SCIENTIFIC REPORTS

OPEN

Isolation, Structure Elucidation, and Absolute Configuration of Germacrane Isomers from *Carpesium divaricatum*

Received: 16 November 2017
Accepted: 25 April 2018
Published online: 20 August 2018

Tao Zhang[1], Jia-Huan Chen[1,2], Jin-Guang Si[1], Gang Ding[1], Qiu-Bo Zhang[1], Hong-Wu Zhang[1], Hong-Mei Jia[1] & Zhong-Mei Zou[1]

Five sets of germacrane isomers (1/8/17, 2/7/10/11/13/16/18, 3/4/5/14/20, 6/12/15, and 9/19) with different skeletal types, including seven new ones (1–3, 8–9, and 15–16) were isolated from the whole plant of *Carpesium divaricatum*. Among them, there are six pairs of stereoisomers (1/8, 2/13, 4/14, 6/12, 7/11 and 10/11). The planar structures and relative configurations of the new compounds were elucidated by detailed spectroscopic analysis. The absolute configurations of 4, 10, 11, and 17 were established by circular dichroism (CD) spectra and X-ray crystallographic analyses, and the stereochemistry of the new compounds 1–3, 8–9, and 15–16 were determined by similar CD spectra with 4, 10, 11, and 17, respectively. The confusion in the literature about subtypes I and II of germacranolides was clarified in this paper. The NMR data of 10–11, and the absolute configurations of the known compounds 4–6, 13–14, and 17–20 were reported for the first time. Compounds 13, 17, and 18 showed cytotoxicity against human cervical (HeLa), colon (LoVo) and stomach cancer (BGC-823) cell lines with IC_{50} values in the range 4.72–13.68 μM compared with the control *cis*-platin (7.90–15.34 μM).

The genus *Carpesium* (Asteraceae) includes 25 species worldwide, most of which are distributed across Asia and Europe, particularly in southwest China[1,2]. The plant *Carpesium divaricatum*, as a Chinese folk medicine, has been used for the treatment of fevers, colds, bruises, and inflammatory diseases[3–7]. Previous investigations indicated that a series of diverse compounds were isolated, including sesquiterpenoid lactones, acyclic diterpenes, and thymol derivatives, with the sesquiterpenoid lactones being the major constituents[5–11].

Germacranolides are one of the main sesquiterpene lactones, reported with broad bioactivities including cytotoxicity, anti-inflammation and anti-malaria[2,4,10–13]. So far, 54 germacranolides from the *Carpesium* plants have been reported[2,12,14]. The parent nucleus of the germacranolides contains a 5-membered γ-lactone ring fused to a circular 10-membered carbocycle, with different post-modifications to produce complex and diverse structural features. In addition, these germacranolides contain as many as nine stereogenic centers, creating the problem of stereo configuration. The relative configurations of 2, 5-hemiacetal-linked germacranolides are often deduced by NOESY analysis[7–9,11,15–20], but their absolute configurations have rarely been reported[12,14]. The undefined absolute configuration and the incorrect depiction of the epoxy bonds cause significant confusion within the structures of this kind of compounds[7–9,11,15–20].

In our continuing effort to search for bioactive constituents from *C. divaricatum*, germacrane isomers attracted our attention due to their structural diversity. Five sets of germacrane isomers (**1/8/17**, **2/7/10/11/13/16/18**, **3/4/5/14/20**, **6/12/15**, and **9/19**), including seven new ones (**1–3**, **8–9**, and **15–16**) were indentified in the current investigation. Notably, these germacranolides include six pairs of stereoisomers (**1/8**, **2/13**, **4/14**, **6/12**, **7/11** and **10/11**). Structurally, these germacrane isomers belong to different skeletal types. According to the configuration of 2,5-hemiacetal group, linkage site of the ketone group and the position of 5-membered γ-lactone ring, the germacranolides could be further divided into several subtypes (Fig. 1). Compounds **1–7** together with the reported compounds ineupatolide B–C[21] are attributable to subtype I (named 2β,5β-epoxygermacranolide), which

[1]Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100193, P. R. China. [2]School of Traditional Chinese Medicine, Shenyang Pharmaceutical University, Shenyang, 110016, P. R. China. Correspondence and requests for materials should be addressed to Z.-M.Z. (email: zmzou@implad.ac.cn)

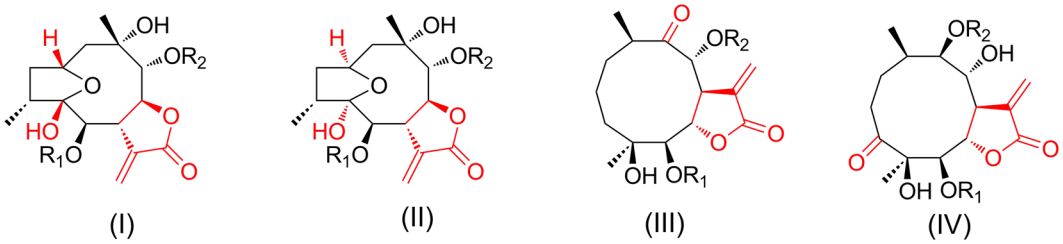

Figure 1. Four subtypes of germacranolides.

contains a 5-membered α-methylene-γ-lactone ring linkage at C-7 and C-8, and the 2β,5β-hemiacetal group on the macro-ring system. Compounds **8**–**14** and the known compounds divaricin A–C[8] are assigned as subtype II (named 2α,5α-epoxygermacranolide), having a 7,8-α-methylene-γ-lactone ring and the 2α,5α-hemiacetal group. The known compounds incaspitolide A–C[22,23] and eight germacranolides we previously reported from *C. divaricatum*[12] are classified as subtype III (named 9-oxo-germacranolide) with structural features of one 6,7-α-methylene-γ-lactone ring and the 9-ketone group. Compounds **15**–**20** represent subtype IV (named 3-oxo-germacranolide), possessing a 6,7-α-methylene-γ-lactone ring and the 9-ketone group. Both subtypes I and II are 2,5-hemiacetal-linked germacranolides, but their configurations at the bridgehead carbons between 5-membered ring and 9-membered ring are opposite. Subtypes III and IV have similar skeletons, but linkage sites of the ketone group are different (9-ketone group in subtype III and 3-ketone group in subtype IV). Twenty analogues here we further isolated from same species represent other three subtypes of germacranolides (subtypes I–II and IV). NOESY spectrum, circular dichroism (CD) method and X-ray data analysis were used to confirm their relative and absolute configurations. In this paper, the isolation, the structural elucidation, the absolute configuration and bioactive evaluation of these compounds were present. The confusion in the literature about subtypes I and II of germacranolides is also discussed.

Results and Discussion

Structural Elucidation of Compounds from Subtype I. Compound **1** (Fig. 2) was obtained as white needles. The molecular formula was assigned as $C_{23}H_{34}O_9$ on the basis of the positive-ion HRESIMS ion at *m/z* 477.2104 $[M+Na]^+$, together with its ^1H and ^{13}C NMR data (Tables 1 and 2). The IR spectrum showed the presence of hydroxyl (3493 cm^{-1}) and carbonyl (1767 and 1737 cm^{-1}) functional groups. The ^1H NMR spectrum of **1** displayed the presence of two isobutyryloxy groups, which was further confirmed by the HRESIMS data of **1** with fragment ions at *m/z* 367.1758 $[M+1\text{-HOiBu}]^+$ and 261.1127 $[M+1\text{-HOiBu-HOiBu-H}_2O]^+$. The ^1H and ^{13}C NMR spectra of **1** also showed an α-methylene-γ-lactone moiety at δ_H 5.68 (1 H, dd, $J=3.0$, 1.2 Hz, Ha-13) and 6.13 (1 H, dd, $J=3.6$, 1.2 Hz, Hb-13), δ_C 134.8 (C-11), 124.2 (C-13) and 170.2 (C-12); two lactone carbonyl carbons at δ_C 176.6 (C-1′) and 178.0 (C-1″); a dioxygenated carbon at δ_C 105.6 (C-5); one oxygenated tertiary carbon at 72.0 (C-10); five methines including four oxygenated ones at δ_H 4.30 (1 H, m, H-2), 4.87 (1 H, d, $J=7.2$ Hz, H-6), 3.90 (1 H, m, H-7), 4.70 (1 H, dd, $J=6.0$, 6.0 Hz, H-8) and 5.07 (1 H, d, $J=4.8$ Hz, H-9), δ_C 70.9 (C-2), 74.4 (C-6), 45.9 (C-7), 78.1 (C-8), and 80.3 (C-9); and two methyl groups at δ_H 1.33 (3 H, s, CH_3-14), 0.99 (3 H, d, $J=7.2$ Hz, CH_3-15). These observations and analyses of ^1H-^1H COSY, HSQC, and HMBC spectra (Fig. 3) suggested that the structure of **1** was similar to that of 2β,5-epoxy-5,10-dihydroxy-6α-angeloyloxy-9β-isobutyryloxy-germacran-8α,12-olide (**4**)[9], except for an isobutyryloxy group in **1** compared to the angeloyloxy group at C-6 in **4**.

