

Communication

A New Sesquiterpene Glycoside from the Aerial Parts of *Saussurea triangulata*

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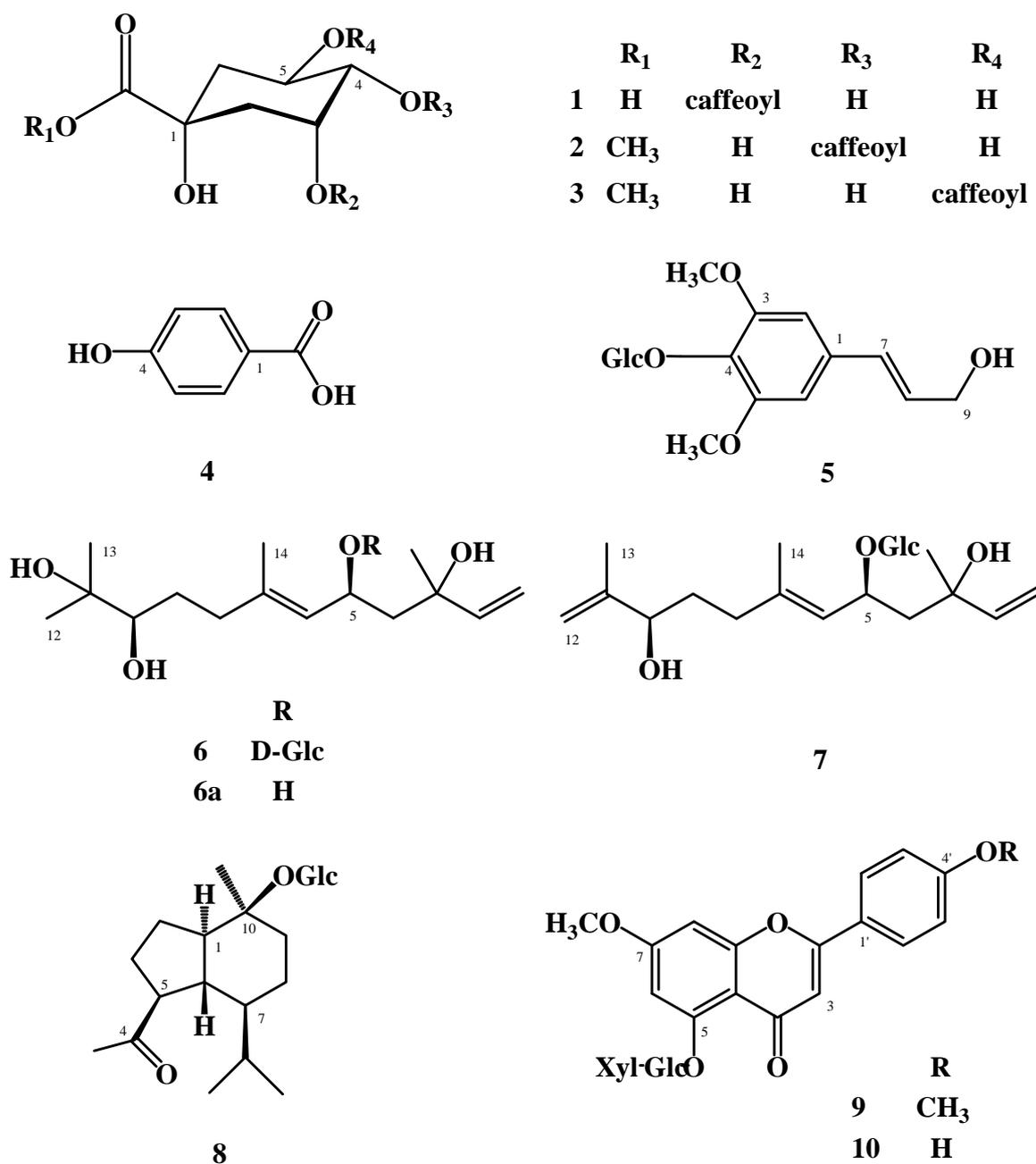
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Abstract: Column chromatographic separation of a MeOH extract of the aerial parts of *Saussurea triangulata* led to the isolation of a new sesquiterpene glycoside **6**, together with three quinic acid derivatives, two phenolics, two sesquiterpene glycosides and two flavonoids. The new compound **6** was identified as amarantholidol A glycoside by spectroscopic and chemical methods.

