Two Novel Guaiane Sesquiterpenes from the Whole Plant of *Youngia japonica*

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Abstract

Two new guaiane-type sesquiterpenes (**1** and **2**) together with eight related sesquiterpenoidal constituents (**3**–**10**) were isolated from the whole extract of *Youngia japonica*. The chemical structures of **1**–**10** were established by spectroscopic analyses as 4-*epi*-isolipidiol (**1**), 4′-*p*-hydroxyphenylacetyl crepiside E (**2**), isolipidiol (**3**), isoambeboin (**4**), grosheimin (**5**), annuionone D (**6**), loliolide (**7**), youngiajaponicoside A (**8**), crepiside H (**9**), and crepiside E (**10**), respectively. Among the isolated components, **5** exhibited a significant inhibitory effect on the proliferation of cultured human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15).

Key words

Youngia japonica · Asteraceae · guaiane sesquiterpene · cytotoxicity

Supporting information available online at http://www.thieme-connect.de/products

Youngia japonica (L.) DC. (Asteraceae) is a biannual herbaceous plant, native to Korea, China, and Japan that is widely spread on roadsides, gardens, and waste areas [1]. The young leaves and stalks are employed as a wild vegetable and are also used as wild health food for salads in North America. It is frequently used in traditional Chinese medicine for the treatment of inflammatory diseases, such as angina, leucorrhea, and rheumatoid arthritis. Moreover, it is utilized as an internal remedy for colds and sore throats, and the paste is used for external application to relieve the symptoms of boils, mastitis, and bruises due to sprain [2,3]. Several sesquiterpenoidal constituents [4–6] were reported to be purified from *Y. japonica*, which were considered of great interest to show an effect on the regulation and prevention of diverse biological damage [7–9]. In the course of a phytochemical investigation of *Y. japonica*, two new guaiane-type sesquiterpenes (**1** and **2**) together with eight related known sesquiterpenes (**3**–**10**) were isolated (**Fig. 1**). In the present paper, we describe briefly the identification of two new compounds, **1** and **2**, as well as the inhibitory effect of isolated sesquiterpenes on the proliferation of four cultured human tumor cell lines, *in vitro*.

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ +11.7 (*c* 0.20, CH_3OH). The molecular formula of **1** was established as $C_{15}H_{22}O_4$ at *m/z* 289.1426 (calcd. 289.1410) $[M + Na]^+$ by HR-ESI-MS, identical with that of isolipidiol (**3**). The chemical shifts and coupling constants of all of the proton signals of **1** were highly similar to those of **3**, which implied that **1** might be an isomer of **3**. Thus, all proton signals and carbon signals of **1** were completely identified by the aid of two-dimensional NMR experiments such as COSY, DEPT, HMQC, HMBC, and ROESY (**Table 1**), which concluded that the planar structure of **1** was identical with that of **3**, i.e., **1** was a stereoisomer of **3**. Compound **3** is a representative sesquiterpene found in the species and the absolute configuration of **3** was completely established by 2-D NMR experiment and by X-ray diffraction analysis as depicted in **Figs. 2** and **3**. Actually, three stereoisomers of isolipidiol (**3**), i.e., lipidiol (11-*epi*-isolipidiol) [10], 8-*epi*-isolipidiol [11], and 4,8-*epi*-isolipidiol [11], have been reported so far. However, the proton signals of **1** were not matched with those of **3** or with those of the other three reported stereoisomers either. When the proton NMR spectrum of **1** was compared with that of **3**, the signals of H-

