Terpenoids and Related Compounds - Structure, Synthesis, and Biological Activity-Original Article

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Bisabolane, Oplopane, and Lignan Constituents of *Cremanthodium campanulatum* Collected in China

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Abstract

The chemical constituents in the roots of 3 *Cremanthodium campanulatum* Diels (Asteraceae) samples collected in Yunnan and Sichuan Provinces, China, were chemically investigated. A total of 8 bisabolane-type sesquiterpenoids, 2 of which were new, and a new oplopane-type sesquiterpenoid, along with a new lignan were isolated and their structures elucidated based on spectroscopic analyses. This is the first report on the chemical study of *C. campanulatum*.

Keywords

Cremanthodium campanulatum, Asteraceae, sesquiterpenoid, bisabolane-type, oplopane-type, lignan

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Plants in the Hengduan Mountains area can provide good materials for the study of plant diversity and evolution. We have been studying the inter- and intraspecific diversity of *Ligularia* species in this area using a combination of chemical and genetic analyses and have demonstrated various modes of diversity in many species.[1,2] The genus *Cremanthodium* (Asteraceae) is taxonomically very close to *Ligularia*, growing on high mountains (usually more than 4000 m in altitude) of the Tibetan plateau and adjacent areas.[3] Most *Cremanthodium* species are small in size[4,5] and it is challenging to collect sufficient amounts for chemical analysis. Hence, chemical studies have been limited to a few species, for example, *Cremanthodium ellisii,*[6-10] *Cremanthodium discoideum*,[11,12] *Cremanthodium potaninii*,[13] and *Cremanthodium pleurocaule.*[14] Bisabolane- and oplopane-type sesquiterpenoids, triterpenoids, and other compounds have been isolated from these species. We have investigated chemical constituents of *Cremanthodium helianthus*,[15] *Cremanthodium stenactinium*,[16] *Cremanthodium rhodocephalum*,[17] and *Cremanthodium lineare*,[18] and bisabolane, oplopane, and eremophilane sesquiterpenoids have been isolated. These results suggested that the root chemicals in *Cremanthodium* are close to those in *Ligularia*. As part of our ongoing studies on the interspecific diversity of *Cremanthodium* species, we focused on *Cremanthodium campanulatum* Diels. There have been no reports on the chemical composition of this plant. We report here that *C. campanulatum* growing in Yunnan and Sichuan Provinces produces highly oxygenated bisabolane sesquiterpenoids bearing an epoxide in the cyclohexane ring as major constituents.

The roots of 3 samples collected in Yunnan and Sichuan Provinces (Table 1) were dried and extracted with EtOAc. The extracts were subjected to silica gel column chromatography and high performance liquid chromatography (HPLC) to isolate 2 new, **1** and **2**, and 6 known, **3** to **8**, bisabolane-type sesquiterpenoids, as well as 1 new oplopane-type sesquiterpenoid **9** and a new lignan **10** (Figure 1).

The molecular formula of compound **1** was determined to be $C_{30}H_{40}O_9$ on the basis of high resolution mass spectrometry (HRMS) data [*m/z* 545.2744 $(M+H^+)$ Δ −0.6 mmu]. The infrared (IR) spectrum showed an absorption band at 1718 cm^{-1} for carbonyl group(s). The presence of 3 angeloyloxy (OAng) groups was revealed by ^1H [δ_H 5.73, 5.68, and 5.69 (each qq) for H-3', H-3'', and H-3'''] and ^{13}C nuclear magnetic resonance (NMR) spectra (Table 2). Their presence was supported by the appearance of a series of fragment ion peaks at

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Table 1. Localities of 3 Samples and Isolated Compounds.

Sample no.	Specimen no.	Locality	Altitude (m)	Bisabolanes						Others	
1	2007-56	[a]	4400	**4**	**5**					**10***	
2	2007-120	Daxueshan[b]	4300	**1***	**2***	**4**	**5**			**9***	**10***
3	2008-12	Hongshan[c]	4500	**1***	**3**	**5**	**6**	**7**	**8**	**10***	

Asterisks denote new compounds and underlines the major constituents.

[a] Border between Jiulong and Kangding counties, Sichuan.

