Four New Cytotoxic Germacranolides from *Carpesium divaricatum*

Dae Keun Kim,[†] Nam In Baek,[‡] Sang Un Choi,[§] Chong Ock Lee,[§] Kang Ro Lee,* and Ok Pyo Zee

Natural Products Laboratory, College of Pharmacy, Sung Kyun Kwan University, Suwon 440-746, Korea

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In a bioassay-guided search for cytotoxic compounds from higher plants of South Korea, four new sesquiterpenes of the germacranolide type, named cardivins A (**1**), B (**2**), C (**3**), and D (**4**), have been isolated from the aerial parts of *Carpesium divaricatum*. Structures of these compounds were elucidated on the basis of spectroscopic techniques. Compounds **1**, **2**, **3**, and **4** showed cytotoxicity to the human tumor cells, A-549 (nonsmall cell lung), SK-OV-3 (ovary), SK-MEL-2 (skin), XF-498 (central nervous system), and HCT-15 (colon).

Carpesium divaricatum S. et Z. (Compositae), widely distributed in South Korea, has been used in Korean traditional medicine for its antipyretic, analgesic, vermifugic, and antiinflammatory properties.[1] Maruyama et al. reported the isolation of several sesquiterpene lactones from the genus *Carpesium*; granilin,[2] carpesiolin, carabrone,[3] carabrol, ivaxillin,[4] and ineupatolides A and B[5] from *Carpesium abrotanoides* and divaricins A, B, and C[6] from *C. divaricatum*. In our continuing search for cytotoxic compounds derived from higher plants, we have demonstrated that the MeOH extract of *C. divaricatum* exhibited significant cytotoxicity against cultured human tumor cell lines, A-549, SK−OV-3, SK-MEL-2, XF-498, and HCT-15. The cytotoxicity of the MeOH extract was found to be mainly concentrated in the CH_2Cl_2-soluble fraction. Activity-guided column chromatography of the CH_2Cl_2 fraction on the basis of the inhibitory activity upon the growth of tumor cells in vitro afforded four cytotoxic compounds. In this paper, we report the isolation and structure elucidation of four novel sesquiterpene lactones, cardivins A−D (**1**−**4**) as well as their cytotoxic activities.

Cardivins A (**1**), B (**2**), C (**3**), and D (**4**) have very similar patterns in their UV, IR, and NMR spectra. The NMR resonances for their germacranolide skeletons are essentially identical with each other. From the ^{1}H-NMR, ^{13}C-NMR (Tables 1 and 2), and ^{1}H−^{1}H COSY spectra, it is apparent that the only difference between compounds **1**−**4** lies in the ester substituents to the 6- and 9-hydroxy groups of the sesquiterpene skeletons: 2-methylbutanoic and angelic acids in compound **1**, isobutyric and 2-methylbutanoic acids in **2**, angelic and isobutyric acids in **3**, and two isobutyric acids in **4**.

Compound **1** was isolated as colorless needles. Its IR spectrum showed the presence of hydroxy (3410 cm^{-1}), γ-lactone (1753 cm^{-1}), and ester (1739 cm^{-1}) groups. Its molecular formula was established as $C_{25}H_{36}O_9$ by HRMS (*m*/*z* 480.2360). The ^{1}H-NMR spectrum exhibited signals at 6.45 and 5.65 for the presence of an exocyclic methylene group[7] (partial structure A). By 2D NMR (^{1}H−^{1}H and HMBC) and DEPT experiments, the partial structure B was revealed; the proton signals at 2.17 and 2.18 showed correlations with the signal at 3.76, the 3.76 proton signal correlated with 1.88, which also correlated with 2.23 in the ^{1}H−^{1}H COSY spectrum. The partial structure C was deduced in the same way; the proton signal at 4.68 showed a correlation with the signal at 3.03. This signal correlated with 4.33, which in turn correlated with 5.05 in the ^{1}H−^{1}H COSY spectrum. Comparison of the NMR data of **1** with those reported in the literature, confirmed the partial structures D (2-methylbutanoic acid) and E (angelic acid).[8] The partial structures A, B, C, D, and E (Figure 1) were built-up into a complete planar structure from ^{1}H−^{13}C long-range correlations observed in the HMBC spectrum (Figure 2).