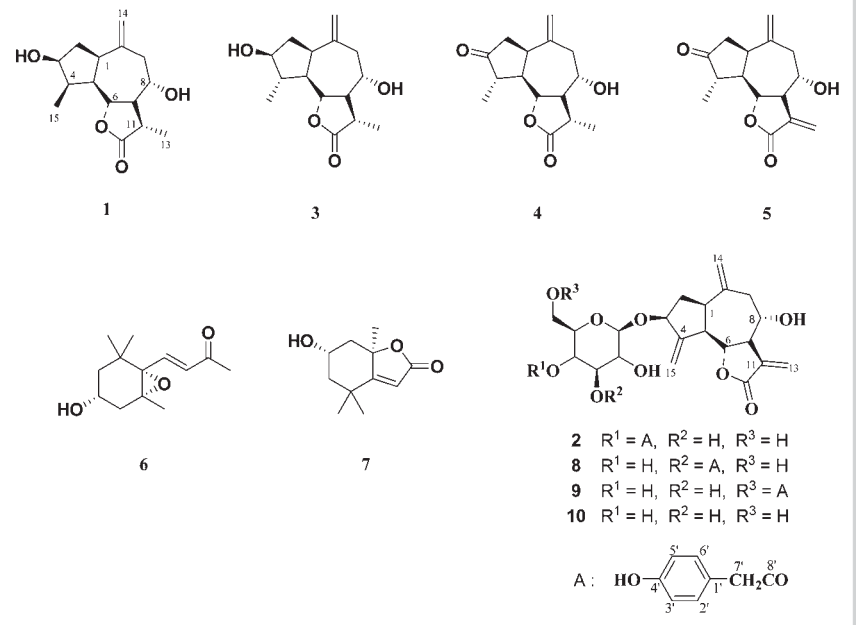

Fig. 1 Structures of isolated compounds (**1**–**10**) from *Y. japonica*.

Pos.	1 (δ_H)	3 (δ_H)	1 (δ_C)	3 (δ_C)
1	2.75 (1 H, m)	2.79 (1 H, m)	41.9	43.0
2	2.13 (1 H, m)	2.02 (1 H, m)	35.8	39.6
	2.27 (1 H, m)	2.24 (1 H, m)		
3	4.46 (1 H, m)	3.91 (1 H, m)	73.6	78.1
4	2.50 (1 H, m)	2.14 (1 H, m)	42.0	47.9
5	2.29 (1 H, dd, *J* = 6.4, 11.2 Hz)	1.98 (1 H, m)	48.5	51.5
6	4.24 (1 H, dd, *J* = 11.0, 11.2 Hz)	3.93 (1 H, m)	79.1	82.5
7	2.20 (1 H, ddd, *J* = 9.6, 10.0, 10.4 Hz)	2.18 (1 H, ddd, *J* = 9.6, 10.0, 10.4 Hz)	57.9	59.2
8	3.89 (1 H, m)	3.83 (1 H, m)	75.9	76.3
9	2.27 (1 H, dd, *J* = 11.8, 12.0 Hz)	2.35 (1 H, dd, *J* = 11.8, 12.8 Hz)	51.1	48.9
	3.08 (1 H, dd, *J* = 4.0, 12.0 Hz)	3.00 (1 H, dd, *J* = 4.8, 12.8 Hz)		
10			145.5	146.1
11	2.90 (1 H, m)	2.77 (1 H, m)	42.6	42.9
12			179.7	179.5
13	1.71 (3 H, d, *J* = 7.2 Hz)	1.68 (3 H, d, *J* = 7.2 Hz)	17.4	19.0
14	5.05 (1 H, br s)	4.99 (1 H, br s)	113.5	114.2
	5.16 (1 H, br s)	5.09 (1 H, br s)		
15	1.25 (3 H, d, *J* = 7.2 Hz)	1.44 (3 H, d, *J* = 6.4 Hz)	9.2	17.0

Table 1 ^{1}H- and ^{13}C-NMR spectroscopic data of **1** and **3**. Assignments are based on HMQC and HMBC experiments, and chemical shifts are given in ppm.

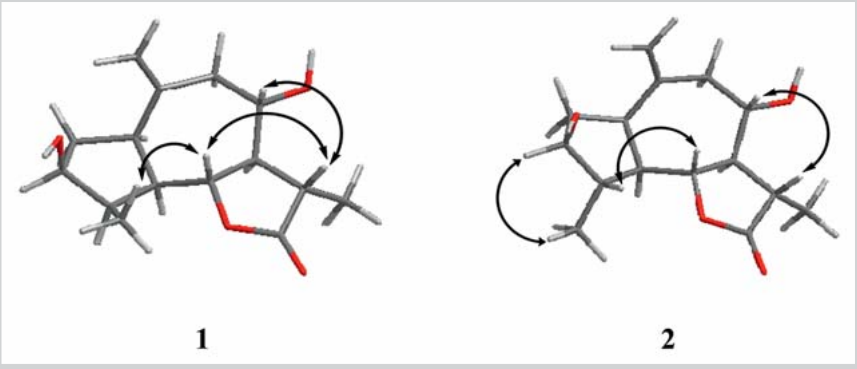

Fig. 2 Correlations observed in selected ROESY spectra of **1** and **2**.