[b] Xiangcheng County, Sichuan.

[c] Shangrila County, Yunnan.

m/z 445 (M–AngOH+H$^+$), 345 (M–2×AngOH+H$^+$), and 245 (M–3×AngOH+H$^+$) in the mass spectrometry (MS) spectrum. Fifteen of the 30 carbons were assigned to 3 angeloyloxy moieties. The connectivity of the remaining 15 carbons was investigated using analyses of correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) spectra, and a highly oxygenated cyclohexane ring was deduced (Figure 2). The ring contained 1 nonoxygenated methine carbon (H-6, δ_H 3.25; C-6, δ_C 46.2) through which HMBC correlations from exomethylene protons (H$_2$-14, δ_H 5.30 and 5.34) were observed. These exomethylene protons correlated with an oxymethine group (H-8, δ_H 5.78; C-8, δ_C 73.6), which was in turn attached to a methylene group (H$_2$-9, δ_H 1.90 and 1.96; C-9, δ_C 34.3) followed by an oxymethine group (H-10, δ_H 2.73; C-10, δ_C 60.3). The oxymethine proton (H-10) correlated with a carbon (C-11, δ_C 57.5), which was a part of a 2-methylpropylene oxide moiety. On the basis of these observations, it was concluded that compound **1** had a highly oxygenated bisabolane type skeleton, including an exomethylene moiety as well as 3 angeloyloxy groups.

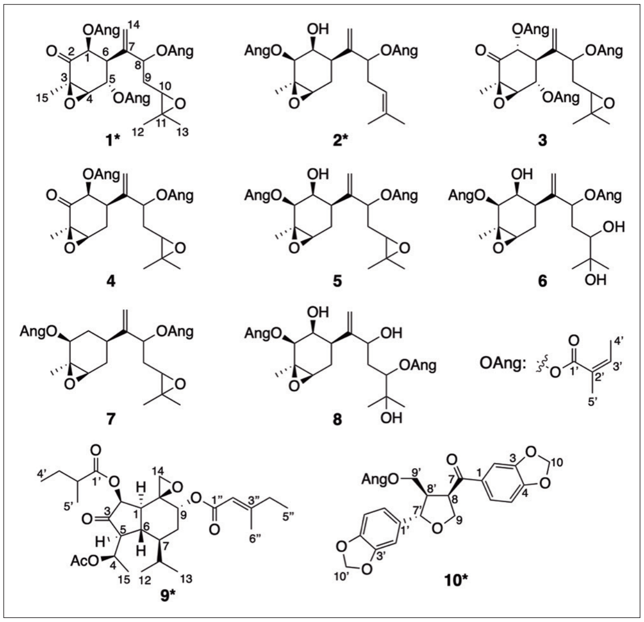

Figure 1. Compounds isolated from *Cremanthodium campanulatum* (asterisks denote new compounds).

Compound **1** had 11 degrees of unsaturation based on its molecular formula. Nine degrees of unsaturation were assigned to 3 angeloyl groups, a carbonyl group at C-2, an exomethylene

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2** (in C_6D_6).

	1		**2**
Position	δ_H	δ_C	δ_H
1	5.66 (d, 4.2)	73.5	4.30 (dd, 11.0, 3.9)
2	-	198.8	5.15 (d, 3.9)
3	-	58.3	-
4	3.05 (d, 1.2)	63.5	2.58 (d, 5.1)
5	6.23 (dd, 6.4, 1.2)	67.6	1.81 (m)
	-		1.92 (m)
6	3.25 (dd, 6.4, 4.2)	46.2	2.13 (dd, 12.4, 6.2)
7	-	142.6	-
8	5.78 (dd, 7.1, 5.7)	73.6	5.36 (t, 6.9)
9	1.96 (m)	34.3	2.50 (dt, 14.3, 6.9)
	1.90 (m)		2.39 (dt, 14.3, 6.9)
10	2.73 (t, 6.0)	60.3	5.20 (br t, 6.9)
11	-	57.5	-
12	1.09 (s)	24.5	1.64 (br s)
13	1.14 (s)	18.8	1.57 (br s)
14	5.34 (s)	116.8	5.45 (s)
	5.33 (s)		5.32 (s)
15	1.17 (s)	14.6	1.06 (s)
1'	-	166.3	-
	-	166.1	-
	-	166.1	-
2'	-	127.0	-
	-	126.9	-
	-	126.9	-
3'	5.73 (qq, 7.6, 1.6)	140.6	5.73 (qq, 7.2, 1.6)
	5.69 (qq, 7.6, 1.6)	140.5	5.71 (qq, 7.2, 1.6)
	5.68 (qq, 7.6, 1.6)	139.4	-
4'	1.97 (dq, 7.6, 1.6)	15.9	2.02 (dq, 7.2, 1.6)
	1.96 (dq, 7.6, 1.6)	15.9	2.00 (dq, 7.2, 1.6)
	1.93 (dq, 7.6, 1.6)	15.8	-
5'	1.89 (quint, 1.6)	20.7	1.93 (quint, 1.6)
	1.85 (quint, 1.6)	20.6	1.92 (quint, 1.6)
	1.85 (quint, 1.6)	20.5	-
OH	-	-	3.41 (d, 11.0)