Keywords: *Saussurea triangulata*, quinic acid, phenolics, sesquiterpene glycosides.

Introduction

In a continuation of our study on biologically active compounds from Korean Compositae plants [1,2], the phytochemical constituents of the aerial parts of *Saussurea triangulata* were investigated. *S. triangulata* is widely distributed in Korea, and has been used in Korean folk medicine for the treatment of inflammation, hypertension and hepatitis [3]. However, there have been no phytochemical or bioactivity studies of this plant. Purification of the MeOH extract of the aerial parts from *S. triangulata* by column chromatography yielded a new compound **6**, together with nine known compounds: three quinic acid derivatives **1** - **3**, two phenolics **4** and **5**, two sesquiterpene glycosides **7** and **8**, and two flavonoids **9** and **10** (Figure 1). Their structures were determined by spectroscopic means. Compounds **1** - **4**, **7**, **9** and **10** were isolated from the genus *Saussurea* for the first time.

Figure 1. The structures of compounds **1** - **10**.

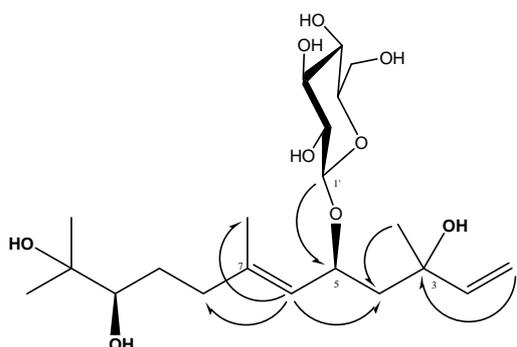
Results and Discussion

By comparison of their spectral data (¹H-, ¹³C-NMR and MS) with that reported in the literature, compounds **1-5** and **7-10** were identified as 3-caffeoylquinic acid (**1**) [4], methyl 4-caffeoylquinic acid (**2**) [5], methyl 5-caffeoylquinic acid (**3**) [6], 4-hydroxybenzoic acid (**4**) [7], syringin (**5**) [8], amarantholidoside II (**7**) [9], (-)-oplopan-4-one-10-*α*-*O*-β-D-glucoside (**8**) [10], 7,4'-di-*O*-methylapigenin 5-*O*-*α*-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**9**) [11,12] and 7-*O*-methylapigenin 5-*O*-*α*-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**10**), respectively [11].

Compound **6** was obtained as a colorless oil and [α]_D value was -17.3° (c 0.85, MeOH). Its IR spectrum revealed absorption bands for hydroxyl (3397 cm⁻¹) and C=C double bond functional groups (1642 cm⁻¹), and the UV spectrum exhibited an absorption band at λ_{max} 200 nm. The HRESI-MS