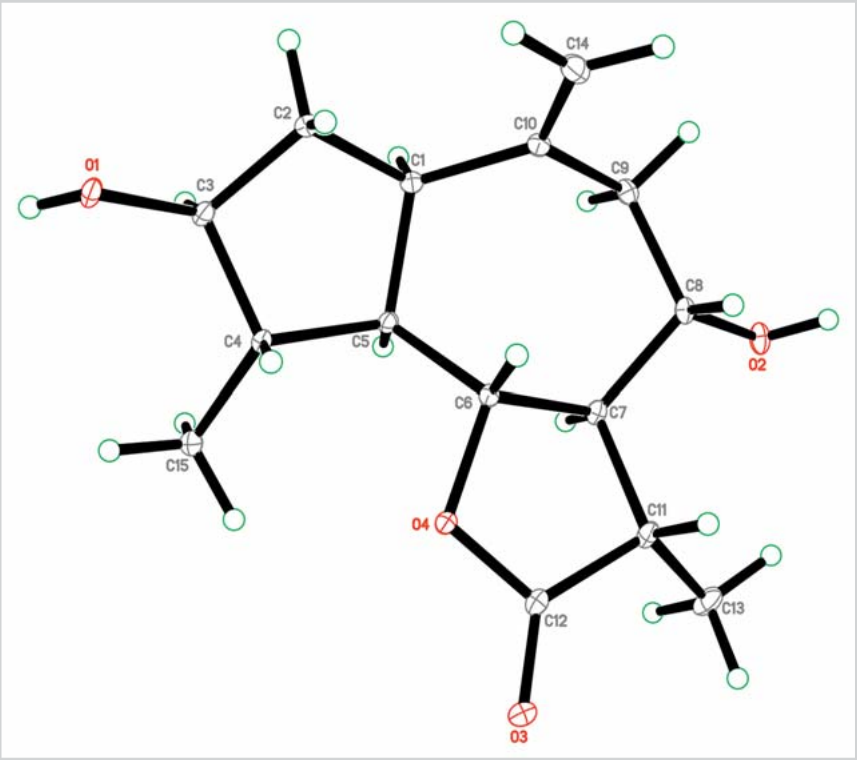

Fig. 3 X-ray crystallographic analysis of **2**.

3, H-4, and H-5 of **1** were shifted downfield ($\Delta\delta$ + 0.6, + 0.4, + 0.3), whereas that of H-15 was shifted to upfield ($\Delta\delta$ – 0.2) (**Table 1**). Moreover, the ROESY spectra of **1** and **3** displayed different correlations. Particularly, H-6 of **1** was correlated with methyl protons (H-15) attached at C-4, whereas H-6 of **3** was correlated with H-4 (**Fig. 2**). These spectroscopic evidences suggested that methyl group attached at C-4 of **1** had the opposite orientation compared with **3** and concluded that **1** was a 4-*epimer* of **3**. Thus, the chemical structure of **1** was established as 4-*epi*-isolipidiol as depicted in **Figs. 1** and **2**.

Compound **2** was obtained as a white powder, $[\alpha]_D^{20}$ + 3.0° (*c* 0.185, MeOH). The molecular formula of **2** was established as $C_{29}H_{34}O_{11}$ at *m/z* 581.2003 (calcd. 581.1993) $[M + Na]^+$, identical with that of **8** and **9**. The NMR spectrum of **2** also displayed high similarity with those of **8** and **9**, which implied that **2** might be an isomer of **8** and **9**. The HMBC experiment of **2** showed two distinct correlations, i.e., the anomeric proton (Glc H-1, δ 5.06) with C-3 (δ 80.9), and Glc H-4 (δ 5.69) with C-8′ (δ 172.3), which supported the connectivity between the glucose moiety with the 3-hydroxy group of the sesquiterpene skeleton and also the linkage point of the *p*-hydroxyphenylacetyl group on the G-4 position. Therefore, the structure of **2** was identified as 4′-*p*-hydroxyphenylacetyl crepiside E.

