

# A New Cytotoxic Acyclic Diterpene from *Carpesium divaricatum*

Ok Pyo Zee<sup>1</sup>, Dae Keun Kim<sup>2</sup>, Sang Un Choi<sup>3</sup>, Chong Ock Lee<sup>3</sup> and Kang Ro Lee<sup>1</sup>

<sup>1</sup>Natural Products Laboratory, College of Pharmacy, Sung Kyun Kwan University, Suwon 440-746, Korea, <sup>2</sup>Natural Products Laboratory, College of Pharmacy, Woosuk University, Cheonju 565-800, Korea and <sup>3</sup>Pharmaceutical Screening Team, Korea Research Institute of Chemical Technology, Taejeon 305-606, Korea

(Received November 20, 1998)

A new acyclic diterpene (**1**) and a known acyclic diterpene, 12(*S*)-hydroxygeranylgeraniol (**2**) were isolated from the aerial parts of *Carpesium divaricatum*. The structure of **1** was determined to be (2*E*,10*E*)-1,12-dihydroxy-18-acetoxy-3,7,15-trimethylhexadeca-2,10,14-triene (**1**) on the basis of spectroscopic studies. Compounds **1** and **2** exhibited cytotoxicity against cultured human tumor cell lines, A549, SK-OV-3, SK-MEL-2, XF498, and HCT15, with ED<sub>50</sub> values ranging from 4.3-10.2 µg/ml and 4.1-8.3 µg/ml, respectively.

**Key words** : *Carpesium divaricatum*, Acyclic diterpene, Cytotoxicity

## INTRODUCTION

In our previous phytochemical studies of *Carpesium divaricatum* *S. et Z.* (Compositae), we reported four new cytotoxic sesquiterpene lactones (Kim *et al.*, 1997b) and two new and two known sesquiterpene lactones (Kim *et al.*, 1997a). Our continuing investigation on the constituents of this plant afforded two cytotoxic acyclic diterpenes, a new diterpene derivative (compound **1**) and a known diterpene, 12(*S*)-hydroxy geranylgeraniol (**2**). This paper reports the isolation and structural elucidation of compound **1**. Further, the cytotoxicity of compounds **1** and **2** was tested using cultured human tumor cell lines, A549, SK-OV-3, SK-MEL-2, XF498 and HCT15.

## MATERIALS AND METHODS

### General experimental procedures

Melting points were measured on a Gallenkamp melting point apparatus (uncorr.). <sup>1</sup>H- and <sup>13</sup>C-NMR were recorded on a Bruker AMX-500 spectrometer. UV spectra were obtained on a Shimadzu UV<sub>240</sub> UV-Visible recording spectrophotometer. IR spectra were measured on Shimadzu IR-435 Infrared spectrophotometer. The EI-MS was measured on VG70-VSEQ and CI-MS was recorded with isobutane as reactant gas on Finnigan/MAT HSQ/30 mass spectrometer.

### Plant material

*Carpesium divaricatum* was collected in August 1994 on Samyeong Mt., Kangwondo, Korea. A voucher specimen is deposited in the herbarium of College of Pharmacy, Sung Kyun Kwan University (SKKU-94-005).

### Biological assay

Cytotoxicity of compounds **1** and **2** was measured against A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon) cell lines *in vitro* using sulforhodamine B (SRB) assay (Giard *et al.*, 1973).

### Extraction and isolation

The air-dried plant materials (3.5 kg) were finely ground and extracted at room temperature with MeOH for 2 weeks (3×2 L). The resultant MeOH extract (110 g) was suspended in H<sub>2</sub>O (1 L), and partitioned with CH<sub>2</sub>Cl<sub>2</sub> (2×1 L) and *n*-BuOH (2×1 L), successively, to give CH<sub>2</sub>Cl<sub>2</sub> (30 g), *n*-BuOH (22 g) and water (50 g) soluble fractions. The CH<sub>2</sub>Cl<sub>2</sub> soluble fraction was subjected to column chromatography over silica gel (800 g), eluted sequentially with *n*-hexane-EtOAc (5:1, 800 ml), *n*-hexane-EtOAc (3:1, 650 ml), *n*-hexane-EtOAc (1:1, 600 ml), and EtOAc (500 ml). The eluents were fractionated by TLC to yield fractions designated as MC1-MC5: void volume (450 ml), MC1 (520 ml), MC2 (350 ml), MC3 (410 ml), MC4 (380 ml), and MC5 (420 ml). The eluent (3.5 g) obtained from fraction MC4 was further chromatographed with silica gel (220 g) eluting with *n*-hexane-CH<sub>2</sub>Cl<sub>2</sub>-EtOAc (1:1:1) to give four subfractions (a-d). Subfraction c (1.6 g) was purified

Correspondence to: Kang Ro Lee, Natural Products Laboratory, College of Pharmacy, SungKyunKwan University, Chonchondong 300, Jangan-ku, Suwon, 440-746, Korea

