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A New Steroidal Glycoside from *Allium macrostemon* Bunge

Yun Sik Kim, Joon Min Cha, Dong Hyun Kim, Tae Hyun Lee, and Kang Ro Lee*

Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Korea

Abstract – A phytochemical investigation of *Allium macrostemon* Bunge (Liliaceae) afforded the new pregnane steroidal glycoside, named allimacroside F (**1**), along with three known glycosides, benzyl-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**2**), phenylethyl-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**3**), (*Z*)-3-hexenyl-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**4**). The identification and structural elucidation of a new compound (**1**) was carried out based on spectral data analyses (^{1}H-NMR, ^{13}C-NMR, ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY) and HR-FAB-MS.

Keywords – *Allium macrostemon*, Liliaceae, Steroidal glycoside, Allimacroside F.

Introduction

Allium macrostemon Bunge (Liliaceae), known as wild onion, is widely distributed in East Asian countries.[1] Its dried bulbs have been known as a traditional Chinese medicine "Xiebai", and used for treatment of heart diseases such as thoracic pain, stenocardia, and heart asthma.[2] Various steroidal glycosides with medicinal properties have been reported from the genus *Allium*,[3] and previous phytochemical investigations on *A. macrostemon* demonstrated the presence of steroidal glycosides, including macrostemonosides A-S.[4-8] In the course of our search for the new steroidal glycosides from this plant, we reported the isolation of allimacrosides A–E.[9] In continuing study of this source, we isolated further a new pregnane-type steroidal glycoside, namely allimacroside F (**1**), together with three known compounds (**2 - 4**).

Experimental

General experimental 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. UV spectra were recorded using a Schimadzu UV-1601 UV-visible spectrophotometer. High resolution (HR)-fast atom bombardment (FAB) mass spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra, including ^{1}H-^{1}H correlated spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), heteronuclear single quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC) and nuclear overhauser effect spectroscopy (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 (YMC GEL ODS-A, 12 nm, S-75 μm) were used for column chromatography. TLC was performed using percolated Silica gel F_{254} plates and RP-18 F_{254s} plates (Merck). Spots were detected by 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 was controlled by the Enhanced ChemStation Version B.01.00 software. 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 – *A. mactostemon* was collected in Taeback, Gangwon province, Korea in April, 2010, and the plant was identified by one of the authors (K. R. Lee). A voucher specimen (SKKU-NPL 1202) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation – Dried whole plants of *A. macrostemon* (1.5 kg) were extracted with 80% MeOH three times at room temperature and evaporated under

*Author for correspondence
Kang Ro Lee, Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Korea
Tel: +82-31-290-7710; E-mail: krlee@skku.edu