The other purified components **3**–**10** were identified as isolipidiol (**3**) [12], isoamberboin (**4**) [13], grosheimin (**5**) [14], annuionone D (**6**) [15], loliolide (**7**) [16], youngiajaponicoside A (**8**) [17], crepiside H (**9**) [18], and crepiside E (**10**) [18] by direct comparison of their spectral data with those in the literature.

All isolated compounds (**1**–**10**) from *Y. japonica* were examined for inhibitory effects on proliferative of human cancer cell lines A549 (nonsmall cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT15 (colon adenocarcinoma), according to the SRB assay *in vitro* [19]. Among the tested compounds, only **5**, which holds the partial structure of the exomethylene γ-lactone moiety, exhibited a potent cytotoxicity against the A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines with IC_{50} values of 1.74 µM, 0.96 µM, 1.66 µM, and 1.51 µM, respectively. However, compounds **2** and **8**–**10**, which lacked the exomethylene γ-lactone moiety, exhibited poor inhibition on the tested tumor cells (IC_{50} > 30 uM).

Materials and Methods

General experimental procedures

NMR spectra were obtained by a Bruker AM 300, 500 and Bruker AVANCE II 800 spectrometers using TMS as an internal standard for ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, and HMBC. HRESIMS was recorded by the Applied Biosystems Mariner time-of-flight mass spectrometer with an electrospray interface. Preparative-HPLC was performed on a Futecs P-4000 system with a Shimpack prep-ODS(H) kit column (5 µm, 20 mm × 25 cm).

Plant material

The whole plants of *Y. japonica* were collected in May 2007 at the herbarium of the Korea Research Institute of Chemical Technology (KRICT) and were authenticated by Dr. Young Sup Kim. A voucher specimen (KR0498) was deposited at the herbarium of KRICT, Korea.

Extraction and isolation

The whole plants (9 kg) of *Y. japonica* were soaked in methanol (MeOH) (2 × 40 L) at room temperature for 7 days. The MeOH extract was filtered and evaporated to dryness under reduced pressure. The concentrated extract (1.4 kg) was suspended in 20 L of water and then extracted successively with an equal volume of dichloromethane (MC), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH), which afforded 278 g of the MC fraction, 20 g of the EtOAc fraction, 120 g of the *n*-BuOH fraction, and 890 g of aqueous layer. The repeated chromatographic purification of the MC fraction and EtOAc fraction resulted in the isolation of **1**–**10**. The detailed purification procedures of compounds **1**–**10** are available in the Supporting Information.

4-epi-isolipidiol (**1**): White amorphous powder; $[\alpha]_D^{20}$ + 11.7 (*c* 0.20, CH_3OH; ^{1}H NMR (pyridine-d_5, 800 MHz); ^{13}C NMR (pyridine-d_5, 200 MHz) (**Table 1**); HRESIMS *m/z* 289.1426 $[M + Na]^+$ (calcd. for $C_{15}H_{22}O_4Na$, 289.1410).

