Notes

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

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Key Words : Pimpinella brachycarpa, Umbelliferae, Sesquiterpene

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 ¹H-NMR spectrum of **1** (Table 1) displayed signals for two oxygenated methine proton signals at $\delta_H = 4.22$ (1H, m) and 4.33 (1H, m), two exomethylenes at $\delta_H = 5.05$, 5.07, 5.13, and 5.24 (each 1H, s), one isopropenyl proton $\delta_H =$

1.73 (3H, s) and 4.74 (2H, s), a methine proton $\delta_{\rm H} = 2.42$, and five methylene protons. Fifteen carbon signals appeared in the ¹³C-NMR spectrum, including one methyl carbon at $\delta_{\rm C}$ = 19.9, two oxygenated carbons at $\delta_{\rm C}$ = 72.4 and 74.3, six olefinic carbons at $\delta_{\rm C} = 110.2$, 111.4, 113.5, 150.5, 150.7, and 151.0, five methylene carbons at $\delta_{\rm C} = 25.6$, 29.8, 32.4, 33.2, and 37.4, and a methine carbon at $\delta_c = 39.9$. The ¹H-¹H COSY spectrum of **1** showed correlation signals at $\delta_{\rm H} = 1.95$ 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, CHCl₃) was almost the same value but of the opposite sign to that of sinugibberodiol (3) ($[\alpha]_{D}^{25}$ -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}	$\delta_{\rm C}$	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 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 ($\delta_{\rm H} = 4.22$) and H-7 ($\delta_{\rm H} = 2.42$), and H-9 ($\delta_{\rm H} = 4.33$) and H-7 ($\delta_{\rm H} = 2.42$) 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 (3S,7S,9S)-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 [$\delta_H = 4.25$ (H-3); $\delta_C = 75.5$ (C-3) in **2**; $\delta_H = 4.22$ (H-3); $\delta_C = 72.4$ (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 ($\delta_H = 2.52$) and H-9 ($\delta_H = 4.27$), but no correlations were found between H-3 ($\delta_H = 4.25$) and H-7 ($\delta_H = 2.52$) (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 δ_{S} - δ_{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 3R and 9R using the modified Mosher's method (Figure 3). Thus, the structure of **3** was established as (3R,7R,9R)-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-1 β -ol (**4**),¹⁴ *6a*-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 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. brachy-carpa* (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, CHCl₃, 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.

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

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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).

(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, 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).

Acknowledgments. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20110028285). We thank Drs. E. J. Bang, S. G. Kim, and J. J. Seo at the Korea Basic Science Institute for their aid in obtaining the NMR and mass spectra.

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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