The relative configuration of **1** was determined by analysis of the NOESY data (Fig. 4). The NOE correlations of H_3-15/H-6 and H-6/H-8 indicated they were cofacial and were arbitrarily assigned as α-orientations, whereas the correlations of H-7/H-9 and H-9/H_3-14 showed their β-orientations. The NOE correlations of H-2/H-4 and H-6/H_3-15, and the lack of correlation of H-4 with H-7, suggested that H-2, and 5-OH were β-oriented. Thus, the relative configuration of **1** was established.

Compound **2** possessed molecular formula of $C_{25}H_{36}O_9$ based on the HRESIMS ion at *m/z* 503.2261 $[M+Na]^+$. The ^1H and ^{13}C NMR data of **2** were similar to those of **1**, except that the isobutyryloxy groups at C-6 and C-9 in **1** were replaced by an angeloyloxy group at C-6 and a 3-methylbutyryloxy group at C-9 in **2**. The ^1H-^1H COSY, HSQC, and HMBC spectra supported the structure of **2** as shown. The NOE correlations of H-2/H-4, H-6/H_3-15, H-6/H-8, H-7/H-9 and H-9/H_3-14 in **2** indicated that **2** had the same relative configuration as **1**.

Compounds **3**–**4** shared the same molecular formula $C_{24}H_{34}O_9$ from their HRESIMS at *m/z* 489.2112 $[M+Na]^+$ and *m/z* 489.2100 $[M+Na]^+$. The ^1H and ^{13}C NMR data of **3** showed a great similarity with those of **4**, except for the ester residues at C-6. The angeloyloxy group at C-8 in **4** was placed by a tigloyloxy group in **3**[24]. The ^1H-^1H COSY, HSQC and HMBC spectra of **3** confirmed this observation, leading to the assignment of its planar structure. The relative configuration of **3** was deduced to be the same as **4**, on the basis of similar ROESY data.

Compounds **4**–**7** were identified as 2β,5-epoxy-5,10-dihydroxy-6α-angeloyloxy-9β-isobutyryloxy-germacran-8α,12-olide (**4**)[9], ineupatolide A (**5**)[21], 2β,5-epoxy- 5,10-dihydroxy-6α,9β-diangeloyloxy-germacran-8α,12-olide (**6**)[15–20] and ineupatolide (**7**)[8,14,15] by comparison of their MS, ^1H NMR, and ^{13}C NMR spectroscopic data, as well as optical rotation data with reported data. The conclusions were also confirmed by the ^1H-^1H COSY, HSQC, HMBC, and NOESY spectra. However, only compound **7** has been reported the absolute configuration before.

Fortunately, a single crystal of **4** was obtained from MeOH. The X-ray crystallographic analysis [flack parameter:−0.10(12)] unambiguously established the absolute configuration of **4** as 2*R*, 4*R*, 5*S*, 6*R*, 7*S*, 8*S*, 9*R*, and

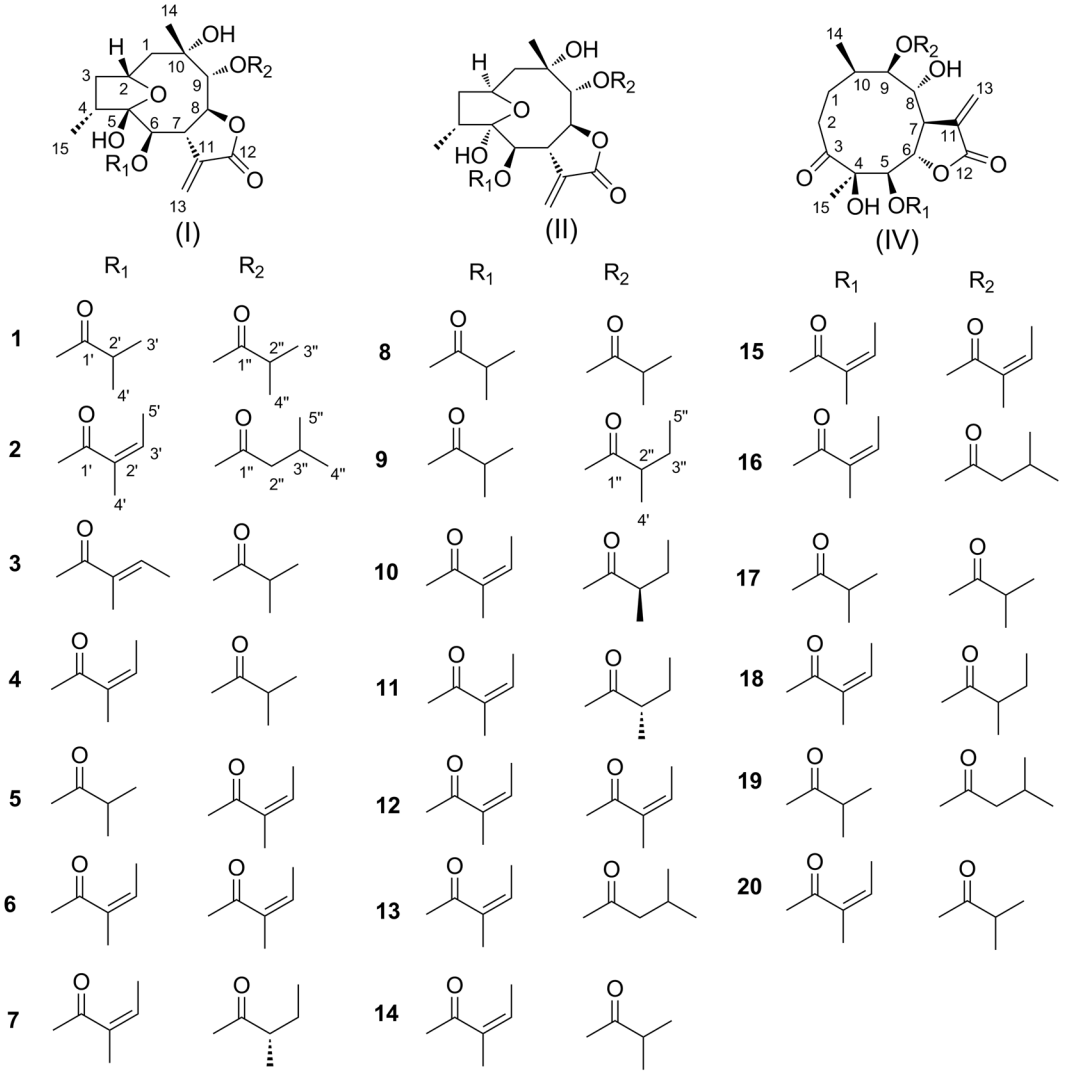

Figure 2. Chemical structures of compounds **1**–**20**.

10*S* (Fig. 5). Based on the established absolute configuration of **4**, we deduced the absolute configurations of **1**–**3** and **4**–**6** from similar CD data (supplementary information Fig. C1). Due to the fact that there are some differences in the CD spectra of **1** and **5**, the absolute configurations of **1** and **5** were further confirmed by using quantum chemical electronic circular dichroism (ECD) calculations. It was clear that the calculated ECD spectra of (2*R*, 4*R*, 5*S*, 6*R*, 7*S*, 8*S*, 9*R*, 10*S*)-**1** and **5** were matched very well with the experimental ECD spectra of **1** and **5** (supplementary information C2). Thus, the structures of compounds **1**–**6** were defined as shown, named (2*R*, 5*S*)-cardivarolide A (**1**), (2*R*, 5*S*)-cardivarolide B (**2**), (2*R*, 5*S*)-ciscardivarolide C (**3**), (2*R*, 5*S*)-cardivarolide C (**4**), ineupatolide A (**5**), and (2*R*, 5*S*)-cardivarolide D (**6**), respectively.

This type of germacranolides has significant confusion because almost all of the compounds have been determined by comparison of their spectroscopic data with those of ineupatolide (**7**)[15]. In addition to the previous incorrect assignment of the absolute configuration of ineupatolide (**7**)[15,21], the depiction of the epoxy bonds, in which the bonds of 2,5-epoxy group were depicted with bold or dashed lines, is also incorrect[15,16]. Thus, it is suggested that the structures of germacranolides of this type (subtype I) should be depicted as shown.

