Sieboldii saponin A

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 C₄₂H₆₆O₁₅ from the molecular ion peak [M + H]⁺ at *m/z* 811.4480 (calcd. for C₄₂H₆₇O₁₅: 811.4480) in the positive-ion HR-FABMS. The IR spectrum at 3421 cm⁻¹ indicated that **1** possessed hydroxyl group. The ¹H-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 ¹³C-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 ¹³C-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

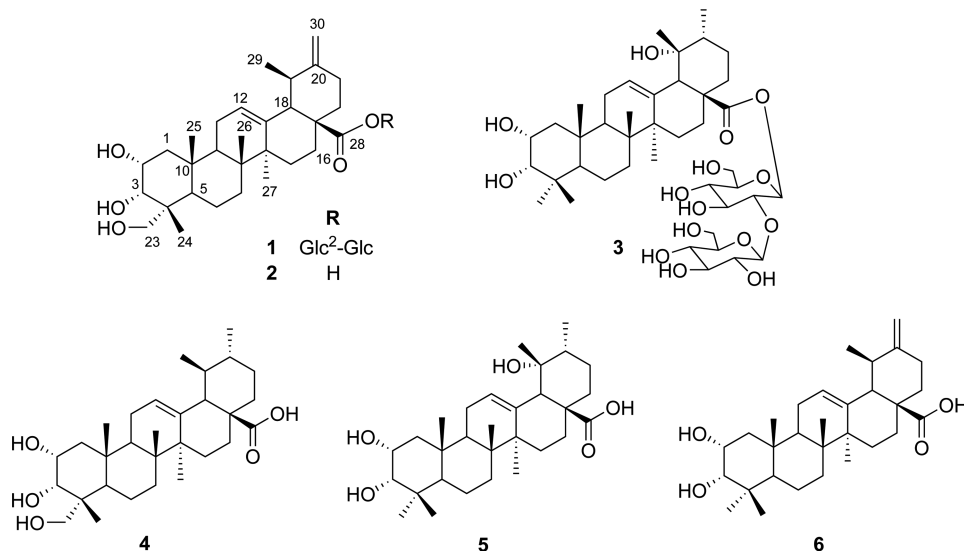


Figure 1. Structures of compounds 1-6.

Table 1. ^1H - 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 ^1H - 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 2 α ,3 α ,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 ^1H - ^1H 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 ^1H -NMR and MS data were in good agreement with values reported previously,¹¹ and D-glucose ($[\alpha]_{\text{D}}^{25} +49.4^\circ c = 0.04$ 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

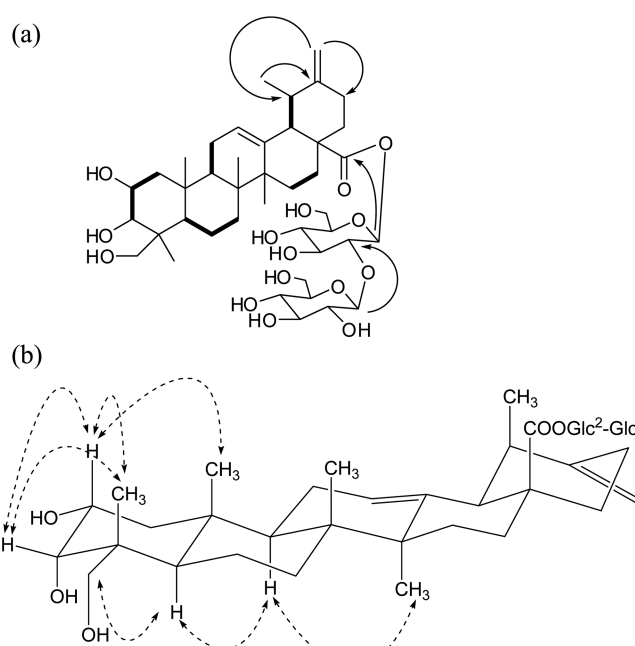


Figure 2. Key HMBC (H→C), ^1H - ^1H COSY (—) correlations (a) and NOESY (⋯) correlations of **1** (b).

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]-2 α ,3 α ,23-trihydroxyursa-12,20(30)-dien-28-oic acid, named sieboldii saponin A.

The known compounds, 2 α ,3 α ,23-trihydroxyursa-12,20(30)-dien-28-oic acid (**2**),¹¹ pruvuloside A (**3**),¹⁴ esculentic acid (**4**),¹⁵ euscaphic acid (**5**),¹⁶ and 2 α ,3 α -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 ^1H - ^1H COSY, DEPT, HMQC, HMBC and NOESY experiments, were recorded on a Varian UNITY INOVA 700 NMR spectrometer operating at 700 MHz (^1H) 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-C₁₈ 10 μm column (250 \times 10 mm). Silica gel 60 (Merck, 70-230 mesh and 230-400 mesh) and RP-C₁₈ 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 $\text{C}_2\text{H}_5\text{OH}$ (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 Chem-Station 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), CHCl₃ (20 g), EtOAc (12 g), and *n*-BuOH (24 g) layers. The EtOAc-soluble layer (12 g) was separated on a RP-C₁₈ silica gel column (230-400 mesh, 400 g), and eluted gradually with MeOH-H₂O (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 CHCl₃-MeOH (20:1) and then purified by reversed-phase preparative HPLC with MeOH-H₂O (12:7) at a flow rate of 2.0 mL/min (Econosil RP-C₁₈ 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 CHCl₃-soluble layer (20 g) was chromatographed on a RP-C₁₈ silica gel open column (230-400 mesh, 550 g) eluting with a gradient solvent system of MeOH-H₂O (1:1 and 1:0), yielding nine subfractions (fr. C1-C9). Fr. C4 (8 g) was separated on a RP-C₁₈ 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% CH₃CN 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% CH₃CN 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 (CD₃OD, 700 MHz) and ¹³C-NMR (CD₃OD, 175 MHz) see Table 1; HR-FABMS *m/z* 811.4480 [M + H]⁺ (calcd. for C₄₂H₆₇O₁₅: 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 CHCl₃ 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-H₂O = 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 (C₃D₅N, 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 C₃₀H₄₇O₅: 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 H₂O (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.

Acknowledgments. This research 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 (2012R1A5A2A28671860). We thank the Korea Basic Science Institute (KBSI) for the MS spectral measurements.

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