

Two New Sesquiterpenes from the Aerial Parts of *Pimpinella brachycarpa* NAKAI

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Pimpinella brachycarpa (Umbelliferae) is one of the most favored and increasingly popular wild vegetables grown in Asian regions.^{1,2} In particular, this plant has been used in Korean folk medicine for treating gastrointestinal disturbances, bronchial asthma, insomnia, and persistent cough.³ Terpenes, flavonoids, and essential oil components have been isolated from the herbs.³⁻⁵ Several biological activities of *P. brachycarpa* have been reported, including antibacterial, antioxidative, *anti*-proliferative, antifungal, and antithrombotic activities.⁶⁻⁹ We have recently reported the isolation of quinic acid derivatives with an *anti*-inflammatory effect from this plant.¹⁰ In continuing research on this source, two new sesquiterpenes (**1** and **2**) and ten known terpenes (**3-12**) were further isolated from the MeOH extracts. The structures were elucidated by means of spectroscopic methods and chemical evidence.

Compound **1** was obtained as a colorless gum, and its molecular formula C₁₅H₂₄O₂ was inferred from the positive ion HR-FAB MS *m/z* 237.1858 [M + H]⁺ (calcd. for 237.1855). The ¹H-NMR spectrum of **1** (Table 1) displayed signals for two oxygenated methine proton signals at δ_H = 4.22 (1H, m) and 4.33 (1H, m), two exomethylenes at δ_H = 5.05, 5.07, 5.13, and 5.24 (each 1H, s), one isopropenyl proton δ_H =

1.73 (3H, s) and 4.74 (2H, s), a methine proton δ_H = 2.42, and five methylene protons. Fifteen carbon signals appeared in the ¹³C-NMR spectrum, including one methyl carbon at δ_C = 19.9, two oxygenated carbons at δ_C = 72.4 and 74.3, six olefinic carbons at δ_C = 110.2, 111.4, 113.5, 150.5, 150.7, and 151.0, five methylene carbons at δ_C = 25.6, 29.8, 32.4, 33.2, and 37.4, and a methine carbon at δ_C = 39.9. The ¹H-¹H COSY spectrum of **1** showed correlation signals at δ_H = 1.95 and 2.27 (H-2)/1.95 and 2.40 (H-1) and 4.22 (H-3), 1.58 and 1.64 (H-6)/2.19 and 2.22 (H-5) and 2.42 (H-7), 1.82 and 1.93 (H-8)/2.42 (H-7) and 4.33 (H-9), indicating the presence of partial structures (see bold lines in Figure 2). In the HMBC spectrum of **1**, long-range correlations were observed between the following protons and carbons: H-1 and C-3, C-9, C-14; H-3 and C-5, C-15; H-7 and C-9, C-12, C-13; H-9 and C-7, C-14; H-13 and C-7, C-12 (Figure 2). These spectral data led us to conclude that the planar structure of **1** is sinugibberodiol (**3**), which was isolated from *Simularia gibberosa*.¹¹ The optical rotation of **1** ([α]_D²⁵ +8.6, CHCl₃) was almost the same value but of the opposite sign to that of sinugibberodiol (**3**) ([α]_D²⁵ -5.0, CHCl₃), suggesting that compound **1** could be a stereoisomer of sinugibberodiol (**3**).^{11,12} The relative configurations of the hydroxyl groups at

Table 1. ¹H- (500 MHz) and ¹³C-NMR (125 MHz) spectral data of **1-3** in CDCl₃ (δ in ppm)

Position	1		2		3	
	δ _H	δ _C	δ _H	δ _C	δ _H	δ _C
1	1.95 m, 2.40 m	25.6	2.08 m, 2.28 m	27.8	2.10 m, 2.30 m	24.3
2	1.95 m, 2.27 m	33.2	2.04 m, 2.09 m	32.4	2.30 m	32.7
3	4.22 m	72.4	4.25 m	75.5	4.22 m	74.5
4		151.0		149.5		149.7
5	2.19 m, 2.22 m	32.4	2.05 m, 2.40 m	29.9	1.55 m, 2.40 m	30.6
6	1.58 m, 1.64 m	29.8	1.58 m, 1.65 m	31.7	1.59 m, 1.64 m	32.0
7	2.42 m	39.9	2.52 m	38.8	2.11 m	41.1
8	1.82 m, 1.93 m	37.4	1.89 m, 1.95 m	36.6	1.64 m, 1.85 m	37.0
9	4.33 m	74.3	4.27 m	73.6	4.00 m	76.8
10		150.7		150.3		150.2
11		150.5		151.8		148.8
12	4.74 s	110.2	4.73 s, 4.78 s	110.1	4.76 s, 4.69 s	110.2
13	1.73 s	19.9	1.73 s	19.4	1.69 s	19.0
14	5.05 s, 5.13 s	111.4	5.07 s, 5.19 s	111.6	5.05 s, 5.10 s	114.7
15	5.07 s, 5.24 s	113.5	5.00 s, 5.18 s	114.9	5.05 s, 5.19 s	114.0