Structural Elucidation of Compounds from Subtype II. Compound **8** had the same molecular formula ($C_{23}H_{34}O_9$, *m*/*z* 477.2101 $[M+Na]^+$ in HRESIMS) and the same planar structure as **1** according to the ^{1}H-^{1}H COSY, HSQC, and HMBC spectra (Fig. 3). Comparing the ^{1}HNMR data of **8** with **1**, the main difference was downfield signals of H-7 (δ 3.30 in **8** and 3.90 in **1**), H-9 (δ 4.59 in **8** and 5.07 in **1**), and H-8 (δ 5.24 in **8** and 4.70 in **1**) in **8** (see Table 1). These results suggested that **8** and **1** had different configurations[8]. The relative configuration of **8** was elucidated by NOESY analysis (Fig. 4). The NOE correlations of H-4/H-7, H_3-15 (4-CH_3) /H-3a, H-3a/H-2 and H-2/H-1b, indicated that H-2, H_3-15, and 5-OH had α-orientations[8]. The β-orientations of H-7, H-9 and H_3-14, and α-orientations of H-6 and H-8 could be deduced from the correlations of H-4/H-7, H-7/H-9, H-9/H_3-14 and H-8/H-6. Compared with **1**, compound **8** had the opposite configurations of H-2 and 5-OH linkage to the bridgehead carbons between 5-membered ring and 9-membered ring. Therefore, H-4 in space was much closer to H-7 but H_3-15 was far away from H-6 in **8**, compared to those of **1** (supplementary information

No.	1[a]	2[a]	3[b]	4[a]	5[b]	6[a]	7[a]	8[a]
1a	1.90 dd (15.6, 12.0)	1.92 m	1.98 m	1.98 m	1.96 o[c]	1.95 o	1.98 m	2.04 dd (15.6,12.0)
1b	1.83 dd (15.6, 4.2)	1.84 dd (15.0, 4.2)	1.87 m	1.89 dd (15.6, 4.2)	1.89 m	1.86 o	1.90 dd (15.6, 4.2)	1.61 dd (15.6, 4.2)
2	4.30 m	4.30 m	4.33 m	4.36 m	4.35 m	4.32 m	4.36 m	4.55 m
3a	2.56 m	2.57 m	2.69 m	2.72 m	2.62 o	2.59 m	2.63 m	1.92 m
3b	1.40 m	1.40 m	1.40 m	1.46 m	1.45 m	1.41 m	1.47 m	1.75 dd (12.0, 6.6)
4	2.30 m	2.31 m	2.32 m	2.36 m	2.36 m	2.32 m	2.37 m	2.69 m
6	4.87 d (7.2)	4.99 d (7.8)	4.98 d (7.0)	5.04 d (7.2)	4.94 d (7.0)	5.01 d (6.6)	5.04 d (7.2)	5.02 d (10.8)
7	3.90 m	3.92 m	3.94 m	3.97 m	4.03 m	4.00 m	3.97 m	3.30 dd (10.8,1.2)
8	4.70 dd (6.0, 6.0)	4.74 dd (5.4, 5.4)	4.75 dd (6.5, 5.0)	4.80 dd (6.0, 5.4)	4.80 dd (6.0, 5.5)	4.79 dd (5.4, 5.4)	4.80 dd (6.0, 5.4)	5.24 dd (10.2,1.2)
9	5.07 d (6.0)	5.12 d (5.4)	5.14 d (5.0)	5.18 d (5.4)	5.23 d (5.5)	5.22 d (5.4)	5.18 d (5.4)	4.59 d (10.2)
13a	6.13 dd (3.6, 1.2)	6.06 dd (3.0, 0.6)	6.04 dd (3.5, 1.0)	6.11 d (3.0)	6.19 d (3.0)	6.07 dd (3.6, 1.2)	6.11 dd (3.0, 0.6)	6.17 br s
13b	5.68 dd (3.6, 1.2)	5.60 dd (3.0, 0.6)	5.62 dd (3.5, 1.0)	5.65 d (3.0)	5.75 d (3.0)	5.60 dd (3.6, 1.2)	5.65 dd (3.0, 0.6)	5.73 d (1.2)
14	1.33 s	1.40 s	1.36 s	1.40 s	1.41 s	1.36 s	1.40 s	1.19 s
15	0.99 d (7.2)	0.99 d (7.2)	0.99 d (7.5)	1.04 d (7.2)	1.06 d (7.0)	1.00 d (7.2)	1.04 d (7.2)	1.14 d (6.6)
2′	2.54 m				2.61 o			2.53 m
3′	1.15 d (7.2)	6.21 qq (7.2, 1.8)	7.02 q (8.5)	6.25 qq (7.8, 1.8)	1.20 d (7.0)	6.21 qq (7.2, 1.8)	6.25 qq (7.2, 1.8)	1.10 d (7.2)
4′	1.07 d (7.2)	1.90 dq (1.8, 1.2)	1.82 br s	1.96 dq (1.8, 1.2)	1.13 d (7.0)	1.91 dq (1.8, 1.2)	1.96 dq (1.8, 1.2)	1.13 d (7.2)
5′		1.96 dq (7.2, 1.2)	1.84 d (8.5)	2.01 dq (7.8, 1.2)		1.97 dq (7.2, 1.2)	2.01 dq (7.2, 1.2)	
2″	2.56 m	2.27 o, 2.27 o	2.59 m	2.62 m			2.53 m	2.64 m
3″	1.15 d (7.2)	2.08 m	1.18 d (7.5)	1.21 d (7.2)	6.08 qq (7.0, 1.5)	6.04 qq (7.2, 1.8)	1.79 m,1.46 m	1.16 d (7.2)
4″	1.14 d (7.2)	0.94 d (7.2)	1.17 d (7.5)	1.20 d (7.2)	1.97 d (1.5)	1.93 dq (1.8, 1.2)	1.20 d (7.2)	1.20 d (7.2)
5″		0.93 d (7.2)			1.92 dq (7.0, 1.0)	1.86 dq (7.2, 1.2)	0.96 t (7.8)	
No.	**9[b]**	**10[a]**	**11[a]**	**12[b]**	**13[a]**	**14[a]**	**15[b]**	**16[b]**
1a	2.07 dd (15.5, 12.5)	2.05 dd (15.6, 12.6)	2.06 dd (15.6, 12.6)	2.13 dd (15.5, 12.0)	2.05 dd (15.6, 12.0)	2.05 dd (15.6, 12.0)	1.87 m	1.87 m
1b	1.63 dd (15.5, 4.0)	1.62 dd (15.6, 4.2)	1.62 dd (15.6, 4.2)	1.67 dd (15.5, 4.0)	1.61 dd (15.6, 4.2)	1.61 dd (15.6, 4.2)	1.74 m	1.65 m
2	4.57 m	4.56 m	4.57 m	4.62 m	4.56 m	4.56 m	3.87 m, 2.24 o	3.88 m, 2.23 o
3a	1.94 m	1.94 m	1.95 m	1.97 o	1.93 m	1.93 m		
3b	1.78 m	1.77 dd (12.0, 6.6)	1.78 dd (12.0, 6.6)	1.83 dd (12.0, 7.0)	1.77 dd (12.0, 7.2)	1.77 dd (12.0,7.2)		
4	2.72 m	2.72 m	2.72 m	2.77 m		2.72 m		
5							5.51 dd (10.0, 2.0)	5.52 dd (9.5, 2.0)
6	5.04 d (11.5)	5.17 d (10.8)	5.17 d (10.8)	5.22 d (11.0)	5.16 d (10.8)	5.17 d (10.8)	4.73 dd (10.0, 6.5)	4.73 dd (9.5, 6.5)
7	3.34 dd (11.5,1.5)	3.32 dd (10.8, 1.2)	3.33 dd (10.8, 1.2)	3.41 br d (11.0)	3.36 dd (10.8,1.2)	3.33 dd (10.2, 1.2)	3.08 m	3.06 m
8	5.26 br d (10.0)	5.28 dd (10.2, 1.2)	5.27 dd (10.2, 1.2)	5.33 br d (10.0)	5.26 dd (10.2,1.2)	5.27 br d (9.6)	4.48 d (10.5)	4.44 d (10.5)
9	4.61 d (10.0)	4.60 d (10.2)	4.60 d (10.2)	4.73 d (10.0)	4.59 d (10.2)	4.60 d (9.6)	5.27 d (10.5)	5.18 d (10.5)
10							2.24 o	2.23 o
13	6.19 br s, 5.76 br s	6.11 d (1.2), 5.67 d (1.2)	6.11 d (1.2), 5.67 d (1.2)	6.17 br s, 5.73 br s	6.11 br s, 5.67 d (1.2)	6.11 br s, 5.67 br s	6.34 d (3.0), 5.71 d (3.0)	6.34 d (3.0), 5.69 d (3.0)
14	1.22 s	1.21 s	1.21 s	1.26 s	1.20 s	1.20 s	0.99 d (6.5)	0.99 d (6.5)
15	1.17 d (6.5)	1.13 d (6.6)	1.13 d (6.6)	1.18 d (6.5)	1.13 d (6.6)	1.13 d (6.6)	1.25 s	1.26 s
2′	2.56 m							
3′	1.13 d (7.0)	6.10 qq (6.6, 1.8)	6.10 qq (7.2, 1.8)	6.17 o	6.10 qq (5.4,1.8)	6.10 qq (6.0, 1.2)	6.18 o	6.20 qq (7.0, 1.5)
4′	1.16 d (7.0)	1.89 dq (1.8, 1.2)	1.89 dq (1.8, 1.2)	1.97 s	1.89 dq (1.8,1.2)	1.90 dq (1.2, 1.2)	2.00 s	1.99 q (1.5)
5′		1.90 dq (6.6, 1.2)	1.91 dq (7.2, 1.2)	1.95 dq (5.5, 1.5)	1.90 dq (5.4,1.2)	1.89 dq (6.0, 1.2)	1.96 br d (10.5)	2.02 dq (7.0, 1.5)
2″	2.47 m	2.46 m	2.47 m		2.28 o, 2.28 o	2.64 m		2.32 o, 2.32 o
3″	1.76 m,1.48 m	1.70 m,1.45 m	1.77 m,1.47 m	6.17 o	2.10 m	1.16 d (7.2)	6.18 o	2.13 o
4″	1.17 d (7.0)	1.19 d (6.6)	1.16 d (7.2)	1.97 s	0.97 d (7.8)	1.20 d (7.2)	2.00 s	1.01 d (5.5)
5″	0.97 t (7.5)	0.90 t (7.8)	0.96 t (7.8)	1.95 dq (5.5, 1.5)	0.96 d (7.8)		1.96 d (10.5)	1.01 d (5.5)