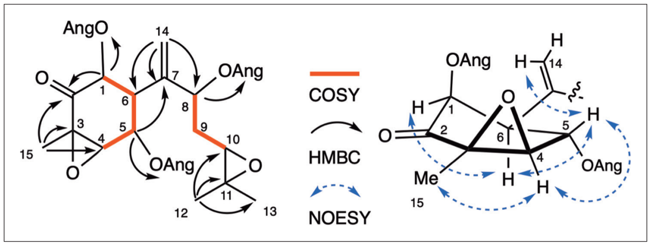

Figure 2. Major 2D correlations detected for compound **1**.

group, and a 6-membered ring. The remaining 2 degrees of unsaturation were attributed to 2 epoxides, C-3/C-4 and C-10/C-11, because these carbons resonated at slightly higher fields [δ_C 58.3 (C-3), 63.5 (C-4), 60.3 (C-10), and 57.5 (C-11)] than typical oxygenated carbons. The 3 angeloyloxy groups were deduced to be attached to C-1, C-5, and C-8, because H-1, H-5, and H-8, respectively, correlated with ester carbonyl carbons in the HMBC (Figure 2). Thus, the planar structure of **1** was established.

The relative configuration of the cyclohexanone ring in **1** was deduced from the ^{1}H NMR coupling constants and the nuclear overhauser effect spectroscopy (NOESY) spectrum (Figure 2). The vicinal coupling constant $J_{1,6}$ (4.2 Hz) and NOE between H-1/H-6 indicated that H-1 and H-6 should be on the same side (Table 2). When H-6 is assumed to be α-oriented, H-1 should also be α. Because $J_{4,5}$ (1.2 Hz) is almost zero, the dihedral angle H-4/C-4/C-5/H-5 should be close to 90°. NOE between H-5/H-14 suggested that H-5 was β-oriented. Therefore, H-4 should be α, and the epoxide at C-3/C-4 was determined to be β. The configurations at C-8 and C-10 were not determined. Consequently, the structure of **1** was determined to be 1β,5α,8-trisangeloyloxy-3β,4β,10,11-bisepoxybisabol-7(14)-en-2-one.

The molecular formula of compound **2** was determined to be $C_{25}H_{36}O_6$ by HRMS. The molecular and fragment ion peaks were detected at *m/z* 432 (M^+), 333 ($M–AngOH+H^+$), and 233 ($M–2\times AngOH+H^+$), indicating the presence of 2 angeloyloxy groups. The ^{1}H NMR spectrum was similar to that of compound **1**. The difference was the presence of a proton resonating at δ_H 5.20 assignable to an olefinic proton in compound **2**. Exomethylene protons (δ_H 5.32, 5.45) were observed as well. Therefore, 6 degrees of unsaturation out of 8 were attributed to 2 angeloyloxy groups and 2 double bonds. The rest of 2 degrees of unsaturation was ascribed to 2 rings. COSY correlations revealed that this compound also had a bisabolane skeleton with angeloyloxy groups at C-2 (H-2, δ 5.15) and C-8 (H-8, δ 5.36), and a hydroxy group at C-1 (H-1, δ 4.30) (Figure 3). In difference NOE experiment, the enhancement of H-1 and H-2 signals was observed when H-6 was irradiated (Figure 3), indicating that a side chain attached to C-6, a hydroxy group at C-1, and an angeloyloxy group at C-2 were β-oriented. Because H-4 was doublet ($J_{4,5}\alpha$ = 5.1 Hz, $J_{4,5}\beta$ = 0 Hz), the dihedral angle H-4/C-4/C-5/H-5β should be 90°. Furthermore, the coupling constant of hydroxy proton (*J* = 11.0 Hz) suggested the presence of hydrogen bond between the 1-OH and the epoxide at C-3/C-4.[19] These data are in good agreement with those of compound **5**.[20] The structure was established to be 2β,8-bisangeloyloxy-3β,4β-epoxybisabola-7(14),10-dien-1β-ol.