Assignments were based on 2D NMR including COSY, HMQC and HMBC (Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses).

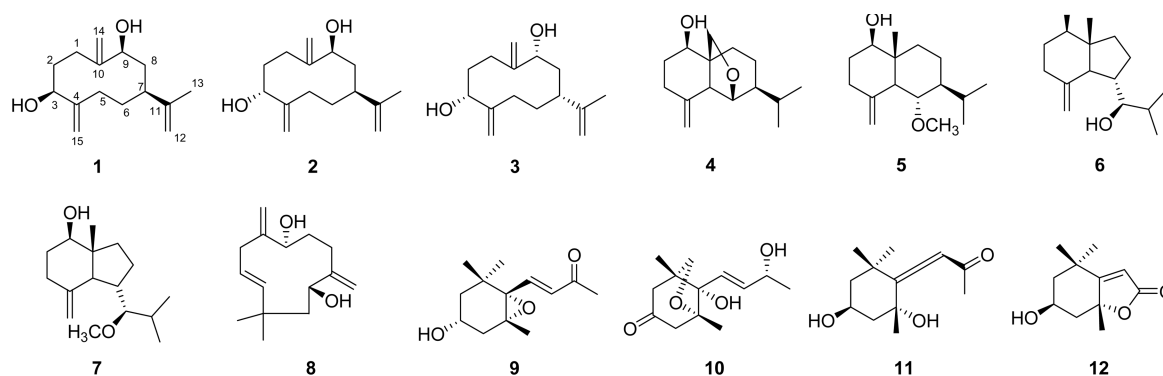
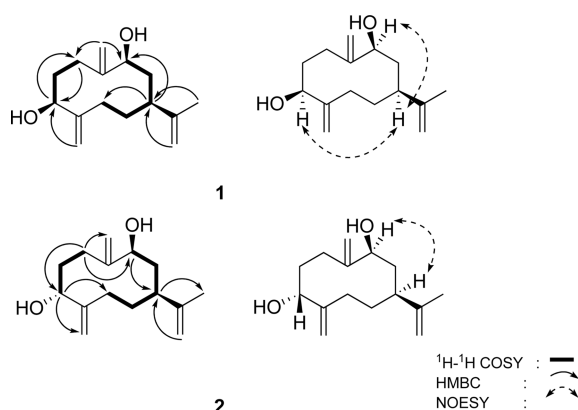


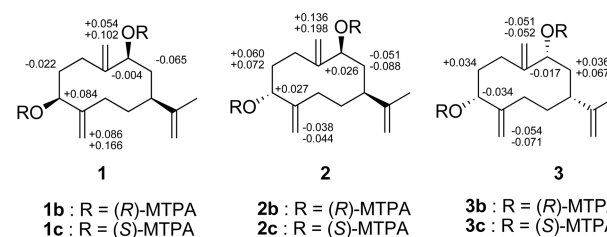
Figure 1. Chemical structures of 1-12.

Figure 2. Key ^1H - ^1H COSY, HMBC, and NOESY correlations of 1 and 2.

C-3 and C-9 were established by the NOESY experiment (Figure 2), in which correlations between H-3 ($\delta_{\text{H}} = 4.22$) and H-7 ($\delta_{\text{H}} = 2.42$), and H-9 ($\delta_{\text{H}} = 4.33$) and H-7 ($\delta_{\text{H}} = 2.42$) were observed. The absolute configurations at C-3 and C-9 were determined by applying the modified Mosher's method (Figure 3).¹³ The results indicated that the absolute configurations of C-3 and C-9 were *S* and *S*, respectively. Thus, the structure of 1 was established as (3*S*,7*S*,9*S*)-3,9-dihydroxygermacra-4(15),10(14),11(12)-triene.

