A New Steroidal Glycoside from *Allium macrostemon* Bunge

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**Abstract** – A phytochemical investigation of *Allium macrostemon* Bunge (Liliaceae) afforded the new pregane 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 (1H-NMR, 13C-NMR, 1H–1H 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 1H–1H 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 (1H) and 175 MHz (13C) 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 × 10 mm). Silica gel 60 (Merck, 70 - 230 mesh and 230 - 400 mesh) and RP-C18 silica gel (YMC GEL ODS-A, 12 μm, S-75 μm) were used for column chromatography. TLC was performed using percolated Silica gel F254 plates and RP-18 F254s plates (Merck). Spots were detected by TLC under UV light or by heating after spraying with 10% H2SO4 in C2H5OH (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. macrostemon* was collected in Taebuck, 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
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), CHCl₃ (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 CHCl₃-
MeOH-H₂O (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-C₁₈ open column (2.5 × 30.0 cm, 60.0 g),
eluting with 50% aqueous MeOH to give nine subfrac-
tions (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% H₂O and 100% MeOH, yielding subfrac-
tions B71 and B72. Subfraction B72 (1.5 g) separated on
a RP-C₁₈ 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;
[α]_D^25° = -59.3 (MeOH); UV (MeOH) λ_max: 239 nm; IR
(KBr) ν_max: 3385, 2925, 1664, 1160, 1070 cm⁻¹; ¹H-
NMR (Pyridine-d₅, 700 MHz) and ¹³C-NMR (Pyridine-d₅,
175 MHz) see Table 1; HR-FAB-MS m/z 1147.4785 [M+
Na]+ (calcd. for C₅₁H₈₀NaO₂₇: 1147.4785).

**Benzyl-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopy-
ranoside (2)** – Colorless gum; IR (KBr) ν_max: 3385, 2923,
1049 cm⁻¹; ¹H-NMR (700 MHz, Pyridine-d₅): 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’’); ¹³C-
NMR (175 MHz, Pyridine-d₅): 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-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopy-
ranoside (3)** – Colorless gum; UV (MeOH) λ_max: 254, 212 nm; IR (KBr) ν_max: 3385, 2925, 1047 cm⁻¹; ¹H-
NMR (700 MHz, Pyridine-d₅): 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’’); ¹³C-NMR (175
MHz, Pyridine-d₅): 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

![Fig. 1. The structures of 1 - 4 isolated from A. macrostemon.](image-url)
(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-\( \alpha \)-L-rhamnopyranosyl-(1 \( \rightarrow \) 6)-\( \beta \)-D-glucopyranoside (4) – Colorless gum; IR (KBr) \( \nu \) max: 3385, 2933, 1048 cm\(^{-1}\); \( ^1\)H-NMR (700 MHz, Pyridine-\( d_5 \)): \( \delta \) 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-\( d_5 \)): \( \delta \) 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\(^\circ\)C for 2 h. The hydrolysate was extracted with CH\(_2\)Cl\(_2\), 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-\( d_5 \). (\( \delta \) in ppm, 700 MHz for \( ^1\)H and 175 MHz for \( ^{13}\)C)

<table>
<thead>
<tr>
<th>Position</th>
<th>( \delta_1 )</th>
<th>( \delta_2 )</th>
<th>Position</th>
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<th>( \delta_2 )</th>
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<td>38.6</td>
<td>Gal 1'</td>
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<td>2.06 m, 1.66 m</td>
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<td>4.07 m</td>
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<td>4</td>
<td>2.65 dd (13.3, 2.4), 2.40 t-like (12.2)</td>
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<tr>
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<td>-</td>
<td>142.8</td>
<td>5'</td>
<td>3.94 m</td>
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<td>6</td>
<td>5.29 br d (5.2)</td>
<td>122.6</td>
<td>6'</td>
<td>4.65 m, 4.15 m</td>
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<td>Glc 1''</td>
<td>5.12 d (7.8)</td>
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<tr>
<td>8</td>
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<td>2''</td>
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<td>Glc 1'''</td>
<td>5.55 d (7.7)</td>
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<td>16</td>
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<td>197.6</td>
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<tr>
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<td></td>
<td>6''''</td>
<td>4.51 m, 4.22 m</td>
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<td></td>
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<td>6''''</td>
<td>4.55 m, 4.36 m</td>
<td>63.7</td>
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\( J \) values are in parentheses and reported in Hz; the assignments were based on \( ^1\)H-\( ^1\)H COSY, HMQC, and HMBC experiments.
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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 H2O (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 1H-, 13C-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 C51H80O27 from the molecular ion peak [M+Na]+ at m/z 1147.4785 (calcd. for C51H80NaO27 : 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 1H-NMR spectrum of 1 (Table 1) displayed the signals of two olefinic protons at δH 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 δH 3.85 (m, H-3), and three methyl singlet signals at δH 2.23 (s, H-21), 0.90 (s, H-18), and 0.86 (s, H-19) of aglycone, and five anemic 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 = 7.5 Hz, H-1'''') and 5.11 (d, J = 7.5 Hz, H-1''''') of five sugar moieties. The 13C-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 13C-NMR and DEPT spectra displayed 21 signals for the aglycone, which are composed of one ketone carbon at δC 197.6, four olefinic carbons at δC 156.5, 146.0, 142.8 and 122.6, one oxygenated methine carbon at δC 79.4, two quaternary carbons at δC 47.5 and 38.3, three methine carbons at δC 57.7, 52.0, and 31.6, seven methylene carbons at δC 40.5, 38.6, 36.4, 33.6, 33.0, 31.4 and 22.2, and three methyl carbons at δC 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 13C-NMR, 1H–1H 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 [δH 5.11 (d, J = 7.5, H-1''''') and δC 106.3, 76.1, 80.0, 72.2, 79.1, and 63.7]. In the HMBC

Fig. 2. Key HMBC (HC), 1H–1H COSY (—) correlations 1 (A), and NOESY (→) correlations of 1 (B).
spectrum, the key correlations from $\delta^{1} 4.87$ (H-1') to $\delta^{1} 79.4$ (C-3), from $\delta^{1} 5.12$ (H-1'') to $\delta^{1} 81.4$ (C-4'), from $\delta^{1} 5.55$ (H-1''') to $\delta^{1} 89.2$ (C-3'') and $\delta^{1} 5.11$ (H-1''''') to $\delta^{1} 82.7$ (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 ($^{3}J_{H1, H2} \geq 7.5$ Hz) for anomeric protons revealed the $\beta$-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. Thus, the structure of 1 was established as pregna-5,16-dien-3$\beta$-ol-20-one 3-O- $\beta$-D-glucopyranosyl (1$\rightarrow$4)-$\beta$-D-glucopyranosyl (1$\rightarrow$3)-[$\beta$-D-glucopyranosyl (1$\rightarrow$2)]-$\beta$-D-galactopyranoside, named allimacroside F.

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