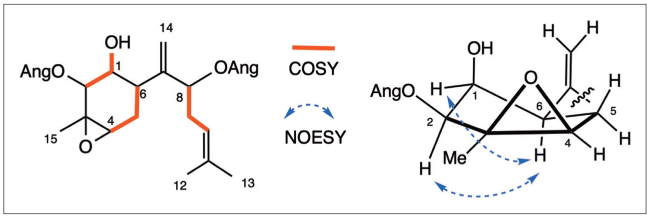

Figure 3. Major 2D correlations detected for compound **2**.

The absolute configuration of the 6-membered ring in bisabolanes was determined as depicted in the formula by the positive Cotton effect (θ: +13 000) at 298 nm in the electronic circular dichroism (ECD) spectrum of compound **3** (*vide infra*)[21] and in consideration of biosynthetic relationship with it.

The molecular formula of compound **9** was determined to be $C_{28}H_{42}O_8$ using HRMS and ^{13}C NMR data (Table 3). The IR spectrum showed carbonyl absorptions at 1756 and 1730 cm^{-1}, which was supported by ^{13}C NMR signals at δ 170.4, 174.8, and 207.8. The ^{1}H NMR spectrum indicated the presence of 8 methyls [δ 0.63 (d), 0.76 (t), 0.81 (t), 0.85 (d), 1.11 (d), 1.33 (d), 1.82 (s), and 2.16 (d)] and 3 oxymethine groups [δ 4.76 (t), 5.07 (d), and 5.24 (qd)] (Table 3). A pair of slightly downfield shifted protons at δ 2.75 and 2.94 (each d) were detected suggesting the presence of an epoxide. The COSY correlations H_3-15/H-4/H-5/H-6/H-1/H-2, H-6/H-7/H_2-8/H-9, and H_3-12/H-11/H_3-13 were observed (Figure 4). The HMBC correlations between the respective pairs of H-5 and C-3, H-6 and C-3, H-1 and C-10, H-14 and C-9/C-10, and H_3-12/H_3-13 and C-7/C-11 were detected indicating the oplopane skeleton as shown in Figure 4. The 2-methylbutanoyloxy group was deduced to be attached to C-2 (δ_C 71.5), because an HMBC correlation between H-2 and C-1" was observed. The positions of acetate and 3-methylpent-2-enoyloxy groups were distinguished by NOEs (*vide infra*).

Coupling constants $J_{1,6}$ (12.1 Hz), $J_{5,6}$ (10.1 Hz), and $J_{6,7}$ (10.1 Hz) and NOEs between H-1/H-7 and H-5/H-7 suggested that the ring junction for rings A and B was *trans*, and that H-1, H-5, and H-7 were all on the same side. The 2-methylbutanoyloxy group at C-2 was determined to be β-orientation by detecting the NOE between H-1 and H-2. The acetate group should be at the C-4 (δ_C 69.9) position, because the NOE between H-4 and the acetyl methyl group was detected. Therefore, the 3-methylpent-2-enoyloxy group was indicated to be attached at C-9 (δ_C 74.3). The proton at C-9 resonated at δ 4.76 as a triplet indicating the equatorial nature of H-9. The NOEs between H-9/(H-8α and H-8β) and H-9/H-14 (δ_H2.75)

Table 3. ^{1}H and ^{13}C NMR Data of Compounds **9** and **10** (in C_6D_6).