Compound 2 was obtained as a colorless gum, and the molecular formula was determined to be $\text{C}_{15}\text{H}_{24}\text{O}_2$ from the $[\text{M} + \text{H}]^+$ peak at m/z 237.1853 (calcd. for 237.1855) in the HR-FAB MS spectrum. The NMR spectral data of 2 were very similar to those of compound 1, except for the chemical shift in C-3 [$\delta_{\text{H}} = 4.25$ (H-3); $\delta_{\text{C}} = 75.5$ (C-3) in 2; $\delta_{\text{H}} = 4.22$ (H-3); $\delta_{\text{C}} = 72.4$ (C-3) in 1], which suggested that they have different stereochemistry of the hydroxyl group at C-3. The NOESY correlations were observed between H-7 ($\delta_{\text{H}} = 2.52$) and H-9 ($\delta_{\text{H}} = 4.27$), but no correlations were found between H-3 ($\delta_{\text{H}} = 4.25$) and H-7 ($\delta_{\text{H}} = 2.52$) (Figure 2). The absolute configurations at C-3 and C-9 were determined using the modified Mosher's method to be 3*R* and 9*S* (Figure 3). Therefore, the structure of 2 was established as (3*R*,7*S*,9*S*)-3,9-dihydroxygermacra-4(15),10(14),11(12)-triene.

Although compound 3 (sinugibberodiol) has been reported previously,¹¹ the absolute configuration of the compound

Figure 3. Values of $\delta_{\text{S}}\text{-}\delta_{\text{R}}$ (data obtained in pyridine- d_5) for the MTPA esters of 1-3.

was not determined. The absolute configurations at C-3 and C-9 in 3 were determined to be 3*R* and 9*R* using the modified Mosher's method (Figure 3). Thus, the structure of 3 was established as (3*R*,7*R*,9*R*)-3,9-dihydroxygermacra-4(15),10(14),11(12)-triene.

The structures of the other known compounds (4-11) were identified as 6 β ,14-epoxyeudesm-4(15)-en-1 β -ol (4),¹⁴ 6 α -methoxyeudesm-4(15)-en-1 β -ol (5),¹⁴ (7*R**)-opposit-4(15)-ene-1 β ,7-diol (6),¹⁴ 7 β -methoxy-4(14)-oppositen-1 β -ol (7),¹⁵ (2*R**,6*S**)-2,6-dihydroxyhumlaobtusa (8),¹⁶ 3 α -hydroxy-5,6-epoxy-7-megastigmen-9-one (9),¹⁶ (1*R*,6*R*,9*R*)-6,9,11-trihydroxy-4-megastigmen-3-one (10),¹⁷ grasshopper ketone (11),¹⁸ and lolilide (12)¹⁸ by comparing their spectroscopic data with data in the literature.

Experimental Section

Plant Material. The aerial parts of *P. brachycarpa* were collected at Taebaek mountain in Gangwon-Do province, Korea in May 2009 and the plant was identified by one of the authors (K.R. Lee). A voucher specimen (SKKU-09-09) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The aerial parts of *P. brachycarpa* (5 kg) were extracted with 80% MeOH three times at room temperature. The resulting MeOH extracts (480 g) were suspended in distilled water (800 mL \times 3) and then successively partitioned with *n*-hexane, CHCl_3 , EtOAc, and *n*-BuOH, yielding residues weighing 43 g, 5 g, 13 g, and 33 g, respectively. The purification of twelve compounds (1-12) is described in Supplementary Material.

(3*S*,7*S*,9*S*)-3,9-Dihydroxygermacra-4(15),10(14),11(12)-

triene (1). Colorless gum; $[\alpha]_D^{25} +8.6$ (*c* 0.15, CHCl₃); IR (KBr) ν_{\max} 3380, 2946, 2833, 1663, 1452, 1115, 1032, 677 cm⁻¹; ¹H-NMR and ¹³C-NMR data, see Table 1; FAB-MS *m/z* 273 [M + H]⁺; HR-FAB-MS *m/z* 273.1858 [M + H]⁺; (calcd. for C₁₅H₂₅O₂, 273.1855).