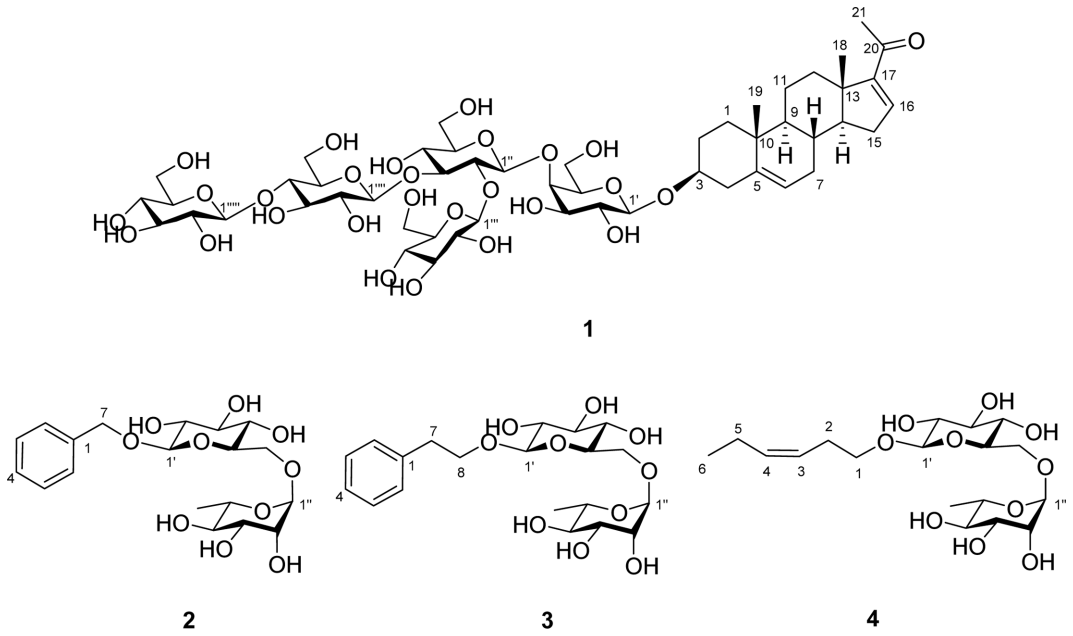

Fig. 1. The structures of **1** - **4** isolated from *A. macrostemon.*

reduced pressure to give a residue (210.0 g), which was dissolved in water (800 ml) and partitioned with solvents to give *n*-hexane (10.0 g), $CHCl_3$ (5.5 g), EtOAc (1.9 g), and *n*-BuOH (12.2 g) soluble layers. The *n*-BuOH-soluble layer (12.2 g) was chromatographed on a silica gel column (diameter × height: 5.5 × 35.0 cm, 300.0 g) with a $CHCl_3$-MeOH-H_2O (20:10:1 to 2:3:1) to give 10 fractions (B1-B10) based on a TLC analysis. Fraction B4 (1.3 g) was separated on a RP-C_{18} open column (2.5 × 30.0 cm, 60.0 g), eluting with 50% aqueous MeOH to give nine subfractions (B41-B49). Subfraction B42 (43 mg) was purified by an RP-C18 semi-prep. HPLC (2 mL/min, 35% aqueous MeOH) to afford **2** (7 mg, t_R = 17.8 min). Subfraction B44 (27 mg) was purified by an RP-C18 semi-prep. HPLC (2 mL/min, 35% aqueous MeOH) to afford **3** (6 mg, t_R = 33.7 min). Subfraction B45 (30 mg) was purified by an RP-C18 semi-prep. HPLC (2 mL/min, 40% aqueous MeOH) to afford **4** (8 mg, t_R = 21.3 min). Fraction B7 (2.6 g) was separately chromatographed on a Diaion HP-20 column (2.5 × 35.0 cm, 80.0 g) eluting with a gradient solvent system of 100% H_2O and 100% MeOH, yielding subfractions B71 and B72. Subfraction B72 (1.5 g) separated on a RP-C_{18} silica gel open column (2.5 × 30.0 cm, 60 g), eluting with 40% aqueous MeOH to give six subfractions (B721-B726). Subfraction 726 (22 mg) was purified by an RP-C18 semi-prep. HPLC (2 mL/min, 36% aqueous MeCN) to afford **1** (3 mg, t_R = 15.2 min).

Allimacroside F (1) – White amorphous powder; $[\alpha]_D^{25}$ -59.3 (MeOH); UV (MeOH) λ_{max}: 239 nm; IR (KBr) ν_{max}: 3385, 2925, 1664, 1370, 1160, 1070 cm^{-1}; ^{1}H-NMR (Pyridine-d_5, 700 MHz) and ^{13}C-NMR (Pyridine-d_5, 175 MHz) see Table 1; HR-FAB-MS *m/z* 1147.4785 $[M+Na]^+$ (calcd. for $C_{51}H_{80}NaO_{27}$: 1147.4785).

Benzyl-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (2) – Colorless gum; IR (KBr) ν_{max}: 3385, 2923, 1049 cm^{-1}; ^{1}H-NMR (700 MHz, Pyridine-d_5): δ 7.53 (2H, d, *J* = 7.3 Hz, H-2, 6), 7.27 (2H, m, H-3, 5), 7.22 (1H, m, H-4), 5.16 (1H, d, *J* = 11.8 Hz, H-7a), 5.56 (1H, d, *J* = 1.2 Hz, H-1"), 4.91 (1H, d, *J* = 7.8 Hz, H-1'), 4.83 (1H, d, *J* = 11.8 Hz, H-7b), 1.63 (3H, d, *J* = 6.2 Hz, H-6"); ^{13}C-NMR (175 MHz, Pyridine-d_5): δ 138.5 (C-1), 128.4 (C-2, 6), 128.4 (C-3, 5), 127.6 (C-4), 103.5 (C-1'), 102.4 (C-1"), 78.3 (C-5'), 77.0 (C-3'), 74.9 (C-2'), 73.9 (C-4"), 72.6 (C-3"), 72.1 (C-2"), 71.7 (C-4'), 70.7 (C-1), 69.6 (C-5"), 68.2 (C-6'), 18.5 (C-6"); FAB-MS (positive mode) *m/z* = 417.23 $[M+H]^+$.