Table 1. ^{1}H NMR Spectroscopic Data for Compounds **1**–**16** (δ in ppm, J in Hz). [a]Measured at 600 MHz in methanol-d_4; [b]Measured at 500 MHz in methanol-d_4; [c]Overlapped with other signals.

Fig. C3). Thus, NOE correlations of H_3-15/H-6 and H-4/H-6 were not observed in **8**. On the basis of these data, the relative configuration of **8** was established.

The molecular formula of compound **9** was assigned as $C_{24}H_{36}O_9$ by the positive-ion HRESIMS ion at m/z 491.2271 $[M+Na]^+$, the same as that of **20**. The ^{1}H and ^{13}C NMR data of **9** were similar to those of **8**, except for the ester residues at C-9. The 2-methylbutyryloxy group at C-9 appeared in **9** instead of the isobutyryloxy group in **8**. The ^{1}H-^{1}H COSY, HSQC, and HMBC spectra supported the structure of **9** as shown. The NOE correlations of H-4/H-7, H-8/H-6, H-7/H-9 and H-9/H_3-14 in **8** and **9** indicated that **9** had the same relative configuration as **8**.

Compounds **10**–**14** were identified as (2*S*, 4*R*, 5*R*, 6*R*, 7*S*, 8*S*, 9*R*, 10*S*, 2″*R*) -2,5-epoxy-5,10-dihydroxy-6-angeloyloxy-9-2-methylbutyryloxy-germacran-8,12-olide (**10**)[14,25,26], (2*S*, 4*R*, 5*R*, 6*R*, 7*S*, 8*S*, 9*R*, 10*S*, 2″*S*)-2,5-epoxy-5,10-dihydroxy-6-angeloyloxy-9-2-methylbutyryloxy-germacran-8,12-olide (**11**)[14,25,26], (2*S*, 4*R*, 5*R*, 6*R*, 7*S*, 8*S*, 9*R*, 10*S*)-2,5-epoxy-5,10-dihydroxy-6-angeloyloxy-9-angeloyloxy-germacran-8,12-olide (**12**)[8,14,25,26], 2α,5-epoxy-5,10-dihydroxy-6α-angeloyloxy-9β-3-methylbutyryloxy-germacran-8,12-olide (**13**)[18–20,25,26], and 2α,5-epoxy-5,10-dihydroxy-6α-angeloyloxy-9β-isobutyryloxy-germacran-8,12-olide (**14**)[9,19,20,26] by comparison of their MS, ^{1}H NMR, and ^{13}CNMR spectral with reported data. The conclusions were also confirmed by the ^{1}H-^{1}H COSY, HSQC, HMBC, and NOESY spectra. However, the absolute configurations of **13**–**14** have not been concluded in the literature.

Herein, we further confirmed the absolute configurations of **10** and **11** by X-ray crystallographic analysis. Although a suitable single crystal of compound **10** or **11** was not obtained through many attempts with different solvents, mixed trigonal crystals of **10** and **11** (1:1) were obtained from MeOH. Both compounds have the same nuclear structures, and the only difference is that the absolute configuration of 2-methylbutyryloxy group at C-9 is 2″*R* or 2″*S*. Because the minor difference does not affect their crystal structures, the X-ray diffraction experiment of the mixture crystal supported the absolute configuration of the nuclear structure. The X-ray crystallographic analysis [flack parameter = −0.0(2)] unambiguously established the absolute configurations of **10** and **11** as 2*S*, 4*R*, 5*R*, 6*R*, 7*S*, 8*S*, 9*R*, and 10*S* (Fig. 6).

Considering similar CD data of **8**–**11** and **13**–**14** resulted in the conclusion of the absolute configurations of **8**–**9** and **13**–**14** as 2*S*, 4*R*, 5*R*, 6*R*, 7*S*, 8*S*, 9*R*, and 10*S* (supplementary information Fig. C4). Thus, the structures of new compounds **8**–**9** and known compounds **10**–**14** were defined as shown and named (2*S*, 5*R*)-isocardivarolide A (**8**), (2*S*, 5*R*)-isocardivarolide E (**9**), (2*S*, 5*R*, 2″*R*)-ineupatolide (**10**), (2*S*, 5*R*, 2″*S*)-ineupatolide (**11**), ent-divaricin B (**12**), (2*S*, 5*R*)-isocardivarolide B (**13**), and (2*S*,5*R*)-isocardivarolide C (**14**), respectively.

In addition, compounds **10** and **11** were usually reported as a mixture from *C. triste*[25,26]. Although both of them were separated successfully in the literature[14], the NMR data have not been reported. Herein, the NMR data of **10** and **11** were reported for the first time. The MS, ^{1}H NMR, and ^{13}CNMR spectroscopic data of compound **12** were consistent or superposable with those of divaricin B[8,19,20,26]. These data indicated that both of them shared the same relative configurations. However, all isolated compounds **1**–**11** and **13**–**14** had negative optical rotation, but divaricin B has the opposite optical rotation ($[\alpha]_D^{20}$ − 15.6 of **12** and $[\alpha]_D^{20}$ + 18 of divaricin B[19]), which suggested that divaricin B and compound **12** could be enantiomers and have the opposite absolute configuration. Herein, in order to distinguish two compounds, compound **12** was named ent-divaricin B. The MS, NMR, CD and optical rotation data of **12** was also reported in the paper.

Similarly, there is confusion in the literature about this class of compounds (subtype II) due to the incorrect depiction of the epoxy bonds. Thus, the structures of germacranolides of this type (subtype II) were depicted correctly in this paper.

Structural Elucidation of Compounds from Subtype IV. The molecular formula of compound **15** was assigned as $C_{25}H_{34}O_9$ by positive-ion HRESIMS ion at *m/z* 501.2101 $[M+Na]^+$, the same as those of **6** and **12**. However, the ^{1}H and ^{13}C NMR data implied that the structure of **15** was similar to that of **17**[22,25] (supplementary information S24), except that two isobutyryloxy groups of **17** were replaced by two angeloyloxy groups in **15**, which was further confirmed by the ^{1}H-^{1}H COSY, HSQC, and HMBC spectra (Fig. 3). The relative configuration of **15** was determined by analysis of ROESY data. The key NOE correlations of H-8/H-6, H-7/H-5, H-5/H_3-15, H-7/H-9, and H-9/H-10 indicated that **15** had the same relative configuration as **17** (Fig. 4).

Compound **16** was the isomers of **2**, **7**, **10**, **11**, **13**, **18** and **19**, based on its HRESIMS (*m/z* 503.2263 $[M+Na]^+$, $C_{25}H_{36}O_9Na$). The ^{1}H and ^{13}C NMR data were similar to those of **17**, except for an angeloyloxy group at C-6 and the 3-methylbutyryloxy groups at c-9 in **16** instead of two isobutyryloxy groups in **17**. The conclusion was confirmed by analysis of relevant ^{1}H-^{1}H COSY, HSQC and HMBC data. The relative configuration of **16** was determined to be the same as that of **15** by comparison of their ROESY data.

Compounds **17**–**20** were identified as incaspitolide D (**17**)[23,27], 4β,8α-dihydroxy-5β-angeloyloxy-9β-2-methylbutyryloxy-3-oxo-germacran-6α,12-olide (**18**)[17], 4β,8α-dihydroxy-5β-isobutyryloxy-9β-3-methylbutyryloxy-3-oxo-germacran-6α,12-olide (**19**)[22], and 4β,8α-dihydroxy-5β-angeloyloxy-9β-isobutyryloxy-3-oxo-germacran-6α,12-olide (**20**)[28], by comparison of their MS, ^{1}H NMR, and ^{13}CNMR spectroscopic data, as well as optical rotation data with reported data. However, their absolute configurations have not been reported.