**Table I.**  $^{13}\text{C}$ -NMR (125 MHz) and  $^1\text{H}$ -NMR (500 MHz) data for compound **1** (in  $\text{CDCl}_3$ )

Position	$\delta^{13}\text{C}$	DEPT	$\delta^1\text{H}^a$	$^1\text{H}$ - $^1\text{H}$ COSY	HMBC <sup>b</sup>
1	59.3	$\text{CH}_2$	4.14 (d, 6.0)	2	C-2, 3
2	124.2	CH	5.41 (t, 6.0)	1	
3	138.5	C			
4	39.4	$\text{CH}_2$	obs*		
5	25.7	$\text{CH}_2$	obs		
6	32.4	$\text{CH}_2$	obs		
7	38.1	CH	1.55 (m)	19	
8	31.6	$\text{CH}_2$	obs		
9	25.6	$\text{CH}_2$	obs	10	
10	130.3	CH	5.40 (t, 6.0)	9	
11	135.2	C			
12	75.5	CH	3.39 (m)	13	
13	35.5	$\text{CH}_2$	obs	12, 14	
14	120.6	CH	5.17 (m)	16, 17	
15	134.1	C			
16	25.9	$\text{CH}_3$	1.74 (s)	14	C-14, 15, 17
17	18.0	$\text{CH}_3$	1.65 (s)	14	C-14, 15, 16
18	61.9	$\text{CH}_2$	4.58 (s)		C-10, 12, 1'
19	15.5	$\text{CH}_3$	0.91 (d, 7.0)	7	C-6, 7, 8
20	16.2	$\text{CH}_3$	1.67 (s)		C-2, 3, 4
1'	171.1	C			
2'	21.0	$\text{CH}_3$	2.07 (s)		

<sup>a</sup>Chemical shifts (ppm), multiplicity and coupling constants (Hz) in parentheses.

<sup>b</sup>Major  $^1\text{H}$ - $^{13}\text{C}$  long range correlations determined from HMBC experiment.

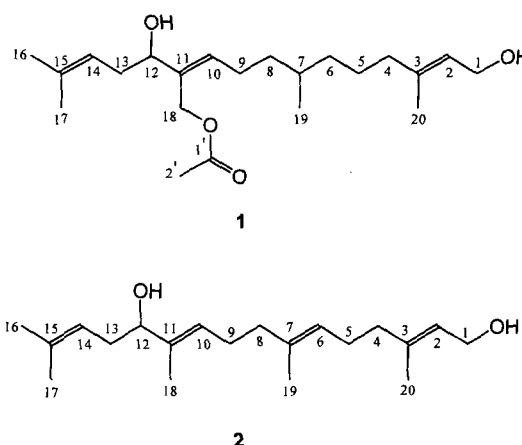
\*Obscure

on Lobar<sup>®</sup>-A column (Merck) ( $\text{CH}_2\text{Cl}_2$ -EtOAc, 8:1) followed by reverse phase Lobar<sup>®</sup>-A column (MeOH- $\text{H}_2\text{O}$ , 8:2) to yield **1** (30 mg) and **2** (20 mg), respectively.

**(2E,10E)-1,12-dihydroxy-18-acetoxy-3,7,15-trimethylhexadeca-2,10,14-triene (1):** an oil;  $[\alpha]_D^{24}$ : +2.4 (MeOH, c 1.0);  $\text{UV}\lambda_{\text{max}}^{\text{MeOH}}$  nm: 216;  $\text{IR}\nu_{\text{max}}^{\text{Nujol}}$   $\text{cm}^{-1}$ : 3408 (OH), 1738 (C=O); EI-MS  $m/z$  (rel. int. %): 348 ( $\text{M}^+$ - $\text{H}_2\text{O}$ , 2), 306 (4), 289 (24), 219 (77), 109 (98), 83 (100), 54 (97); CI-MS (isobutane)  $m/z$  (rel. int. %): 367 ( $\text{M}^+$ +1, 15), 289 (100);  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data are presented in Table I.

## RESULTS AND DISCUSSION

Compound **1** was obtained as an oil. Its IR spectrum showed hydroxyl and carbonyl absorption bands at  $3410\text{ cm}^{-1}$  and  $1740\text{ cm}^{-1}$ , respectively. The highest mass spectral fragment of compound **1** in EI-MS appeared at  $m/z$  348, corresponding to  $[\text{M}^+ - \text{H}_2\text{O}]$ . The molecular ion as  $\text{M}^+$ +1 was confirmed by CI-MS (isobutane). From the DEPT ( $\text{CH}_3 \times 5$ ,  $\text{CH}_2 \times 8$ ,  $\text{CH} \times 5$ ,  $\text{C} \times 4$ ),  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR (22C), and CI-MS ( $\text{M}^+$ +1, 367) spectra, the molecular formula was deduced to be  $\text{C}_{22}\text{H}_{38}\text{O}_4$ . The formula showed four unsaturated-bond equivalents in the molecule. The  $^{13}\text{C}$ -NMR spectrum showed six olefinic carbons at  $\delta$  138.8, 135.2, 134.1, 130.3, 124.2, and 120.6 and a carbonyl carbon