Phenylethyl-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (3) – Colorless gum; UV (MeOH) λ_{max}: 254, 212 nm; IR (KBr) ν_{max}: 3385, 2925, 1047 cm^{-1}; ^{1}H-NMR (700 MHz, Pyridine-d_5): δ 7.30 (2H, d, *J* = 7.1 Hz, H-2, 6), 7.25 (2H, dd, *J* = 7.1, 7.4 Hz, H-3, 5), 7.19 (1H, dd, *J* = 7.4, 1.3 Hz, H-4), 5.52 (1H, d, *J* = 0.8 Hz, H-1"), 4.84 (1H, d, *J* = 7.8 Hz, H-1'), 4.18 (1H, m, H-8a), 3.93 (1H, dt, *J* = 9.8, 7.4 Hz, H-8b), 2.98 (2H, dd, *J* = 7.2, 7.2 Hz, H-7), 1.62 (3H, d, *J* = 6.2 Hz, H-6"); ^{13}C-NMR (175 MHz, Pyridine-d_5): δ 140.7 (C-1), 130.7 (C-3, 5), 130.0 (C-2, 6), 127.7 (C-4), 106.0 (C-1'), 103.8 (C-1"), 79.8 (C-3'), 78.4 (C-5'), 76.3 (C-2'), 75.3 (C-4"), 74.1 (C-3"), 73.6

(C-2"), 73.1 (C-8), 71.8 (C-4'), 71.1 (C-5"), 69.6 (C-6'), 37.9 (C-7), 20.0 (C-6"); FAB-MS (positive mode) *m/z* = 431.26 $[M+H]^+$.

(*Z*)-3-Hexenyl-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (4) – Colorless gum; IR (KBr) ν_{max}: 3385, 2933, 1048 cm^{-1}; ^{1}H-NMR (700 MHz, Pyridine-d_5): δ 5.51 (1H, d, *J* = 0.8 Hz, H-1"), 5.46 (1H, dtt, *J* = 10.8, 7.3, 1.5 Hz, H-3), 5.38 (1H, dtt, *J* = 10.8, 7.3, 1.5 Hz, H-4), 4.81 (1H, d, *J* = 7.7 Hz, H-1'), 4.12 (1H, dt , *J* = 9.5, 7.1 Hz, H-1a), 3.71 (1H, dt , *J* = 9.4, 7.1 Hz, H-1b), 2.41 (2H, q, *J* = 6.9 Hz, H-2), 1.92 (2H, quin, *J* = 7.2 Hz, H-5), 1.63 (3H, d, *J* = 6.2 Hz, H-6"), 0.82 (3H, t, *J* = 7.5 Hz, H-6); ^{13}C-NMR (175 MHz, Pyridine-d_5): δ 133.2 (C-4), 125.4 (C-3), 104.4 (C-1'), 102.4 (C-1"), 78.3 (C-3'), 76.9 (C-5'), 74.9 (C-2'), 73.8 (C-4"), 72.6 (C-3"), 72.1 (C-2"), 71.6 (C-4'), 69.6 (C-5"), 69.2 (C-1), 68.1 (C-6'), 28.1 (C-2), 20.6 (C-5), 18.4 (C-6"), 14.1 (C-6); FAB-MS (positive mode) *m/z* = 431.25 $[M+Na]^+$.

Acid hydrolysis of 1 and sugar determination – **1** (2.0 mg) was dissolved in 2 mL of 15% HCl. The solution was heated at 80 ºC for 2 h. The hydrolysate was extracted with CH_2Cl_2, and the aqueous layer was neutralized using an Amberlite IRA-67 column to yield the sugars. The sugar acquired from the hydrolysis was dissolved in

Table 1. ^{1}H and ^{13}C NMR data of **1** in Pyridine-d_5. (δ in ppm, 700 MHz for ^{1}H and 175 MHz for ^{13}C)[a]