In the HMBC spectrum, the proton signal H-7 (3.03) showed a ^{1}H−^{13}C long-range correlation with C-11 (132.8), C-10 (81.3), and C-1″ (168.4). The carbon signal C-1′ (177.8) of the 2-methylbutanoate substituent correlated with H-9 (5.05), and C-1″ (168.4) of angelate correlated with H-7 (3.03) and H-6 (4.68). Finally, the whole planar structure was established as that of **1**. The relative stereochemistry of C_6−C_7−C_8−C_9−C_{10} was determined to be similar to that of divaricin,[6] which was isolated from this plant, on the basis of the coupling constants observed in the ^{1}H-NMR spectrum. Furthermore, the stereochemistry of **1** was confirmed from its

* To whom correspondence should be addressed: College of Pharmacy, SungKyunKwan University, 300 Chonchon-dong, Jangan-ku, Suwon 440-746, Korea. Phone: 82-331-290-7710. FAX: 82-331-292-8800.

[†] Present address: Natural Products Laboratory, College of Pharmacy, Woosuk University, Cheonju 565-800, Korea.

[‡] Lab. of Natural Products Chemistry, College of Industry, Kyung Hee University, Yongin 449-701, Korea.

[§] Pharmaceutical Screening Team, Korea Research Institute of Chemical Technology, Taejeon 305-606, Korea.

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Table 1. ^{1}H-NMR Chemical Shifts of Compounds **1**−**4** ($CDCl_3$)[a,b]

	compound			
proton	**1**	**2**	**3**	**4**
1	2.17 m	2.18 m	2.19 m	2.22 m
	2.18 m	2.22 m	2.22 m	2.26 m
2	3.76 m	3.75 m	3.76 m	3.75 m
3	1.88 m	1.88 m	1.86 m	1.87 m
4	2.23 m	2.22 m	2.24 m	2.22 m
6	4.68 d (10.0)	4.69 d (10.0)	4.70 d (9.9)	4.69 d (9.9)
7	3.03 m	3.01 m	3.01 m	3.00 m
8	4.33 dd	4.31 dd	4.36 dd	4.31 dd
	(6.5, 9.8)	(6.5, 10.0)	(6.4, 9.8)	(6.3, 9.7)
9	5.05 d (10.5)	5.04 d (10.6)	5.13 d (10.6)	5.04 d (9.7)
13a	6.45 d (3.1)	5.65 d (3.1)	5.66 d (3.1)	5.64 d (3.1)
13b	5.65 d (3.1)	6.45 d (3.1)	6.45 d (3.1)	6.45 d (3.1)
14	1.30 s	1.29 s	1.30 s	1.30 s
15	1.00 d (6.7)	1.00 d (6.7)	1.02 d (6.7)	1.00 d (6.7)
2′	2.49 m	2.68 sep (7.0)		2.68 sep (7.0)
3′	1.53 m	1.24 d (7.0)	6.17 q (7.2)	1.23 d (7.0)
	1.73 m			
4′	1.00 t (7.2)	1.24 d (7.0)	2.02 d (7.2)	1.23 d (7.0)
5′	1.31 d (7.0)		1.95 s	
2″		2.48 m	2.69 sep (7.0)	2.68 sep
3″	6.18 q (7.2)	1.51 m	1.24 d (7.0)	1.23 d (7.0)
		1.74 m		
4″	2.02 d (7.2)	0.93 t (7.5)	1.24 d (7.0)	1.23 d (7.0)
5″	1.95 s	1.30 d (7.0)		

[a] Recorded at 500 MHz. [b] *J* values (Hz) are provided in parentheses.