spectrum of **6** showed a quasimolecular ion peak at m/z 457.2426 $[M+Na]^+$, suggesting the molecular formula $C_{21}H_{38}O_9$ (calc. for $C_{21}H_{38}O_9Na$: 457.2408) and three degrees of unsaturation. The 1H -NMR spectrum showed an ABX system of olefinic protons [δ 5.26 (1H, dd, $J = 16.5, 2.0$ Hz), 5.03 (1H, dd, $J = 10.5, 2.0$ Hz) and 5.98 (1H, dd, $J = 16.5, 10.5$ Hz)], a single olefinic proton δ 5.16 (1H, d, $J = 10.0$ Hz), which account for two of the three degree of unsaturation, four methyl groups [δ 1.18, 1.15, 1.71 and 1.30 (each 3H, s)], three methylene groups [δ 1.99 (1H, dd, $J = 14.5, 8.0$ Hz), 1.66 (1H, dd, $J = 14.5, 4.0$ Hz), 2.30 (1H, ddd, $J = 13.5, 10.0, 5.0$ Hz), 2.11 (1H, ddd, $J = 13.5, 9.0, 8.0$ Hz), 1.76 (1H, m), and 1.41 (1H, m)], and two oxygenated methine protons [δ 4.92 (1H, ddd, $J = 10.0, 8.0, 4.0$ Hz) and 3.24 (1H, dd, $J = 10.5, 2.0$ Hz)]. The ^{13}C -NMR spectrum showed four methyl groups (δ 26.0, 25.0, 17.2 and 28.7), three methylene groups (δ 48.4, 37.9 and 30.5), four oxygenated carbons (δ 74.2, 71.6, 79.0 and 73.9) and four olefinic carbons (δ 112.2, 146.5, 126.4 and 141.8). The spectral data all suggested that **6** was a nerolidol type sesquiterpene glycoside [9]. This was confirmed by the enzymatic hydrolysis of **6**, which afforded **6a** and a sugar component, which accounted for the last degree of unsaturation. The sugar was identified to be D-glucose by co-TLC (EtOAc-MeOH-H₂O = 9:3:1, R_f value: 0.17) with a D-glucose standard. The 1H - and ^{13}C -NMR spectrum also confirmed the presence of D-glucose [13]. The position of D-glucose was determined to be at C-5, based on the HMBC correlation (Figure 2). The configuration the sugar moiety was determined to be the β form by the presence of an anomeric proton at δ 4.25 (1H, d, $J = 8.0$ Hz, H-1') and an anomeric carbon at δ 100.2 (C-1') in the 1H - and ^{13}C -NMR spectra, respectively. Comparison of the 1H -NMR spectrum (Table 1) and $[\alpha]_D^{25}$ value indicated a strong similarity between **6a** and to the known compound amarantholidol A, which was previously isolated from *Amaranthus retroflexus* [9]. The configurations at C-5 and C-10 were the same (α -H form) as those of amarantholidol A and **7** based on the chemical shifts and J values. Thus, compound **6** was proposed to be amarantholidol A glycoside, which has been isolated from natural sources for the first time.

Figure 2. Important HMBC (H \rightarrow C) correlations of **6**.



Experimental

General

The melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The optical rotations were determined using a Jasco P-1020 polarimeter (Jasco Co., Japan). The IR

spectra were recorded on a Bruker Vector 22 FT-IR spectrometer (Bruker Co., German). The UV spectra were obtained using a Shimadzu UV-1601 UV/Visible instrument (Shimadzu Co., Japan). The NMR spectra were recorded on a Varian VXR-500 instrument. The FAB-MS data were obtained using a JMS700 spectrometer (Jeol Co., Japan). The ESI-MS data were obtained using a Micromass QTOF2 LC/ESI MS (Micromass Co., USA). The semi-preparative HPLC was carried out on a Gemini[®] RP-C₁₈ column (5 μ , 10 \times 250 mm, Phenomenex Co., USA) using an RI detector (Shodex Co., Japan). Open column chromatography was carried out on silica gel (Silica gel 60, 70-230 mesh, Merck Co., Germany). Thin-layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ and RP-18 F_{254s} (Merck Co., Germany). The packing material for the molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co., Sweden). Low pressure liquid chromatography was carried out using a LiChroprep Lobar[®]-A RP-18 column (240 \times 10 mm, Merck Co., Germany) and a Duramat[®] 80 pump (CFG Prominent Co., Germany)

Plant material

The aerial parts of *S. triangulata* were collected at Mt. Odae, Korea in August, 2004. A voucher specimen (SKK-04-081) was deposited at the College of Pharmacy of Sungkyunkwan University.