Position	1		Position	1	
	δ_H	δ_C		δ_H	δ_C
1	1.59 m, 0.90 m	38.6	Gal 1'	4.87 d (7.7)	104.0
2	2.06 m, 1.66 m	31.4	2'	4.41 m	74.5
3	3.85 m	79.4	3'	4.07 m	76.4
4	2.65 dd (13.3, 2.4), 2.40 t-like (12.2)	40.5	4'	4.57 m	81.4
5	-	142.8	5'	3.94 m	76.6
6	5.29 br d (5.2)	122.6	6'	4.65 m, 4.15 m	61.8
7	1.83 m, 1.52 m	33.0	Glc 1"	5.12 d (7.8)	106.4
8	1.49 m	31.6	2"	4.37 m	82.7
9	0.89 m	52.0	3"	4.13 m	89.2
10	-	38.3	4"	3.77 m	72.0
11	1.45 m, 1.45 m	22.2	5"	3.82 m	78.8
12	2.58 m, 1.34 m	36.4	6"	4.45 m, 3.98 m	64.3
13	-	47.5	Glc 1"'	5.55 d (7.7)	106.2
14	1.26 m	57.7	2"'	4.03 m	77.5
15	2.12 ddd (16.9, 6.5, 3.3) 1.85 m	33.6	3"'	4.18 m	79.5
16	6.58 dd (2.9, 1.8)	146.0	4"'	4.13 m	72.9
17	-	156.5	5"'	3.82 m	79.8
18	0.90 s	17.2	6"'	4.55 m, 4.27 m	63.8
19	0.86 s	20.5	Glc 1""	5.25 d (7.7)	105.3
20	-	197.6	2""	4.02 m	76.0
21	2.23 s	28.4	3""	4.18 m	78.0
			4""	4.21 m	82.7
			5""	3.99 m	77.9
			6""	4.51 m, 4.22 m	63.0
			Glc 1""'	5.11 d, (7.5)	106.3
			2""'	4.04 m	76.1
			3""'	3.89 m	80.0
			4""'	4.22 m	72.2
			5""'	4.10 m	79.1
			6""'	4.55 m, 4.36 m	63.7

[a] *J* values are in parentheses and reported in Hz; the assignments were based on ^{1}H-^{1}H COSY, HMQC, and HMBC experiments.

anhydrous pyridine (0.1 mL), and 2.0 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 1.5 h and trimethylsilylated through adding 0.1 mL of 1-trimethylsilylimidazole for 2 h. The mixture was partitioned with *n*-hexane and H_2O (0.3 mL each), and the *n*-hexane layer (1.0 μL) was analyzed through GC/MS. Identification of D-galactose (20.093 min) and D-glucose (22.103 min) were detected in each case by co-injection of the hydrolysate with standard silylated sugars.

Result and Discussion

Structures of **2** - **4** were identified by comparing ^{1}H-, ^{13}C-NMR, and MS spectral data with those in the literatures to be benzyl-*O*-*α*-L-rhamnopyranosyl-(1 →6)-*β*-D-glucopyranoside (**2**),[10] phenylethyl-*O*-*α*-L-rhamnopyranosyl-(1 →6)-*β*-D-glucopyranoside (**3**),[11,12] (*Z*)-3-hexenyl-*O*-*α*-L-rhamnopyranosyl-(1 → 6)-*β*-D-glucopyranoside (**4**).[13] Compounds **2** - **4** were isolated from this source for the first time.

Compound **1** was isolated as a white amorphous powder. The molecular formula was determined to be $C_{51}H_{80}O_{27}$ from the molecular ion peak $[M+Na]^+$ at *m/z* 1147.4785 (calcd. for $C_{51}H_{80}NaO_{27}$: 1147.4785) in the positive-ion HR-FAB-MS. The IR spectrum showed characteristic absorptions for *α*,*β*-unsaturated ketone (1664 cm^{-1}), hydroxyl (3385 cm^{-1}), and glycosidic linkage (1000 - 1160 cm^{-1}).[14] The ^{1}H-NMR spectrum of **1** (Table 1) displayed the signals of two olefinic protons at δ_H 6.58 (dd, *J* = 2.9, 1.8 Hz, H-16) and 5.29 (br d, *J* = 5.2 Hz, H-6), an oxygenated methine proton at δ_H 3.85 (m, H-3), and three methyl singlet signals at δ_H 2.23 (s, H-21), 0.90 (s, H-18), and 0.86 (s, H-19) of aglycone, and five anomeric protons at 4.87 (d, *J* = 7.7 Hz, H-1'), 5.12 (d, *J* = 7.8 Hz, H-1"), 5.55 (d, *J* = 7.7 Hz, H-1'"), 5.25 (d, *J* = 7.7 Hz, H-1""), and 5.11 (d, *J* = 7.5 Hz, H-1""") of five sugar moieties. The ^{13}C-NMR spectrum (Table 1) showed a total of 51 carbon signals, of which 21 carbons were assigned to the aglycone and the remaining 30 carbons to five hexoses. The ^{13}C-NMR and DEPT spectra displayed 21 signals for the aglycone, which are composed of one ketone carbon at δ_C 197.6, four olefinic carbons at δ_C 156.5, 146.0, 142.8 and 122.6, one oxygenated methine carbon at δ_C 79.4, two quaternary carbons at δ_C 47.5 and 38.3, three methine carbons at δ_C 57.7, 52.0, and 31.6, seven methylene carbons at δ_C 40.5, 38.6, 36.4, 33.6, 33.0, 31.4 and 22.2, and three methyl carbons at δ_C 28.4, 20.5 and 17.2. A comparison of the NMR spectral findings of **1** with literature data revealed that the aglycone pair of **1** was identical to that of allimacroside A.[9] Detailed comparison of ^{13}C-NMR, ^{1}H–^{1}H COSY, HSQC, and HMBC spectra of **1** with those of allimacroside A suggested that the sugar moiety of **1** was similar to that of allimacroside A with the exception of presence of an additional glucopyranose [δ_H 5.11 (d, *J* = 7.5, H-1"") and δ_C 106.3, 76.1, 80.0, 72.2, 79.1, and 63.7]. In the HMBC