Table 2. ^{13}C-NMR Chemical Shifts of Compounds **1**−**4** ($CDCl_3$)[a]

	compound			
carbon	**1**	**2**	**3**	**4**
1	34.0	34.0	34.0	34.1
2	71.7	72.0	72.0	72.0
3	25.7	25.7	25.6	25.7
4	29.7	29.7	29.9	29.7
5	215.1	214.9	214.7	214.6
6	78.5	78.7	78.7	78.6
7	42.4	42.1	42.2	42.2
8	78.7	79.1	79.1	79.0
9	78.5	78.7	78.7	78.6
10	81.3	81.3	81.3	81.3
11	132.8	132.7	132.7	132.7
12	168.4	168.3	168.2	168.1
13	124.5	124.5	124.5	124.5
14	25.4	25.5	25.8	25.3
15	20.8	20.8	20.6	20.8
1′	177.8	174.2	168.8	178.1
2′	41.2	34.3	127.4	34.3
3′	26.8	19.0	139.5	19.0
4′	11.6	19.0	15.9	19.0
5′	16.7		20.9	
1″	168.4	177.8	176.0	178.1
2″	127.0	41.4	34.3	34.3
3″	140.0	26.8	19.0	19.1
4″	16.7	11.6	19.0	19.0
5″	20.6	17.0		

[a] Recorded at 125 MHz.

2D NOESY spectrum. The major NOE correlations are shown by arrows in Figure 3 and support the proposed structure of **1**. Compound **1** is therefore characterized as 2,10-dihydroxy-5-oxo-6-angeloyloxy-9-(2-methylbutyloxy)-germacran-8,12-olide, named cardivin A. Compound **1** is a tautomer of ineupatolide,[5] but all the data agreed with the ketone form (compound **1**) only. The presence of the hemiacetal form (ineupatolide) was not detected in the NMR data.

Compound **2** was also obtained as colorless needles. Its IR spectrum showed the presence of hydroxy (3454 cm^{-1}) and γ-lactone (1753 cm^{-1}) groups. HRMS (*m/z*

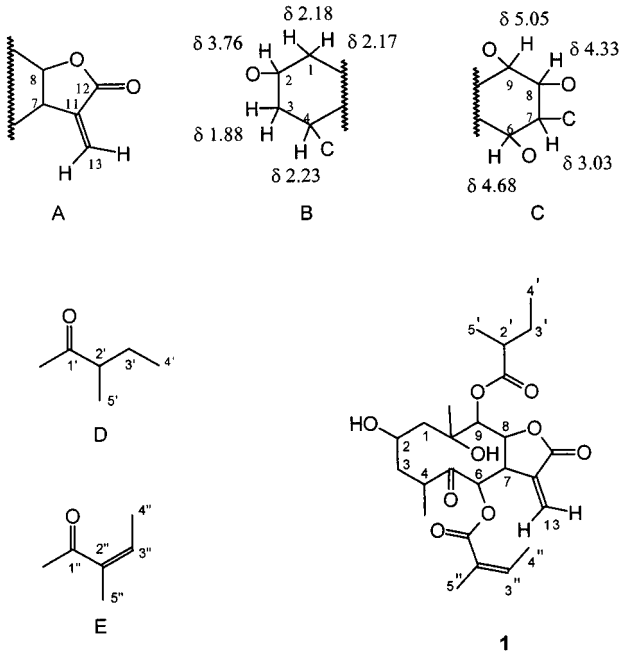

Figure 1. Partial structures of compound **1**.

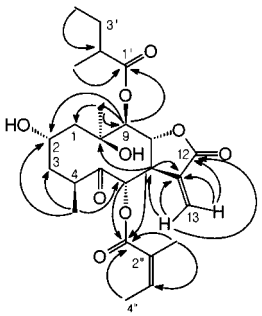

Figure 2. HMBC correlations of **1**.

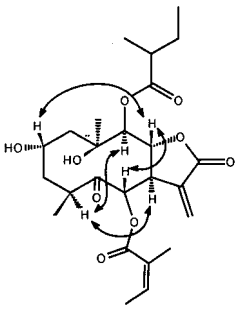

Figure 3. Key NOEs observed for compound **1**.

468.2378) revealed a molecular formula of $C_{24}H_{36}O_9$. The ^{1}H- and ^{13}C-NMR data were nearly identical with those of **1**, except for the side-chain ester substituents. An isobutyrate ester appeared at 174.2, 34.3, and 19.0 in the ^{13}C-NMR spectrum instead of 2-methylbutanoate ester. The position of the ester groups was confirmed by HMBC; the carbon signal C-1′ (174.2) of isobutanoate correlated with the H-9 (5.04), and C-1″ (177.8) of 2-methylbutanoate correlated with H-6 (4.69). The stereochemistry of **2** was determined to be identical with that of **1** on the basis of coupling constants observed in the ^{1}H-NMR spectrum. Compound **2** is characterized as 2,10-dihydroxy-5-oxo-6-[(2-methylbutyl)oxy]-9-(isobutyloxy)germacran-8,12-olide and was named cardivin B.