	9			**10**	
Position	δ_H	δ_C	Position	δ_H	δ_C
1	2.03 (dd, 12.1, 4.0)	43.7	1	-	132.8
2	5.07 (d, 4.0)	71.5	2	7.49 (d, 1.7)	108.2
3	-	207.8	3	-	148.5
4	5.24 (qd, 6.6, 3.7)	69.9	4	-	152.1
5	2.41 (dd, 10.1, 3.7)	55.9	5	6.42 (d, 8.2)	107.9
6	2.29 (dt, 12.1, 10.1)	41.9	6	7.20 (dd, 8.2, 1.7)	124.6
7	1.71 (m)	44.2	7	-	196.2
8	1.78 (m)	28.1	8	3.71 (dt, 9.0, 7.5)	47.4
	1.50 (m)		9	4.39 (dd, 8.8, 7.5)	70.5
9	4.76 (t, 3.0)	74.3		4.05 (dd, 8.8, 7.5)	
10	-	58.0	10	5.14 (d, 1.4)	101.7
11	2.45 (m)	27.7		5.14 (d, 1.4)	
12	0.63 (d, 6.8)	21.5	1'	-	136.2
13	0.85 (d, 6.8)	15.3	2'	7.02 (d, 1.6)	106.8
14	2.94 (d, 4.7)	51.5	3'	-	148.7
	2.75 (d, 4.7)		4'	-	147.7
15	1.33 (d, 6.6)	15.9	5'	6.63 (d, 7.9)	108.4
1'	-	165.1	6'	6.67 (dd, 7.9, 1.6)	120.0
2'	5.84 (sext, 1.2)	114.2	7'	4.87 (d, 7.0)	83.8
3'	-	163.2	8'	2.64 (m)	51.1
4'	1.82 (m)	33.8	9'	4.31 (dd, 11.6, 7.5)	61.9
	1.82 (m)			4.14 (dd, 11.6, 6.0)	
5'	0.81 (t, 7.4)	11.8	10'	5.32 (d, 1.4)	101.0
6'	2.16 (d, 1.2)	18.9		5.29 (d, 1.4)	
1"	-	174.8	1"	-	166.6
2"	2.25 (m)	41.4	2"	-	127.4
3"	1.61 (dquint, 13.7, 7.4)	27.1	3"	5.57 (qq, 7.2, 1.5)	138.8
	1.28 (m)		4"	1.83 (dq, 7.2, 1.5)	15.8
4"	0.76 (t, 7.4)	11.6	5"	1.59 (quint, 1.5)	20.5
5"	1.11 (d, 7.0)	16.5			
1'''	-	170.4			
2'''	1.82 (s)	20.9			

were detected. Therefore, the 3-methylpent-2-enoyloxy group at C-9 was determined to be in the α-orientation from these observations (Figure 4). The configuration at C-4 of other oplopane compounds had been reported to be *R* by X-ray analysis, and their NMR data were used to establish its stereogenic center.[21] The proton resonating at δ_H 5.24 (qd, J = 6.6 and 3.7 Hz, H-4) was in good agreement with the previously reported oplopanes suggesting that C-4 was in an *R* configuration.[21] The stereochemistry of an epoxide at C-10 was deduced to be as shown in Figure 4, because NOEs between H-14a (δ_H 2.94)/H-2 and H-14b (δ_H2.75)/H-9 were observed. If the configuration at C-10 was the opposite, the NOEs as described above would not be observed. Oplopane sesquiterpenoids reported so far have a double bond at C-10/C-14 and those having an epoxide here were quite rare.[22,23] The absolute configuration was established as depicted in the formula from the negative Cotton effect (θ: –6400) at 320 nm in its ECD spectrum (in EtOH).[21,24-27]

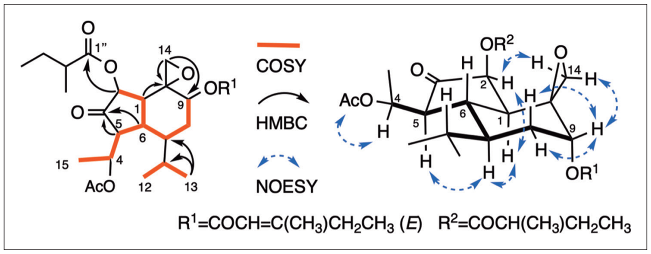

Figure 4. Major 2D correlations for compound **9**.