Therefore, the absolute configuration of **17** was determined to be 4*R*, 5*R*, 6*S*, 7*R*, 8*R*, 9*R*, and 10*R* by X-ray crystallographic analysis [flack parameter: −0.00(11)] (Fig. 7). Similar CD data of **15**–**17** and **18**–**20** assigned the absolute configurations of **15**–**16** and **18**–**20** as 4*R*, 5*R*, 6*S*, 7*R*, 8*R*, 9*R*, and 10*R* (supplementary information Fig. C5). Thus, the structures of new compounds **15**–**16** (named cardivarolide F and cardivarolide G, respectively) and the known compounds **18**–**20** were elucidated as shown.

All compounds were evaluated for their cytotoxic activity against human cervical (HeLa), colon (LoVo), stomach (BGC-823), and breast cancer (MCF-7) cell lines. Compounds **13**, **17**, and **18** exhibited cytotoxicity against HeLa (IC_{50} values of 7.60, 5.76, and 4.72 μM), LoVo (IC_{50} values of 7.81, 8.00, and 7.31 μM), and BGC-823 (IC_{50} values of 12.68, 13.68, and 11.67 μM) cell lines, and the IC_{50} values were lower than that of the positive control *cis*-platin (IC_{50} values of 7.90, 13.03, and 15.34 μM, respectively).

In conclusion, five sets of germacrane isomers (**1/8/17**, **2/7/10/11/13/16/18**, **3/4/5/14/20**, **6/12/15**, and **9/19**) representing three subtypes of germacranolides (subtypes I–II and IV) were isolated from the whole plant of *C. divaricatum*. The isolated germacrane isomers, including seven new ones (**1**–**3**, **8**–**9**, and **15**–**16**), contained a 5-membered γ-lactone ring fused to a circular 10-membered carbocycle. Subtypes I and II have the same planar structure, but the absolute configurations at C-2 and C-5 are different (2*R*, 5*S* in subtype I and 2*S*, 5*R* in subtype II). We obtained six pairs of stereoisomers (**1/8**, **2/13**, **4/14**, **6/12**, **7/11** and **10/11**) from the same plant. The isolation of these stereoisomers is a huge challenge because they are highly oxygenated and have similar structures.

No.	1[a]	2[a]	3[b]	4[a]	5[b]	6[a]	7[a]	8[a]	9[b]	10[a]	11[a]	12[b]	13[a]	14[a]	15[b]	16[b]
1	48.8	48.8	48.9	48.8	49.1	49.2	48.9	43.9	43.9	43.9	43.9	43.8	43.9	43.9	25.7	25.4
2	70.9	70.8	70.9	70.8	71.0	70.8	70.9	73.7	73.7	73.8	73.7	73.7	73.7	73.7	33.0	33.2
3	40.4	40.5	40.5	40.5	40.5	40.5	40.6	37.1	37.1	37.3	37.3	37.1	37.3	37.3	217.9	217.8
4	43.4	43.3	43.4	43.3	43.5	43.4	43.4	35.9	36.0	35.9	35.9	35.8	35.9	35.9	80.4	80.4
5	105.6	105.7	105.7	105.7	105.7	105.7	105.7	105.6	105.6	105.8	105.8	105.6	105.8	105.8	78.1	78.1
6	74.4	74.3	74.7	74.4	74.5	74.4	74.4	75.6	75.6	75.8	75.8	75.7	75.8	75.8	80.0	80.0
7	45.9	45.8	45.8	45.8	45.9	45.8	45.8	44.9	44.9	45.1	45.1	45.0	45.0	45.1	41.7	41.6
8	78.1	78.1	78.1	78.1	78.2	78.2	78.2	78.1	78.0	78.2	78.1	78.2	78.3	78.2	70.5	70.3
9	80.3	80.4	80.3	80.3	80.5	80.5	80.4	78.0	77.9	78.0	77.9	77.8	78.0	78.0	78.5	78.7
10	72.0	72.0	72.1	72.1	72.1	72.1	72.2	71.2	71.3	71.1	71.3	71.1	71.1	71.2	30.1	29.9
11	134.8	135.0	134.9	135.0	135.0	135.0	135.1	134.4	134.4	134.6	134.7	134.5	134.6	134.6	132.8	132.8
12	170.2	170.3	170.3	170.3	170.3	170.3	170.2	169.9	169.8	169.9	169.9	169.9	170.0	170.0	169.7	169.7
13	124.2	124.3	124.3	124.3	124.3	124.3	124.3	125.6	125.4	125.5	125.4	125.4	125.5	125.6	124.0	123.9
14	23.3	23.3	23.3	23.3	23.5	23.4	23.4	29.2	29.3	29.4	29.3	29.2	29.2	29.2	20.0	20.0
15	12.5	12.3	12.1	12.3	12.6	12.3	12.4	13.6	13.6	13.6	13.6	13.5	13.6	13.6	23.4	23.4
1′	176.6	166.7	167.0	166.7	176.6	166.7	166.8	176.1	176.1	166.3	166.3	166.2	166.4	166.4	167.1	167.1
2′	33.9	127.1	128.0	127.1	33.9	127.1	127.1	33.8	33.8	127.1	127.1	127.0	127.1	127.1	127.5	127.5
3′	18.0	140.4	139.2	140.3	18.1	140.4	140.4	18.0	18.0	139.4	139.4	139.3	139.4	139.4	138.4	138.4
4′	17.7	19.1	13.2	19.1	17.6	19.2	19.1	17.8	17.8	19.2	19.3	19.1	19.2	19.3	19.3	19.3
5′		14.7	10.6	14.7		14.7	14.7			14.6	14.6	14.5	14.6	14.6	14.6	14.6
1″	178.0	173.9	178.1	178.0	168.8	168.8	177.6	177.0	176.6	176.7	176.7	167.6	173.1	177.1	167.7	173.2
2″	33.5	42.2	33.5	33.5	127.8	127.8	41.0	33.8	41.1	41.4	41.1	127.7	42.6	33.8	127.8	43.2
3″	18.0	25.2	18.1	18.0	137.0	136.9	26.2	18.0	26.3	26.2	26.3	137.3	25.1	18.0	137.7	25.4
4″	17.9	21.4	18.0	17.9	19.3	19.1	15.9	17.8	15.7	16.0	15.7	19.1	21.4	17.8	19.5	21.4
5″		21.4			14.5	14.4	10.8		10.6	10.7	10.7	14.4	21.4		14.7	21.5

Table 2. ^{13}C NMR Spectroscopic Data for Compounds **1**–**16** (δ in ppm). [a]Measured at 150 MHz in methanol-d_4; [b]Measured at 125 MHz in methanol-d_4.

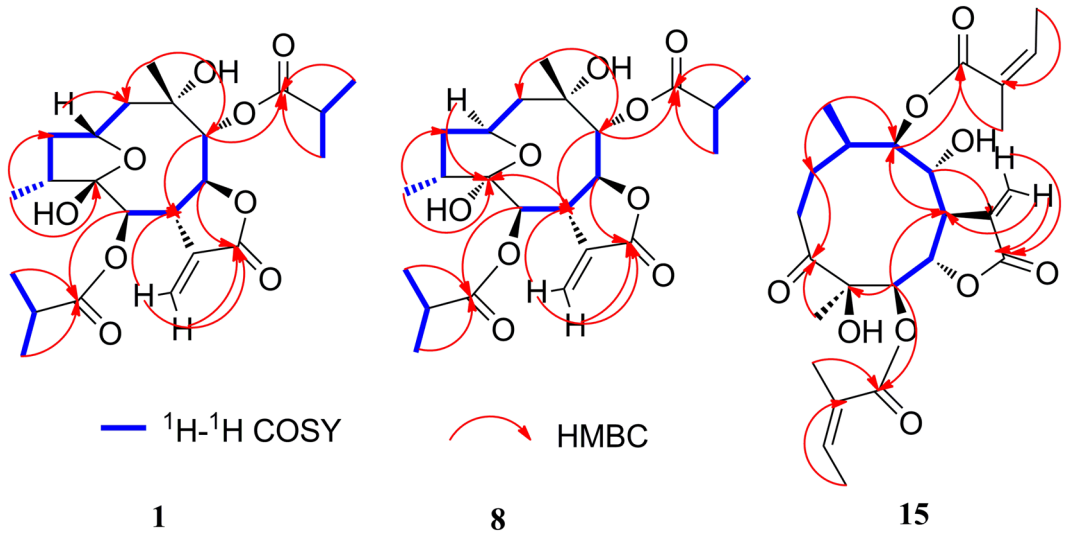

Figure 3. Key ^{1}H-^{1}H COSY and HMBC correlations of compounds **1**, **8**, and **15**.

The absolute configurations of compounds **4**, **10**, **11**, and **17** were unambiguously established by X-ray crystallographic analyses. The other compounds with the same skeleton were determined by comparison of NOESY and CD data with those of **4**, **10**, **11**, and **17**. Our findings have clarified the confusion in the literature about subtypes I and II of germacranolides. Compounds **13**, **17**, and **18** showed significant cytotoxicity against three human tumor cell lines. These findings are an important addition to the present knowledge on the structurally diverse and biologically important germacranolide family.