Extraction and Isolation

The dried aerial parts of *S. triangulata* (1.1 kg) were extracted three times with 80% MeOH at room temperature. The resulting MeOH extract (160.0 g) was partitioned with solvent to give *n*-hexane (35.0 g), CHCl₃ (12.0 g) and *n*-BuOH (22.0 g) soluble fractions. The *n*-BuOH fraction (22.0 g) was chromatographed over a Diaion HP-20 resin column using a gradient solvent system (MeOH - H₂O = 0: 100 \rightarrow 100:0) to give six fractions B1 - B6. The B2 fraction (2.0 g) was subjected to Sephadex LH-20 chromatography (MeOH - H₂O = 80:20) to give five fractions B21 - B25. The B22 fraction (1.0 g) was subjected to RP-C₁₈ silica column chromatography (MeOH - H₂O = 30:70) to afford two fractions B221 and B222. The B221 fraction (600.0 mg) was purified on a RP-18 Lobar[®]-A column (CH₃CN - H₂O = 10:90) to yield compounds **1** (200.0 mg), **2** (25.0 mg) and **3** (180.0 mg). The B222 fraction (300.0 mg) was purified on a RP-18 Lobar[®]-A column (CH₃CN - H₂O = 15:85) to yield compound **4** (10.0 mg). The B24 fraction (200.0 mg) was purified on a RP-18 Lobar[®]-A column (MeOH - H₂O = 20:80) to give compound **5** (50.0 mg). The B3 fraction (3.0 g) was subjected to Sephadex LH-20 chromatography (MeOH - H₂O = 80:20) to give five fractions B31 - B36. The B31 fraction (300.0 mg) was purified by RP-18 Lobar[®]-A chromatography (MeOH - H₂O = 20:80) and semi-preparative HPLC (CH₃CN - H₂O = 20:80) to give **6** (15.0 mg). The B4 fraction (5.0 g) was subjected to Sephadex LH-20 (MeOH - H₂O = 80:20) to give six fractions B41 - B46. The B41 fraction (1.0 g) was separated on a RP-C₁₈ silica column (MeOH - H₂O = 57:43) to give three fractions B411 - B413. The B411 fraction (350.0 mg) was purified by RP-18 Lobar[®]-A chromatography (MeOH - H₂O = 50:50) and semi-preparative HPLC (CH₃CN - H₂O = 27:73) to give compounds **7** (8.0 mg) and **8** (10.0 mg). The B412 fraction (350.0 mg) was purified by recrystallization (100% MeOH) to give **9** (15.0 mg). The B5 fraction (2.0 g) was subjected to Sephadex LH-20 chromatography

(MeOH - H₂O = 80:20) to give five fractions B51 - B56. The B51 fraction (500.0 mg) was purified by recrystallization (100% MeOH) to give **10** (15.0 mg).

Amarantholidol A glycoside (6): Colorless oil; $[\alpha]_D^{25}$: -17.3° (c 0.85, MeOH); UV λ_{\max} (MeOH) nm (log ϵ): 200 (4.04); IR (neat) ν_{\max} cm⁻¹: 3397 (OH), 1642 (C=C); HRESIMS m/z : 457.2426 [M+Na]⁺, (calc. for C₂₁H₃₈O₉Na: 457.2408); ¹H-NMR (CD₃OD, 500 MHz): see Table 1; ¹³C-NMR (CD₃OD, 125 MHz): δ 112.2 (C-1), 146.5 (C-2), 74.2 (C-3), 48.4 (C-4), 71.6 (C-5), 126.4 (C-6), 141.8 (C-7), 37.9 (C-8), 30.5 (C-9), 79.0 (C-10), 73.9 (C-11), 26.0 (C-12), 25.0 (C-13), 17.2 (C-14), 28.7 (C-15), 100.2 (C-1'), 75.3 (C-2'), 78.3 (C-3'), 72.0 (C-4'), 78.2 (C-5'), 63.1(C-6').

Amarantholidoside II (7): Colorless oil; $[\alpha]_D^{25}$: -49.9° (c 0.30, MeOH); FABMS m/z : 439 [M+Na]⁺; ¹H-NMR (CD₃OD, 500 MHz): see Table 1; ¹³C-NMR (CD₃OD, 125 MHz) : δ 112.1 (C-1), 146.5 (C-2), 74.1 (C-3), 48.4 (C-4), 71.5 (C-5), 126.5 (C-6), 141.5 (C-7), 36.8 (C-8), 34.2 (C-9), 76.1 (C-10), 149.0 (C-11), 111.6 (C-12), 17.9 (C-13), 17.0 (C-14), 28.7 (C-15), 100.2 (C-1'), 75.2 (C-2'), 78.3 (C-3'), 72.0 (C-4'), 78.3 (C-5'), 63.1 (C-6').

Table 1. ¹H-NMR (500 MHz, CD₃OD) spectral data of **6**, **6a** and **7**.