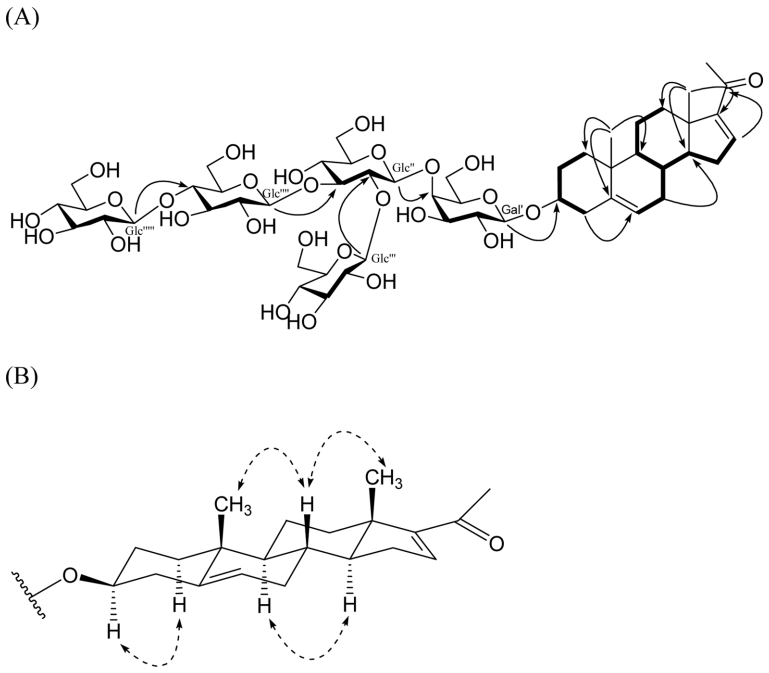

Fig. 2. Key HMBC (HC), ^{1}H-^{1}H COSY (—) correlations **1** (A), and NOESY (◄- -►) correlations of **1** (B).

spectrum, the key correlations from δ_H 4.87 (H-1') to δ_C 79.4 (C-3), from δ_H 5.12 (H-1") to δ_C 81.4 (C-4'), from δ_H 5.55 (H-1"') to δ_C 82.7 (C-2"), from δ_H 5.25 (H-1"") to δ_C 89.2 (C-3") and δ_H 5.11 (H-1""') to δ_C 82.7 (C-4"") suggested that the sequence of sugar moieties and the linkage position between the sugar unit and aglycone was the C-3 hydroxyl group (Fig. 2 A). Large coupling constants ($^3J_{H1, H2} \geq 7.5$ Hz) for anomeric protons revealed the β-configuration of all sugars. The relative stereochemistry of the aglycone was corroborated by NOESY cross-peaks of H_{ax}-1/H-3, H-19/H-8/H-18, and H-9/H-14 (Fig. 2 B). On acid hydrolysis, **1** yielded D-galactose and D-glucose in a ratio of 1:4 by GC analysis after derivatization.[15] Thus, the structure of **1** was established as pregna-5,16-dien-3β-ol-20-one 3-*O*-β-D-glucopyranosyl (1→4)-β-D-glucopyranosyl (1→3)-[β-D-glucopyranosyl (1→2)]-β-D-glucopyranosyl (1→4)-β-D-galactopyranoside, named allimacroside F.

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