4′-p-hydroxyphenylacetyl crepiside E (**2**): white powder; $[\alpha]_D^{20}$ + 3.0 (*c* 0.19, CH_3OH; ^{1}H-NMR (pyridine-d_5, 800 MHz); δ: 2.11 (1 H, m, H-2b), 2.33 (1 H, m, H-2a), 2.38 (1 H, dd, *J* = 13.6, 4.0 Hz, H-9b), 2.70 (1 H, dd, *J* = 13.6, 4.8 Hz, H-9a), 2.80 (1 H, t, *J* = 9.6 Hz, H-5), 2.92 (1 H, q, *J* = 8.8 Hz, H-1), 3.11 (1 H, m, H-7), 3.79 (1 H, d, *J* = 15.5 Hz, H-7′a), 3.76 (1 H, d, *J* = 15.5 Hz, H-7′b), 3.97 (1 H, m, Glc H-5), 4.02 (1 H, m, H-8), 4.04 (1 H, m, Glc H-6b), 4.08 (1 H, dd, *J* = 9.6, 7.2 Hz, Glc H-2), 4.14 (1 H, br d, *J* = 11.2 Hz, Glc H-6a), 4.32 (1 H, dd, *J* = 9.6, 8.8 Hz, Glc H-3), 4.57 (1 H, t, *J* = 9.6 Hz, H-6), 4.82 (1 H, t, *J* = 6.4 Hz, H-3), 5.06 (1 H, d, *J* = 7.2 Hz, Glc H-1), 5.07 (1 H, br s, H-14b), 5.14 (1 H, br s, H-14a), 5.60 (1 H, br s, H-15b), 5.69 (1 H, dd, *J* = 9.6, 8.8 Hz, Glc H-4), 5.76 (1 H, br s, H-15a), 6.40 (1 H, m, H-13b), 6.46 (1 H, m, H-13a), 7.12 (2 H, d, *J* = 8.0 Hz, H-3′, 5′), 7.36 (2 H, d, *J* = 8.0 Hz, H-2′, 6′); ^{13}C-NMR (pyridine-d_5, 200 MHz) (**Table 2**); HRESIMS *m/z* 581.2003 $[M + Na]^+$ (calcd. for $C_{29}H_{34}O_{11}$, 581.1993).

isolipidiol (**3**): White amorphous powder; $[\alpha]_D^{20}$ + 13.1 (*c* 0.16, CH_3OH); ^{1}H NMR (pyridine-d_5, 800 MHz); ^{13}C NMR (pyridine-d_5, 200 MHz) (**Table 1**); EI-MS *m/z*: 266.1 $[M]^+$. X-ray crystallographic analysis (**Fig. 3**)

Tumor cell culture and cytotoxicity assessment

All cell cultures were maintained using RPMI 1640 cell growth medium (Gibco) supplemented with 5% fetal bovine serum (FBS) (Gibco) and grown at 37 °C in a humidified atmosphere containing 5% CO_2. The human cancer cell lines A549 (nonsmall cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT15 (colon adenocarcinoma) were provided by the National Cancer Institute (NCI). The cytotoxicity of isolated compounds (**1**–**10**) against cultured human tumor cell lines was evaluated by the SRB method [19]. All compounds (**1**–**10**) were purified by HPLC (to 95% purity) prior to cytotoxicity assessment. Doxorubicin (Sigma-Aldrich; purity > 98%) was used as the positive control.

Supporting information

A detailed description of the extraction and isolation of compounds **1**–**10**, and spectral graphs of compounds **1**–**3** are available as Supporting Information.

Table 2 ^{13}C-NMR spectroscopic data of **2, 8, 9**, and **10**.

No.	2[a)]	8[b)]	9[a)]	10[a)]
1	46.5	46.6	46.5	46.4
2	38.7	38.7	39.0	38.9
3	80.9	80.8	81.4	81.0
4	150.3	150.2	150.6	150.7
5	52.5	52.5	52.5	52.4
6	79.3	79.1	79.5	79.5
7	51.4	51.2	51.5	51.5
8	72.5	72.6	72.2	72.2
9	43.0	42.5	43.4	43.2
10	144.1	144.8	144.9	144.9
11	141.1	141.0	141.0	141.1
12	170.6	170.0	170.7	170.7
13	122.1	121.5	122.1	122.2
14	116.7	116.6	116.8	116.8
15	115.1	114.7	115	114.8
Sugar moiety				
G-1	103.3	102.3	104.1	104.3
G-2	75.8	73.2	75.6	75.8
G-3	76.1	79.1	75.6	79.1
G-4	73.3	69.9	72.6	72.6
G-5	76.4	77.4	78.7	79.0
G-6	62.5	62.6	65.6	63.3
p-Hydroxyphenylacetic acid moiety				
1′	125.7	126.3	125.8	
2′, 6′	131.5	131.3	131.5	
3′, 5′	116.7	115.9	116.9	
4′	158.4	157.1	158.5	
7′	41.2	40.7	41.1	
8′	172.3	172.1	172.7	

[a)] In pyridine-d_5 solution, [b)] in acetone-d_6 solution

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Conflict of Interest

The authors have no conflict of interest to declare.

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