(3R,7S,9S)-3,9-Dihydroxygermacra-4(15),10(14),11(12)-triene (2). Colorless gum; $[\alpha]_D^{25} -8.0$ (*c* 0.13, CHCl₃); IR (KBr) ν_{\max} 3383, 2947, 2833, 1653, 1453, 1115, 1032, 694 cm⁻¹; ¹H-NMR and ¹³C-NMR data, see Table 1; FAB-MS *m/z* 273 [M + H]⁺; HR-FAB-MS *m/z* 273.1853 [M + H]⁺; (calcd. for C₁₅H₂₅O₂, 273.1855).

Preparation of the (R)-MTPA Ester and (S)-MTPA Ester from Compounds 1-3.¹³ Compound **1** (0.5 mg), in deuterated pyridine (0.2 mL), was transferred to a clean NMR tube. (*S*)-(+)- α -(Trifluoromethyl)phenylacetyl chloride (5 μ L) was immediately added under a N₂ gas stream, and the NMR tube was permitted to stand at room temperature overnight. When the reaction was completed, it afforded the (*R*)-MTPA ester derivative (**1b**) of **1**. In the same manner as described for **1b**, the (*S*)-MTPA ester derivative (**1c**) of **1** was obtained. Similarly, treatment of **2** and **3** with (*S*)- and (*R*)-MTPA afforded the respective Mosher esters **2b**, **2c**, **3b**, and **3c**. The ¹H-NMR spectra of **1b**, **1c**, **2b**, **2c**, **3b**, and **3c** were measured in NMR reaction tubes.

Compound 1b: Colorless gum; ¹H-NMR (Pyridine-*d*₅, 500 MHz) δ 1.760 (2H, m, H-8), 2.350 (2H, m, H-2), 5.024 (1H, s, H_a-14), 5.056 (1H, s, H_a-15), 5.181 (2H, s, H_b-14, 15), 5.630 (1H, m, H-3), 5.812 (1H, m, H-9).

Compound 1c: Colorless gum; ¹H-NMR (Pyridine-*d*₅, 500 MHz) δ 1.695 (2H, m, H-8), 2.328 (2H, m, H-2), 5.126 (1H, s, H_a-14), 5.222 (1H, s, H_a-15), 5.235 (1H, s, H_b-14), 5.267 (1H, s, H_b-15), 5.714 (1H, m, H-3), 5.808 (1H, m, H-9).

Compound 2b: Colorless gum; ¹H-NMR (Pyridine-*d*₅, 500 MHz) δ 1.888 (1H, m, H_a-8), 1.944 (1H, m, H_b-8), 2.049 (1H, m, H_a-2), 2.141 (1H, m, H_b-2), 5.030 (2H, s, H_a-14, 15), 5.049 (1H, s, H_b-14), 5.268 (1H, s, H_b-15), 5.723 (1H, m, H-9), 5.688 (1H, m, H-3).

Compound 2c: Colorless gum; ¹H-NMR (Pyridine-*d*₅, 500 MHz) δ 1.800 (1H, m, H_a-8), 1.893 (1H, m, H_b-8), 2.121 (1H, m, H_a-2), 2.201 (1H, m, H_b-2), 4.986 (1H, s, H_a-15), 5.166 (1H, s, H_a-14), 5.230 (1H, s, H_b-15), 5.247 (1H, s, H_b-14), 5.749 (1H, m, H-9), 5.715 (1H, m, H-3).

Compound 3b: Colorless gum; ¹H-NMR (Pyridine-*d*₅, 500 MHz) δ 1.525 (1H, m, H_a-8), 1.776 (1H, m, H_b-8), 2.100 (2H, m, H-2), 5.097 (1H, s, H_a-14), 5.273 (1H, s, H_a-15), 5.284 (1H, s, H_b-14), 5.346 (1H, s, H_b-15), 5.560 (1H, m, H-9), 5.788 (1H, m, H-3).

Compound 3c: Colorless gum; ¹H-NMR (Pyridine-*d*₅,

500 MHz) δ 1.561 (1H, m, H_a-8), 1.843 (1H, m, H_b-8), 2.134 (2H, m, H-2), 5.046 (1H, s, H_a-14), 5.219 (1H, s, H_a-15), 5.232 (1H, s, H_b-14), 5.275 (1H, s, H_b-15), 5.543 (1H, m, H-9), 5.754 (1H, m, H-3).

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Supporting Information. Spectral data of compounds **1** and **2**, general experimental procedures, and the isolation details are available upon request from the corresponding author.

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