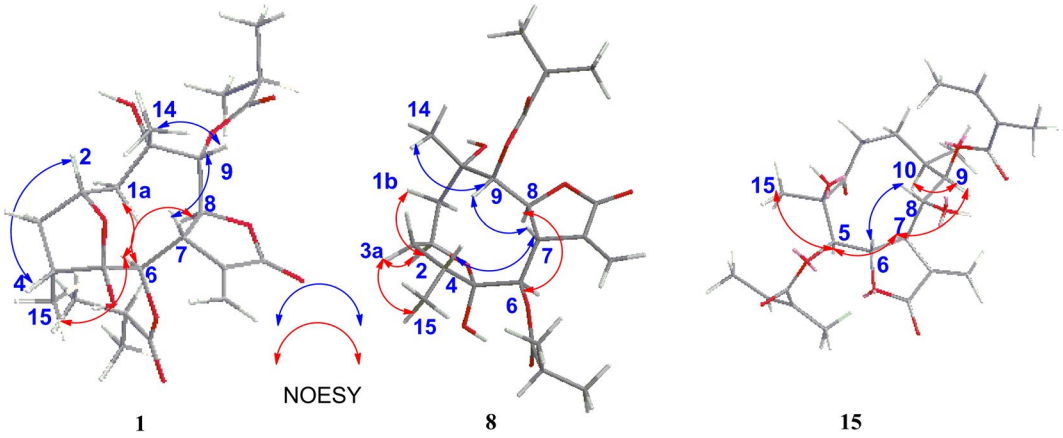

Figure 4. Key NOESY correlations of compounds **1**, **8**, and **15**.

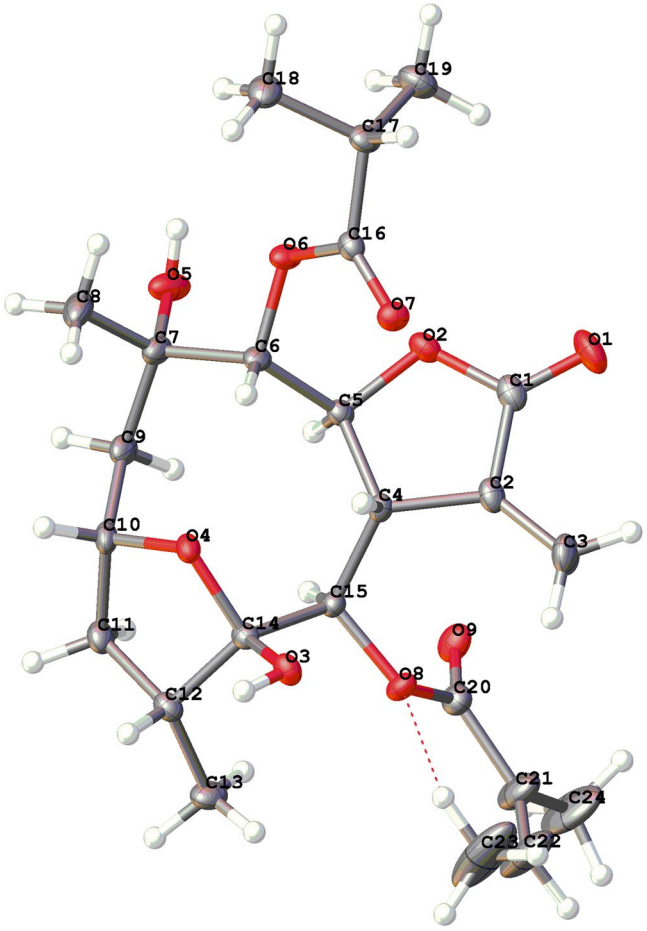

Figure 5. X-ray ORTEP drawing of **4**.

Methods

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter (Perkin-Elmer, Waltham, MA, USA) and UV spectra were recorded on Shimadzu UV-2501 PC (Shimadzu, Kyoto, Japan). IR data were recorded using a Shimadzu FTIR-8400S spectrophotometer (Shimadzu, Kyoto, Japan). ^{1}H and ^{13}C-NMR data were acquired with Bruker 600 and Bruker 500 instruments (Bruker, Rheinstetten, Germany) using the solvent signals (CD_3OD: δ_H 3.31/δ_C 49.0 ppm;) as references. HRESIMS data were acquired using Q-TOF analyzer in SYNAPT HDMS system (Waters, Milford, MA, USA). CD spectra were recorded on a JASCO J-815 Spectropolarimeter (Jasco, Tokyo, Japan). X-ray diffraction data were collected on the Agilent GEMINI^{TM}E instrument (CrysAlisPro software, Version 1.171.35.11; Agilent, Santa Clara, CA, USA). HPLC was

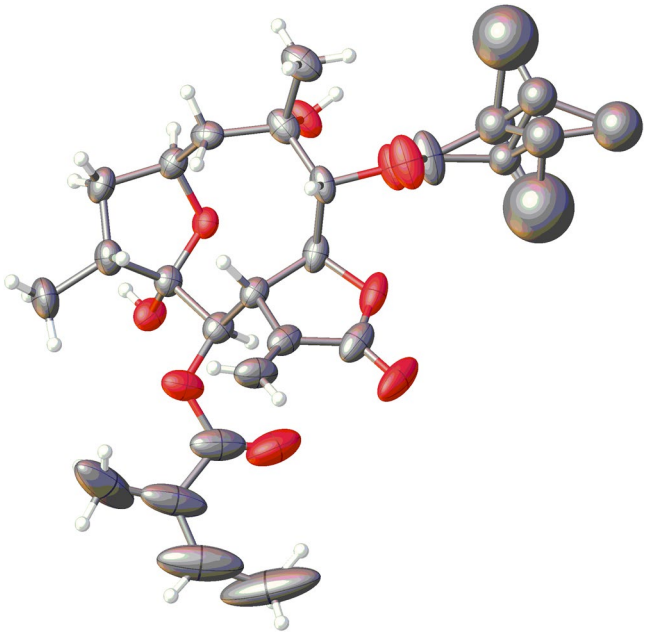

Figure 6. X-ray ORTEP drawing of the mixture crystals of **10** and **11**.

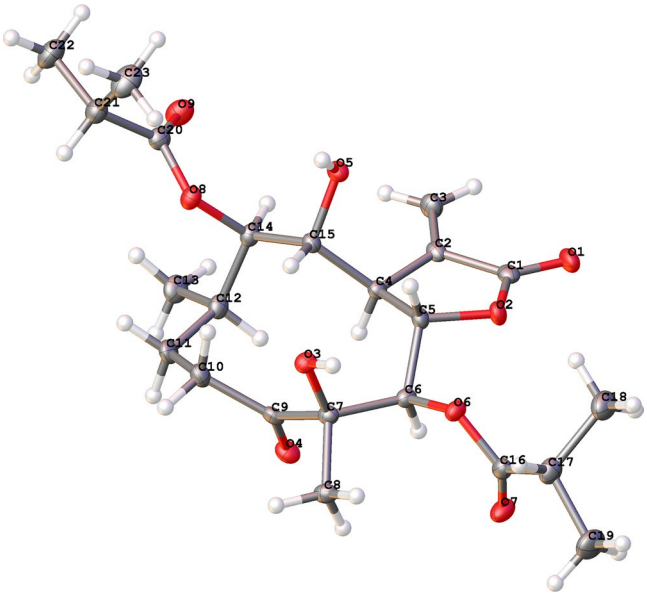

Figure 7. X-ray ORTEP drawing of **17**.

performed using Waters 2535 system (Waters, Milford, MA, USA) with the following components: preparative column, a Daisogel-C_{18}-100A (10 μm, 30 × 250 mm, ChuangXinTongHeng Sci.&Tech., Beijing, China) and a YMC-Pack ODS-A column (5 μm, 10 × 250 mm, YMC, Kyoto, Japan); and detector, Waters 2489 UV. Sephadex LH-20 (40–70 μm, Pharmacia Biotech AB, Uppsala, Sweden), silica gel (60–100, 100–200, and 200–300 mesh) and silica gel GF254 sheets (0.20–0.25 mm) (Qingdao Marine Chemical Plant, Qingdao, China) were used for column chromatography and TLC, respectively. TLC spots were visualized under UV light and by dipping into 5% H_2SO_4 in EtOH followed by heating.

Plant Material. The whole plants of *C. divaricatum* were collected from EnShi, Hubei province of China, in August of 2013. They were identified by Prof. Ben-Gang Zhang of Institute of Medicinal Plant Development. A voucher specimen (No. 20130828) was deposited in the National Compound Library of Traditional Chinese Medicines, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS & PUMC), China.