Position	6	6a	7
1a	5.26 (dd, <i>J</i> = 16.5, 2.0 Hz)	5.33 (dd, <i>J</i> = 16.5, 2.0 Hz)	5.25 (dd, <i>J</i> = 17.5, 2.0 Hz)
1b	5.03 (dd, <i>J</i> = 10.5, 2.0 Hz)	5.12 (dd, <i>J</i> = 10.5, 2.0 Hz)	5.03 (dd, <i>J</i> = 10.5, 2.0 Hz)
2	5.98 (dd, <i>J</i> = 16.5, 10.5 Hz)	5.98 (dd, <i>J</i> = 16.5, 10.5 Hz)	5.98 (dd, <i>J</i> = 17.5, 10.5 Hz)
4a	1.99 (dd, <i>J</i> = 14.5, 8.0 Hz)	1.77 (dd, <i>J</i> = 14.5, 8.0 Hz)	1.98 (dd, <i>J</i> = 14.5, 8.0 Hz)
4b	1.66 (dd, <i>J</i> = 14.5, 4.0 Hz)	1.58 (dd, <i>J</i> = 14.5, 4.0 Hz)	1.64 (dd, <i>J</i> = 14.5, 4.0 Hz)
5	4.92 (ddd, <i>J</i> = 10.0, 8.0, 4.0 Hz)	4.62 (ddd, <i>J</i> = 10.0, 8.0, 4.0 Hz)	4.91 (ddd, <i>J</i> = 10.5, 8.0, 4.0 Hz)
6	5.16 (d, <i>J</i> = 10.0 Hz)	5.16 (d, <i>J</i> = 10.0 Hz)	5.14 (dd, <i>J</i> = 10.5, 2.0 Hz)
8a	2.30 (ddd, <i>J</i> = 13.5, 10.0, 5.0 Hz)	2.25 (ddd, <i>J</i> = 13.5, 10.0, 5.0 Hz)	1.68 (m)
8b	2.11 (ddd, <i>J</i> = 13.5, 9.0, 8.0 Hz)	2.03 (ddd, <i>J</i> = 13.5, 9.0, 8.0 Hz)	
9a	1.76 (m)	1.76 (m)	2.08 (m)
9b	1.41 (m)	1.41 (m)	
10	3.24 (dd, <i>J</i> = 10.5, 2.0 Hz)	3.22 (dd, <i>J</i> = 10.5, 2.0 Hz)	4.01 (t, <i>J</i> = 7.0 Hz)
12	1.18 (3H, s)	1.18 (3H, s)	4.94 (s, H-12a) 4.80 (s, H-12b)
13	1.15 (3H, s)	1.15 (3H, s)	1.73 (3H, s)
14	1.71 (3H, s)	1.67 (3H, s)	1.70 (3H, s)
15	1.30 (3H, s)	1.26 (3H, s)	1.30 (3H, s)
1'	4.25 (d, <i>J</i> = 8.0 Hz)		4.23 (d, <i>J</i> = 8.0 Hz)
2'	3.18 (m)		3.17 (m)
3'	3.28 (m)		3.29 (m)
4'	3.28 (m)		3.29 (m)
5'	3.18 (m)		3.17 (m)
6'a	3.87 (dd, <i>J</i> = 11.5, 2.0 Hz)		3.87 (dd, <i>J</i> = 11.5, 2.5 Hz)
6'b	3.67 (dd, <i>J</i> = 11.5, 6.0 Hz)		3.68 (dd, <i>J</i> = 11.5, 6.0 Hz)

Enzymatic hydrolysis of **6**

Compound **6** (3.0 mg) in distilled water (3.0 mL) was stirred with β -glucosidase (8.0 mg, TCI Co., Japan) in a sealed tube at room temperature for 7 days [2, 14]. The reaction mixture was suspended with CHCl_3 (15 mL) and the CHCl_3 layer was evaporated *in vacuo*. The CHCl_3 extract (1.7 mg) was purified using RP-C₁₈ HPLC (MeOH - H₂O = 50:50) to afford aglycone **6a** (1.0 mg) as a colorless oil, $[\alpha]_{\text{D}}^{25}$: +20.0° (c 0.05, MeOH), ¹H-NMR (CD₃OD, 500 MHz): see Table 1. The sugar in the distilled water layer was identified as D-glucose by co-TLC (EtOAc – MeOH - H₂O = 9:3:1, R_f value: 0.17) with a D-glucose standard (Aldrich Co., USA).

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Sample Availability: Milligram quantities of compounds **1-5** and **8-10** are available from the authors.

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