**Fig. 1.** Structures of compounds **1-2**

at  $\delta$  171.1, thus accounting for four unsaturated-bond equivalents. Therefore, **1** was suggestive of an acyclic compound. The  $^1\text{H}$ -NMR spectrum exhibited three olefinic proton signals at  $\delta$  5.41, 5.40, and 5.17, and three protons attached to the oxygenated carbons at  $\delta$  4.58, 4.14, and 3.39. Compound **1** also showed four singlet methyls at  $\delta$  2.07, 1.74, 1.67, and 1.65, and a doublet methyl  $\delta$  0.91 in the  $^1\text{H}$ -NMR spectrum. These data suggested an acyclic diterpene skeleton for **1**. The  $^1\text{H}$ -NMR spectrum of **1** was very similar to that of compound **2** which was previously reported as an acyclic diterpene (Robert *et al.*, 1986), apart from the replacement of a methine olefinic signal at  $\delta$  5.10 by signal at  $\delta$  1.55, attributable to the saturated aliphatic methine group, and the presence of an acetyl group at  $\delta$  2.07. The downfield shift of C-18 ( $\delta$  4.58) suggested that C-18 was possibly carrying an acetoxy group. The above evidence, together with the  $^1\text{H}$ - $^1\text{H}$  COSY, DEPT and HMQC spectra, led to the gross structure of **1**. The position of the acetyl group was further confirmed by  $^1\text{H}$ - $^{13}\text{C}$  long-range correlation between  $\delta$  4.58 proton signal and  $\delta$  171.1 carbon signal in the HMBC spectrum. The *trans* (*E*) configurations of the double bonds at C-2 and C-10 were established on the basis of the chemical shifts of the vinyl methyl groups in the  $^{13}\text{C}$ -NMR spectrum (Breitmaier *et al.*, 1990) and by comparison of its spectral data with those of compound **2** (Robert *et al.*, 1986). Finally, compound **1** was characterized as (2*E*,10*E*)-1,12-dihydroxy-18-acetoxy-3,7,15-trimethyl-hexadeca-2,10,14-triene (18-acetoxy-12-hydroxy-6,7-dihydrogeranyl geraniol). The compound **1** is optically active ( $[\alpha]_D^{24}$  +2.4), but the configurations at C-7 and C-12 remain uncertain.

The structure of an acyclic known diterpene, 12(*S*)-hydroxy geranylgeraniol (**2**) (Robert *et al.*, 1986) was established by comparing its UV, IR, NMR spectral data and physical properties with those reported in the literature.

Acyclic diterpenes **1** and **2** are cytotoxic *in vitro* in

**Table 2.** Cytotoxicity of compounds **1** and **2**\*

cancer cell lines	A-549	SK-OV-3	SK-MEL-2	XF-498	HCT-15
compound <b>1</b>	9.74	10.20	6.57	7.04	4.38
compound <b>2</b>	8.20	8.33	7.16	8.26	4.17

\*ED<sub>50</sub> value of compounds against each cancer cell line, which was defined as a concentration ( $\mu\text{g/ml}$ ) that caused 50% inhibition of cell growth *in vitro*.

cultured human tumor cellular models, A549, SK-OV-3, SK-MEL-2, XF498, and HCT15, as determined by sulforhodamine B (SRB) assay (Giard *et al.*, 1973). The cytotoxicity of these compounds is shown as ED<sub>50</sub> values in Table II.

#### ACKNOWLEDGEMENTS

We wish to thank Prof. Dr. G. Ruecker, Pharmazeutisches Institut der Universitaet Bonn, Germany, for the CI-MS of compound **1** and E.J. Bang and J.J. Seo, Korea Basic Science Institute, for the NMR spectra.

#### REFERENCES CITED

- Breitmaier, E. and Voeter, W., In: Carbon-13 NMR-Spectroscopy. High Resolution Methods and Applications in Organic Chemistry and Biochemistry, VCH Publishers, New York, pp. 327-337, 1990.
- Giard, D. J., Aaronson, S. A., Todaro, G. J., Arnstein, P., Kersey, J. H., Dosik, H. and Parks, W. P., *in vitro* cultivation of human tumors: Establishment of cell lines derived from a series of solid tumors. *J. Natl. Cancer Inst.* 51, 1417-1421 (1973).
- Kim, D. K., Lee, K. R. and Zee, O. P., Sesquiterpene lactones from *Carpesium divaricatum*. *Phytochemistry* 46, 1245-1247 (1997a).
- Kim, D. K., Baek, N.-I., Choi, S. H., Lee, K. R. and Zee, O. P., Four new cytotoxic germacranolides from *Carpesium divaricatum*. *J. Nat. Prod.* 60, 1199-1202 (1997b).
- Robert, V., Bernard, B., Christian F., Louis, C. and Adrien, C., An acyclic diterpene from the brown alga *Bifurcaria bifurcata*. *Phytochemistry* 25, 751-752 (1986).