Extraction and Isolation. The air-dried plants (9 kg) were extracted three times (7 days each time) with EtOH–H_2O (95:5) at room temperature. The combined extract was concentrated under reduced pressure to furnish a dark brown residue (570 g), which was suspended in H_2O and partitioned in turn with petroleum ether (bp 60–90 °C), EtOAc, and *n*-BuOH. The EtOAc extract (207 g) was separated chromatographically on silica gel column (60–100 mesh, 16 × 20 cm) with a gradient mixture of CH_2Cl_2–MeOH (100:1, 60:1, 30:1, 15:1, and 6:1) as eluent. Five fractions were collected according to TLC analysis. Fraction A (CH_2Cl_2–MeOH, 100:1, 140 g) was separated by silica gel column chromatography (CC) (100–200 mesh, 16 × 20 cm) with petroleum ether–Aceton (50:1, 25:1, 20:1, 15:1, 12:1, 10:1, 7:1, 5:1, 3:1, and 1:1) as eluent to give fractions A_1–A_{11}. Fraction A_{10} (petroleum ether–Aceton, 3:1, 40 g) was separated by Sephadex LH-20 CC (5 × 200 cm, MeOH) to give Fr.$A_{10}S_1$–Fr.$A_{10}S_3$. Fraction $A_{10}S_2$ (20 g) was then subjected to MCI gel CC (6 × 50 cm) with a gradient mixture of MeOH–H_2O (60:40, 80:20, and 100:0, 4000 mL each) to give three fractions (Fr.$A_{10}S_2M_1$–Fr.$A_{10}S_2M_3$).

Fraction $A_{10}S_2M_2$ (MeOH–H_2O, 80:20, 3 g) was purified using preparative HPLC (Daisogel–C_{18}–100 A, 10μm; 250 × 30 mm; 20 mL/min, 60% MeOH in H_2O) to yield **1** (30 mg). Fraction $A_{10}S_2M_2$ (13 g) was further separated chromatographically on silica gel column (200–300 mesh, 5 × 50 cm) with a gradient mixture of CH_2Cl_2– MeOH (150:1, 100:1, 50:1, and 20:1) as eluent, and a total of 86 fractions (Fr.$A_{10}S_2M_2$-1–86, 200 mL each) were collected. Fraction $A_{10}S_2M_2$-56–60 were recrysted with CH_2Cl_2–MeOH (10:1) to yield **17** (200 mg). Fraction $A_{10}S_2M_2$-70 (100 mg) was purified using semipreparative HPLC (YMC–Pack ODS–A column; 5μm; 250 × 10 mm; 2 mL/min, 50% MeOH in H_2O) to yield **13** (20 mg) and **14** (30 mg). Fraction $A_{10}S_2M_2$-69 (100 mg) was purified using semipreparative HPLC with MeOH–H_2O (50:50) to yield **10** (10 mg), **11** (9 mg), and a mixture of **9** and **12** (25 mg). The mixture of **9** and **12** (25 mg) was further purified using semipreparative HPLC (40–80% MeCN in H_2O for 40 min) to yield **9** (4.5 mg) and **12** (6.2 mg). Fraction $A_{10}S_2M_2$-15–19 (140 mg) were purified using semipreparative HPLC (40–60% MeOH in H_2O for 20 min, and followed by 60–80% for 20 min) to yield **6** (10 mg) and **7** (50 mg). Fraction $A_{10}S_2M_2$-20–24 (2 g) were separated by preparative HPLC (65% MeOH in H_2O) and semipreparative HPLC (60% MeOH in H_2O for 10 min, and followed by 60–90% for 25 min; 40–85% MeCN in H_2O for 40 min) to yield **5** (6.8 mg), **3** (4 mg), **15** (5 mg), and **18** (12 mg). Fraction $A_{10}S_2M_2$-34–50 (1.5 g) were separated by preparative HPLC (70% MeOH in H_2O) and semipreparative HPLC (52–75% MeOH in H_2O for 25 min, and followed by 75–95% for 10 min) to yield **4** (50 mg). Fraction $A_{10}S_2M_2$-74–79 (140 mg) were purified using semipreparative HPLC (60–80% MeOH in H_2O for 25 min, and followed by 80–90% for 20 min; 30–70% MeCN in H_2O for 40 min) and to yield **8** (4 mg) and **19** (35 mg).

Fraction A_9 (petroleum ether-Aceton, 5:1, 30 g) was separated by Sephadex LH-20 CC (5 × 200 cm, MeOH) to give Fr.A_9S_1–Fr.A_9S_3. Fraction A_9S_2 (20 g) was then subjected to MCI gel CC (6 × 50 cm) with a gradient mixture of MeOH–H_2O (60:40, 80:20, and 100:0, 4000 mL each) to give three fractions (Fr.$A_9S_2M_1$–Fr.$A_9S_2M_3$). Fraction $A_9S_2M_2$ (10 g) was further separated chromatographically on silica gel column (100–200 mesh, 5 × 50 cm)with a gradient mixture of petroleum ether–Aceton (10:1, 7:1, 5:1, 3.5:1, 2:1, and 1:1) as eluent, and a total of 200 fractions (Fr.$A_9S_2M_2$-1–200, 50 mL each) were collected. Fraction $A_9S_2M_2$-113–123 (1 g) were separated by preparative HPLC (65% MeOH in H_2O) and semipreparative HPLC (68% MeOH in H_2O for 50 min; 40–80% MeCN in H_2O for 40 min) to yield **2** (4.6 mg). Fraction $A_9S_2M_2$-107–112 (2.5 g) were separated by silica gel column chromatography (CC) (200–300 mesh, 5 × 40 cm) with CH_2Cl_2–MeOH (150:1, 75:1, 30:1, and 15:1) as eluent to give Fr. $A_9S_2M_2$-107-112-A_1–Fr. $A_9S_2M_2$-107-112-A_8. Fraction $A_9S_2M_2$-107–112–A_3 (CH_2Cl_2–MeOH, 75: 1, 500 mg) was further purified using semipreparative HPLC(65–90% MeOH in H_2O for 40 min; 40–80% MeCN in H_2O for 40 min) to yield **16** (10 mg) and **20** (10 mg).

X-ray Crystal Structure Analysis. X-ray diffraction data were collected on the Agilent GEMINI^{TM}E instrument (CrysAlisPro software, Version 1.171.35.11), with enhanced Cu Kα radiation (λ = 1.54184 Å). The structure was solved by direct methods and refined by full-matrix least-squares techniques (SHELXL-97). All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were located by geometrical calculations and from positions in the electron density maps. Crystallographic data (excluding structure factors) for **4**, **10**, **11**, and **17** in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition numbers CCDC 1407813, 1407814, and 1407812). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 12 23336033 or e-mail: deposit@ccdc.cam.ac.uk).

A colorless orthorhombic crystal (0.58 × 0.48 × 0.45 mm) of **4** was obtained from MeOH. Crystal data were $C_{24}H_{34}O_9$, $M = 466.51$, $T = 99.5$ K, orthorhombic, space group $P2_12_12_1$, a = 9.05085(16) Å, b = 14.4036(3) Å, c = 19.1015(3) Å, $\alpha = 90.00°$, $\beta = 90.00°$, $\gamma = 90.00°$, $V = 2490.16(8)$ Å^3, $Z = 4$, $\rho = 1.244$ mg/mm^3, μ(Cu Kα) = 0.790 mm^{-1}, measured reflections = 8581, unique reflections = 4706 ($R_{int} = 0.0189$), largest difference peak/hole = 0.247/ −0.197 e Å^{-3}, and flack parameter = −0.10(12). The final R indexes [$I > 2\sigma(I)$] were $R_1 = 0.0338$, and $wR_2 = 0.0884$. The final R indexes (all data) were $R_1 = 0.0345$, and $wR_2 = 0.0890$. The goodness of fit on F^2 was 1.055.

After trying several solvent systems, the mixture trigonal crystal (0.50 × 0.20 × 0.20 mm) of **10** and **11** (1:1) was from MeOH. Main parameters: $C_{25.487355}H_{27}O_{10.25}$, $M = 497.32$, $T = 103.1$ K, trigonal, space group $P3_22_1$, a = 18.3947(4) Å, b = 18.3947(4) Å, c = 28.7027(5) Å, $\alpha = 90.00°$, $\beta = 90.00°$, $\gamma = 120.00°$, $V = 8410.8(3)$ Å^3, $Z = 12$, $\rho = 1.178$ mg/mm^3, μ(Cu Kα) = 0.774 mm^{-1}, measured reflections = 56790, unique reflections = 10785 ($R_{int} = 0.0359$), largest difference peak/hole = 0.857/−0.560 e Å^{-3}, and flack parameter = −0.0(2). The final R indexes [$I > 2\sigma(I)$] were $R_1 = 0.0668$, and $wR_2 = 0.1781$. The final R indexes (all data) were $R_1 = 0.0737$, and $wR_2 = 0.1857$. The goodness of fit on F^2 was 1.027.

