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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 antibacterical, 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 $C_{15}H_{24}O_2$ was inferred from the positive ion HR-FAB MS *m/z* 237.1858 $[M + H]^+$ (calcd. for 237.1855). The ^{1}H-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 ^{13}C-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 ^{1}H-^{1}H 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 *Sinularia gibberosa*.[11] The optical rotation of **1** ($[\alpha]_D^{25}$ +8.6, $CHCl_3$) was almost the same value but of the opposite sign to that of sinugibberodiol (**3**) ($[\alpha]_D^{25}$ −5.0, $CHCl_3$), suggesting that compound **1** could be a stereoisomer of sinugibberodiol (**3**).[11,12] The relative configurations of the hydroxyl groups at

Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectral data of **1-3** in $CDCl_3$ (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).

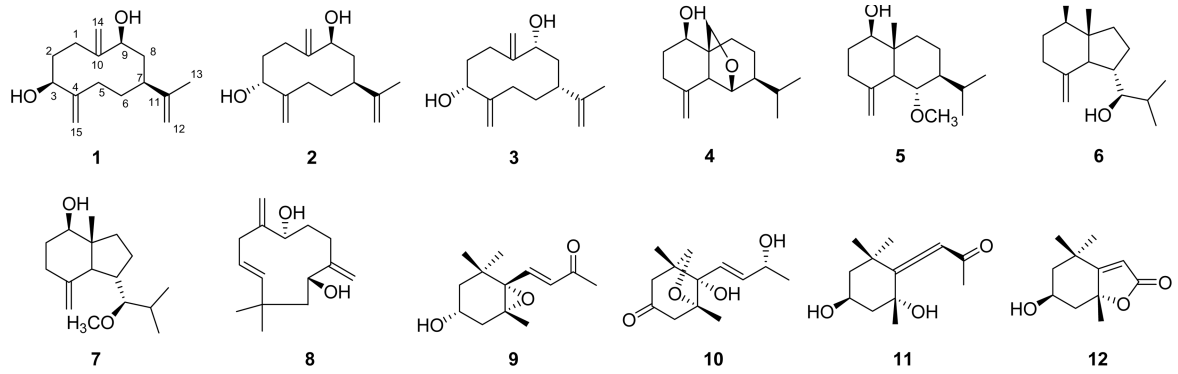

Figure 1. Chemical structures of **1**-**12**.

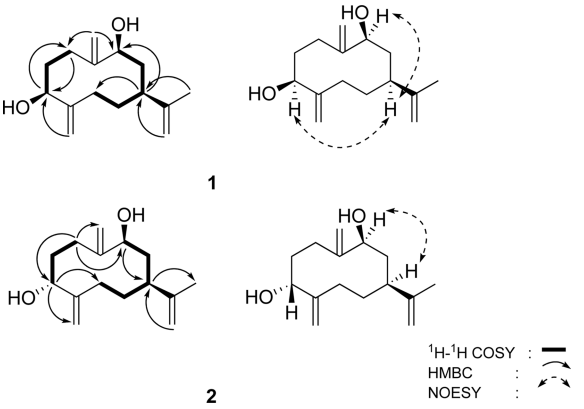

Figure 2. Key ^{1}H-^{1}H COSY, HMBC, and NOESY correlations of **1** and **2**.

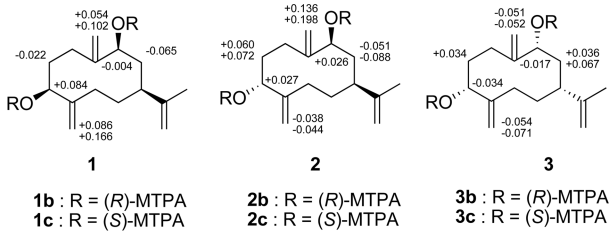

Figure 3. Values of δ_S-δ_R (data obtained in pyridine-d_5) for the MTPA esters of **1**-**3**.

C-3 and C-9 were established by the NOESY experiment (Figure 2), in which correlations between H-3 (δ_H = 4.22) and H-7 (δ_H = 2.42), and H-9 (δ_H = 4.33) and H-7 (δ_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 $C_{15}H_{24}O_2$ from the $[M + H]^+$ 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 [δ_H = 4.25 (H-3); δ_C = 75.5 (C-3) in **2**; δ_H = 4.22 (H-3); δ_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 (δ_H = 2.52) and H-9 (δ_H = 4.27), but no correlations were found between H-3 (δ_H = 4.25) and H-7 (δ_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 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 6β,14-epoxyeudesm-4(15)-en-1β-ol (**4**),[14] 6*a*-methoxyeudesm-4(15)-en-1β-ol (**5**),[14] (7*R**)-opposit-4(15)-ene-1β,7-diol (**6**),[14] 7β-methoxy-4(14)-oppositen-1β-ol (**7**),[15] (2*R**,6*S**)-2,6-dihydroxyhumlaobtusa (**8**),[16] 3α-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 loliolide (**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 × 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, $CHCl_3$); IR (KBr) ν_{max} 3380, 2946, 2833, 1663, 1452, 1115, 1032, 677 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data, see Table 1; FAB-MS *m/z* 273 $[M + H]^+$; HR-FAB-MS *m/z* 273.1858 $[M + H]^+$; (calcd. for $C_{15}H_{25}O_2$, 273.1855).

(3*R*,7*S*,9*S*)-3,9-Dihydroxygermacra-4(15),10(14),11(12)-triene (2). Colorless gum; $[\alpha]_D^{25}$ −8.0 (*c* 0.13, $CHCl_3$); IR (KBr) ν_{max} 3383, 2947, 2833, 1653, 1453, 1115, 1032, 694 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data, see Table 1; FAB-MS *m/z* 273 $[M + H]^+$; HR-FAB-MS *m/z* 273.1853 $[M + H]^+$; (calcd. for $C_{15}H_{25}O_2$, 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 μL) was immediately added under a N_2 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 ^{1}H-NMR spectra of **1b**, **1c**, **2b**, **2c**, **3b**, and **3c** were measured in NMR reaction tubes.

Compound 1b: Colorless gum; ^{1}H-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; ^{1}H-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; ^{1}H-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; ^{1}H-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; ^{1}H-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; ^{1}H-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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