The spectroscopic data of compound **10**, $C_{25}H_{24}O_8$ (by HRMS), were very different from those of compounds **1** to **8**. The ^{1}H NMR spectrum suggested the presence of an

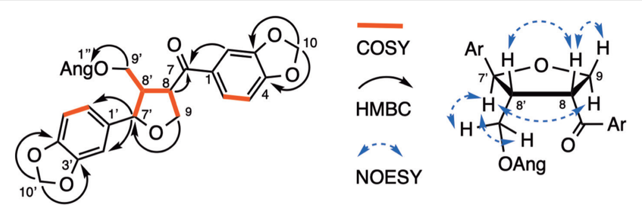

Figure 5. Major 2D correlations for compound **10**.

angeloyloxy group, 2 1,2,4-trisubstituted benzene rings, and 2 methylenedioxy groups [δ 5.14 (2H), 5.29, and 5.32 (each d)] (Table 3). The lignan skeleton was revealed by HMBC correlations between the respective pairs of H-8 and C-7, H-7' and C-2'/C-6', H-9 and C-7', and H-2 and C-7, as well as COSY correlations H-7'/H-8'/H-9', and H-8'/H-8/H-9 (Figure 5). The position of the angeloyloxy group was determined to be at C-9' by observing the HMBC correlation between H-9' and C-1''. Two methylenedioxy groups were also determined at C-3/C-4 and C-3'/C-4' by HMBC correlations between H-10 and C-3/C-4 and between H-10' and C-3'/C-4', respectively. The stereochemistry around a tetrahydrofuran moiety was determined as shown in Figure 5 by NOEs between H-8/H-8', H-8/H-9β, H-7'/H-9α, and H-7'/H-9'.

Other compounds, **3** to **8**, were identified by comparing their spectroscopic data with those in the previous reports (see the "Experimental" section). The composition in each sample is listed in Table 1. Bisabolane-type sequiterpenoids have been isolated from *Ligularia* and other genus.[1,2] Recently, Onuki et al and Hirota et al reported the isolation of compounds **5** and **7** from *Ligularia lankongensis*.[20,28] These compounds have angeloyloxy group(s) at C-1/C-5/C-8 position. Compounds **1** to **7** had an angeloyloxy group at the C-8 position. The absolute configuration of highly oxygenated bisabolane-type sesquiterpenoids with a carbonyl group at the C-2 position was determined previously from ECD data[29] and X-ray crystallography.[30] Kuroda et al recently established the absolute configurations at C-8 and C-10 in a side chain of some bisabolane compounds by total synthesis.[31,32] However, the absolute configuration at the C-8 position and the epoxide at C-10/C-11 in compounds **1** to **8** could not be determined using the spectroscopic methods because the requisite NMR data were not feasible to obtain as part of this study.

Finally, the cytotoxicities of compounds **1** to **8** against HeLa and HL-60 cell lines were evaluated (Table 4). Most of the compounds did not show significant activity against HeLa cells, whereas some were moderately active against HL-60 cells. Compound **2** exhibited potent activity against both cell types (IC_{50} 5.92 μM for HeLa and 1.51 μM for HL-60).

Table 4. Cytotoxicities of Compounds **1** to **8** [IC_{50} (μM)].

Compound	HeLa	HL-60
1	57.4	>100
2	5.92	1.51
3	74.5	26.6
4	>100	30.8
5	>100	45.4
6	>100	87.2
7	>100	38.6
8	>100	83.0

Experimental

General

Specific rotations and ECD spectra were measured on a JASCO P-1030 and a JASCO J-725 auto-recording polarimeter; IR spectra, on a SHIMADZU FT/IR-8400S spectrophotometer; ^{1}H and ^{13}C NMR spectra, on a Varian 500MR (500 and 125 MHz, respectively) spectrometer. Mass spectra, including high-resolution scans, were recorded on a JEOL JMS-700 MStation. Chemcopak Nucleosil 50-5 (4.6 × 250 mm) with a solvent system of hexane-ethyl acetate was used for HPLC (JASCO pump system). Silica gel BW-127ZH (100-270 mesh, Fuji Silysia) was used for column chromatography. Silica gel 60 F_{254} plates (Merck) were used for thin layer chromatography (TLC).