A colorless monoclinic crystal (0.55 × 0.40 × 0.36 mm) of **17** was grown from CH_2Cl_2-MeOH (20:1). Crystal data: $C_{23}H_{34}O_9$, $M = 454.50$, $T = 101.0$ K, monoclinic, space group $P2_1$, a = 13.5135(4) Å, b = 9.5039(2) Å, c = 18.8280(6) Å, $\alpha = 90.00°$, $\beta = 105.051(3)°$, $\gamma = 90.00°$, $V = 2335.13(11)$ Å^3, $Z = 4$, $\rho = 1.293$ mg/mm^3, μ(Cu Kα) = 0.827 mm^{-1}, measured reflections = 16917, unique reflections = 8859 ($R_{int} = 0.0244$), largest

compounds	IC_{50} (μM)			
	HeLa	LoVo	BGC-823	MCF-7
13	7.60 ± 0.54	7.81 ± 0.25	12.68 ± 0.42	>50
17	5.76 ± 0.74	8.00 ± 0.20	13.68 ± 0.63	>50
18	4.72 ± 0.13	7.31 ± 0.21	11.67 ± 0.25	>50
cis-platin	7.90 ± 0.23	13.03 ± 1.49	15.34 ± 0.35	16.38 ± 1.41

Table 3. Cytotoxicity of Compounds **13**, **17** and **18**. Values were mean ± SD. *Cis*-platin, positive control. Cell lines: HeLa: cervical cancer, LoVo: colon cancer, BGC-823: stomach cancer, and MCF-7: breast cancer.

difference peak/hole = 0.759/−0.445 e $Å^{-3}$, and flack parameter = −0.00(11). The final R indexes [$I > 2\sigma(I)$] were $R_1 = 0.0390$, and $wR_2 = 0.1006$. The final R indexes (all data) were $R_1 = 0.0397$, and $wR_2 = 0.1012$. The goodness of fit on F^2 was 1.024.

Cytotoxicity Assays. The assay was run in triplicate. In a 96-well plate, each well was plated with 2×10^4 cells. After cell attachment overnight, the medium was removed, and each well was treated with 100 μL of medium containing 0.1% DMSO or different concentrations of the test compounds and the positive control *cis*-platin. The plate was incubated for 4 days at 37 °C in a humidified, 5% CO_2 atmosphere. Cytotoxicity was determined using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay[29]. After addition of 10 μL MTT solution (5 mg/mL), cells were incubated at 37 °C for 4 h. After adding 150 μL DMSO, cells were shaken to mix thoroughly. The absorbance of each well was measured at 540 nm in a Multiscan photometer. The IC_{50} values were calculated by Origin software and listed in Table 3.

References

1. Mabberley, D. J. *Mabberley's Plant Book* (3.ed), 154 (Cambridge University Press: Cambridge, 2008).
2. Zhang, J. P. *et al.* The genus *carpesium*: a review of its ethnopharmacology, phytochemistry and pharmacology. *J. Ethnopharmacol.* **163**, 173–191 (2015).
3. Editorial board of Chinese Materia Medica. Chinese Materia Medica, Vol. 21 (eds Hu, X. M. *et al.*) 7761 (Shanghai Science & Technology Press, Shanghai, 1999).
4. Kim, E. J. *et al.* Suppression by a sesquiterpene lactone from *Carpesium divaricatum* of inducible nitric oxide synthase by inhibiting nuclear factor-κB activation. *Biochem. Pharmacol.* **61**, 903–910 (2001).
5. Zee, O. P. *et al.* A new cytotoxic acyclic diterpene from *Carpesium divaricatum*. *Arch. Pharm. Res.* **22**, 225–227 (1999).
6. Zee, O. P., Kim, D. K. & Lee, K. R. Thymol derivatives from *Carpesium divaricatum*. *Arch. Pharm. Res.* **21**, 618–620 (1998).
7. Chung, I. M. *et al.* Antiplasmodial activity of isolated compounds from *Carpesium divaricatum*. *Phytother. Res.* **24**, 451–453 (2010).
8. Maruyama, M. Sesquiterpene lactones from *Carpesium divaricatum*. *Phytochemistry.* **29**, 547–550 (1990).
9. Kim, D. K., Lee, K. R. & Zee, O. P. Sesquiterpene lactones from *Carpesium divaricatum*. *Phytochemistry.* **46**, 1245–1247 (1997).
10. Kim, D. K. *et al.* Four new cytotoxic germacranolides from *Carpesium divaricatum*. *J. Nat. Prod.* **60**, 1199–1202 (1997).
11. Xie, W. D. *et al.* Sesquiterpenoids from *Carpesium divaricatum* and their cytotoxic activity. *Fitoterapia.* **83**, 1351–1355 (2012).
12. Zhang, T. *et al.* New highly oxygenated germacranolides from *Carpesium divaricatum* and their cytotoxic activity. *Sci.Rep.* **6**, 27237, https://doi.org/10.1038/srep27237 (2016).
13. Moon, H. I. Antiplasmodial activity of ineupatorolides A from *Carpesium rosulatum*. *Parasitol Res.* **100**, 1147–1149 (2007).
14. Liu, Q. X. *et al.* Isolation, structure elucidation, and absolute configuration of highly oxygenated germacranolides from *Carpesium cernuum*. *J. Nat. Prod.* **10**, 2479–2486 (2016).
15. Baruah, R. N., Sharma, R. P. & Thyagarajan, G. Unusual germacranolides from *Inula eupatorioides*. *J. Org. Chem.* **45**, 4838–4843 (1980).
16. Baruah, N. C. *et al.* Germacranolides of *Inula eupatorioides*. 2. Absolute configuration of the ineupatorolides. *J. Org. Chem.* **47**, 137–140 (1982).
17. Goswami, A. C. *et al.* Germacranolides from *Inula cappa*. *Phytochemistry.* **23**, 367–372 (1984).
18. Lee, H. J. *et al.* A germacranolide sesquiterpene lactone suppressed inducible nitric oxide synthase by downregulating NF-κB activity. *Can. J. Physiol. Pharmacol.* **89**, 232–237 (2011).
19. Moon, H. I. & Zee, O. Antiproliferative effect from sesquiterpene lactones of *Carpesium rosulatum* MlQ consumed in South Korea on the five human cancer cell Lines. *Rec. Nat. Prod.* **4**, 3149–3155 (2010).
20. Moon, H. I. & Zee, O. Sesquiterpene lactones from *Carpesium rosulatum* with potential cytotoxicity against five human cancer cell lines. *Hum. Exp. Toxicol.* **30**, 1083–1087 (2010).
21. Wang, F. Y. *et al.* Sesquiterpene lactones from *Inula cappa*. *Phytochem. Lett.* **5**, 639–642 (2012).
22. Gao, X., Lin, C. J. & Jia, Z. J. Cytotoxic germacranolides and acyclic diterpenoides from the seeds of *Carpesium triste*. *J. Nat. Prod.* **70**, 830–834 (2007).
23. Bohlmann, F., Singh, P. & Jakupovic, J. Further ineupatorolide-like germacranolides from *Inula cuspidata*. *Phytochemistry.* **21**, 157–160 (1982).
24. Lin, Y. L. & Ou, J. C. Napalolides A–D, four new sesquiterpene lactones from *Carpesium nepalense*. *J. Nat. Prod.* **59**, 991–993 (1996).
25. Kim, M. R. *et al.* Sesquiterpene lactones from *Carpesium triste* var. *manshuricum*. *Phytochemistry.* **52**, 113–115 (1999).
26. Kim, M. R. *et al.* Cytotoxic germacranolide sesquiterpene lactones from *Carpesium triste* var. *manshuricum*. *Arch. Pharm. Res.* **30**, 556–560 (2007).
27. Liang, Q. L., Jiang, J. H. & Min, Z. D. A germacrane sesquiterpenoid from *Vernonia patula*. *Chin. J. Nat. Med.* **8**, 104–106 (2010).
28. Gonzalez, A. G. *et al.* Germacranolides from *Allagopappus viscosissimus*. *Phytochemistry.* **31**, 330–331 (1992).
29. Wang, B. J. *et al.* Free radical scavenging and apoptotic effects of Cordyceps sinensis fractionated by supercritical carbon dioxide. *Food Chem. Toxicol.* **43**, 543–552 (2005).

Acknowledgements

This work was financially supported by the CAMS Innovation Fund for Medical Sciences (CIFMS, 2016-I2M-1-010), the Chinese National S&T Special Project on Major New Drug Innovation (2017ZX09301059), and the National Key Research and Development Program of China (2017YFD0201400-2).

Author Contributions

Zhong-Mei Zou designed the study; Tao Zhang performed the experiments with the help of Jia-Huan Chen, Jin-Guang Si, Gang Ding, Qiu-Bo Zhang, Hong-Wu Zhang, and Hong-Mei Jia. The manuscript was prepared by Tao Zhang, and Zhong-Mei Zou. All authors discussed the results and their interpretation and commented on the manuscript at all stages.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-30782-2.

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018