The molecular formula of compound **3** was confirmed as $C_{24}H_{34}O_9$ from the HRMS (*m/z* 466.2200). NMR data were nearly identical with those of **1**, the only difference

Table 3. Cytotoxicity (ED50, μg/mL)[a] of **1**−**4** against Human Tumor Cell Lines

	cell lines[b]				
compound	A-549	SK-OV-3	SK-Mel-2	XF-498	HCT-15
1	3.17	1.47	1.16	1.40	1.28
2	3.71	2.24	1.45	1.69	2.06
3	3.12	1.60	1.08	1.31	1.38
4	8.36	3.78	1.88	3.05	3.00
doxorubicin	0.12	0.13	0.11	0.23	2.40

[a] ED_{50} value of compounds against each cancer cell line, which was defined as a concentration (μg/mL) that caused 50% inhibition of cell growth in vitro. [b] Cell lines: A-549, nonsmall cell lung cancer; SK-OV-3, ovarian cancer; SK-Mel-2, melanoma; XF-498, CNS cancer; and HCT-15, colon cancer.

was that an isobutyrate ester group (176.0, 34.3, and 19.0 in the ^{13}C-NMR spectrum) appeared instead of the 2-methylbutanoate ester. The positions of the two ester substituents were determined by HMBC; the carbon signal C-1′ (168.8) of angelate correlated with the H-9 (5.13), and the C-1″ (176.0) of isobutyrate correlated with H-6 (4.70). In conclusion, the structure of the novel compound **3** was determined to be 2,10-dihydroxy-5-oxo-6-(isobutyloxy)-9-(angeloyloxy)germacran-8,12-olide (cardivin C).

The IR spectrum of compound **4** showed the presence of hydroxy (3430 cm^{-1}) and γ-lactone (1753 cm^{-1}) groups. HRMS (*m/z* 454.2198) of **4** revealed a molecular formula of $C_{23}H_{34}O_9$. The ^{1}H- and ^{13}C-NMR data were very similar to those of **3**, except for the presence of one additional isobutyrate ester (178.1, 34.3, 19.1, and 19.0 in the ^{13}C-NMR spectrum) instead of the angelate ester of **3**. Locations of the two isobutyrate esters were determined by HMBC; the carbon signal C-1′ (178.1) of an isobutyrate correlated with the H-9 (5.04), and the C-1″ (178.1) of the other isobutyrate correlated with H-6 (4.69). Finally, compound **4** is characterized as 2,10-dihydroxy-5-oxo-6,9-bis(isobutyloxy)germacran-8,12-olide, and was named cardivin D.

Cardivins A, B, C, and D are cytotoxic in vitro in several human tumor cellular models as determined by the sulforhodamine B (SRB) assay.[9,10] ED_{50} values of cardivins A−D are shown in Table 3.

Experimental Section

General Experimental Procedures. Melting points were obtained on a Gallenkamp melting point apparatus (uncorrected). Optical rotations were measured with a JASCO DIP-370 instrument. IR spectra were recorded on a Nicolet model 205 FT-IR spectrophotometer. UV spectra were recorded in MeOH on a Shimadzu UV 240 UV-vis recording spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were determined on a Bruker AMX 500 or Bruker AMX 400 spectrometer in $CDCl_3$. MS was recorded on a VG70-VSEQ instrument. Analytical TLC was carried out on Merck aluminum plates precoated with Si gel 60 F_{254} and visualized by a UV lamp. Chromatography was performed on Merck Si gel 60 (230−400 mesh). LPLC was carried out on Duramat 80 with Merck Lichroprep Si 60 (Lobar A) (24 010 mm) or a Merck Lichroprep RP-18 (24 010 mm) column.

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

Extraction and Isolation. The air-dried plant material (3.5 kg) was finely ground and extracted at room temperature with MeOH (2 L × 3) for 2 weeks. The resulting MeOH extract (110 g) was suspended in H_2O (1 L) and partitioned with CH_2Cl_2 (1 L × 2) and *n*-BuOH (1 L × 2), successively, to give CH_2Cl_2 (30 g), *n*-BuOH (22 g), and H_2O (50 g) soluble fractions. The most cytotoxic CH_2Cl_2 fraction was applied to a Si gel column and eluted with hexane−EtOAc mixtures of increasing polarity (5:10:1) to give five subfractions, whose fourth one (3.5 g) was chromatographed with Si gel eluted with $CHCl_3$-EtOAc (15:1) followed by hexane−CH_2Cl_2−EtOAc (1:1:1) to give five fractions A−E. Fractions A (210 mg) and B (430 mg) were purified with Lobar A (Merck) (CH_2Cl_2−EtOAc, 8:1) to yield **1** (15 mg) and **2** (30 mg), respectively. Fraction C (1.6 g) afforded **3** (20 mg) and **4** (30 mg) by Lobar A (MeOH−H_2O, 8:2).

Cardivin A (1): colorless needles (MeOH): mp 228−230 °C; $[\alpha]^{24}_D$ −94.8° (*c* 0.75, MeOH); UV (MeOH) λ_{max} 218; IR (Nujol) ν_{max} 3410 (OH), 1753 (lactone C=O), 1739 (C=O), 1660 (C=C) cm^{-1}; EIMS *m/z* 480 [M$^+$] (6), 462 (2), 378 (8), 350 (7), 250 (9), 97 (10), 83 (100), 57 (81); HREIMS *m/z* 480.2306 (calcd for $C_{25}H_{36}O_9$, 480.2359); ^{1}H NMR ($CDCl_3$, 500 MHz) (Table 1); ^{13}C NMR ($CDCl_3$, 125 MHz) (Table 2).

Cardivin B (2): colorless needles (MeOH); mp 229−231 °C; $[\alpha]^{24}_D$ −115.0° (*c* 0.5, MeOH); UV (MeOH) λ_{max} 218; IR (Nujol) ν_{max} 3454 (OH), 1753 (lactone C=O), 1740 (C=O), 1650 (C=C) cm^{-1}; EIMS *m/z* 468 [M$^+$] (1), 466 (2), 383 (3), 366 (5), 250 (13), 153 (24), 83 (100), 57 (89); HREIMS *m/z* 468.2378 (calcd for $C_{24}H_{36}O_9$, 468.2359); ^{1}H NMR ($CDCl_3$, 500 MHz) (Table 1); ^{13}C NMR ($CDCl_3$, 125 MHz) (Table 2).

Cardivin C (3): colorless needles (MeOH); mp 214−216 °C; $[\alpha]^{24}_D$ −108.6° (*c* 0.2, MeOH); UV (MeOH) λ_{max} 218; IR (Nujol) ν_{max} 3410 (OH), 1763 (lactone C=O), 1738 (C=O), 1650 (C=C) cm^{-1}; EIMS *m/z* 466 [M$^+$] (3), 383 (6), 365 (7), 250 (7), 83 (100), 71 (49); HREIMS *m/z* 466.2200 (calcd for $C_{24}H_{34}O_9$, 466.2202); ^{1}H NMR ($CDCl_3$, 500 MHz) (Table 1); ^{13}C NMR ($CDCl_3$, 125 MHz) (Table 2).

Cardivin D (4): colorless needles (MeOH); mp 215−219 °C; $[\alpha]^{24}_D$ −76.8° (*c* 0.25, MeOH); UV (MeOH) λ_{max} 218; IR (Nujol) ν_{max} 3430 (OH), 1753 (lactone C=O), 1734 (C=O), 1660 (C=C) cm^{-1}; EIMS *m/z* 454 [M$^+$] (1), 366 (8), 338 (8), 250 (14), 194 (23), 153 (25), 97 (19), 71 (100); HREIMS *m/z* 454.2198 (calcd for $C_{23}H_{34}O_9$, 454.2202); ^{1}H NMR ($CDCl_3$, 500 MHz) (Table 1); ^{13}C NMR ($CDCl_3$, 125 MHz) (Table 2).

Cytotoxicity Assay *in Vitro*. All experimental procedures followed the U.S. National Cancer Institute's protocol[9] based on the SRB method as described previously.[11]

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