Plant Materials

Samples 1 to 3 were collected in August 2007 and 2009 at different locations in Sichuan and Yunnan Provinces (Table 1) and were identified by X.G., one of the authors. Voucher specimen numbers of samples 1 to 3 are 2007-56, 2007-120, and 2008-12, respectively (Kunming Institute of Botany).

Isolation of Compounds

The roots of each sample were cut into pieces and extracted with EtOAc. Compounds were separated using silica-gel column chromatography (hexane-EtOAc) and HPLC (hexane-EtOAc) to isolate each compound.

The roots of sample 1 (2007-56) (dry weight 1.8 g) produced an extract (32.2 mg). From this extract, compounds **4**[33,34] (0.5 mg), **5**[20] (1.2 mg), and **10** (0.4 mg) were isolated.

The roots of sample 2 (2007-120) (dry weight 3.4 g) produced an extract (50.5 mg). From this extract, compounds **1** (1.9 mg), **2** (0.6 mg), **4** (0.5 mg), **5** (2.4 mg), **9** (1.8 mg), and **10** (0.6 mg) were isolated.

The roots of sample 3 (2008-12) (dry weight 3.6 g) produced an extract (89.5 mg). From this extract, compounds **1** (1.9 mg), **3**[35] (0.7 mg), **5** (3.4 mg), **6**[36] (0.4 mg), **7**[28] (0.3 mg), **8**[37] (1.2 mg), and **10** (1.3 mg) were isolated.

1β,5α,8-Trisangeloyloxy-3β,4β,10,11-bisepoxybisabol-7(14)-en-2-one (1)

$[\alpha]_D^{21}$: –31.8 (*c* 0.20, EtOH).

Fourier transform infrared spectroscopy (FT-IR) (KBr): 1718, 1647 cm^{-1}.

MS chemical ionization (CI): *m/z* = 545 [$M+H^+$], 445, 375, 345, 245, 83 (100).

HRMS-CI: *m/z* 545.2744 [$M+H^+$], calcd for $C_{30}H_{41}O_9$: 545.2750.

CD [θ] (nm) (EtOH): +7400 (245), –9800 (228), +360 (220).

^1H and ^{13}C NMR data in Table 2.

2β,8-Bisangeloyloxy-3β,4β,10,11-bisepoxybisabol-7(14)-en-1β-ol (2)

Compound deteriorated before measuring the specific rotation and ^{13}C NMR.

FT-IR (KBr): 3491, 1713, 1647 cm^{-1}.

MS-CI *m/z* = 432 [M^+], 363, 333, 263, 233, 83 (100).

HRMS-CI: *m/z* 432.2502 [M^+], calcd for $C_{25}H_{36}O_6$: 432.2511.

^1H NMR data in Table 2.

4-Acetoxy-10,14-epoxy-2β-(2-methylbutanoyl)oxy-9α-(*E*-3-methylpent-2-enoyl)oxyoplopan-3-one (9)

$[\alpha]_D^{21}$: –57.4 (*c* 0.20, EtOH).

FT-IR (KBr): 1757, 1730, 1647 cm^{-1}.

MS (CI): *m/z* = 507 [$M+H^+$] (100), 447, 333, 231, 97.

HRMS-CI: *m/z* 507.2966 [$M+H^+$], calcd for $C_{28}H_{43}O_8$: 507.2958.

CD [θ] (nm) (EtOH): –6400 (320), +3300 (245), –5800 (230), +150 (215), –11 000 (206).

^1H and ^{13}C NMR data in Table 3.

9'-Angeloyloxy-3,4,3',4'-bismethylenedioxy-9,7'-epoxylignan-7-one (10)

$[\alpha]_D^{21}$: +27.7 (*c* 0.10, EtOH).

FT-IR (KBr): 3491, 1713, 987 cm^{-1}.

MS (CI): *m/z* = 453 [$M+H^+$], 435, 353 (100), 335, 203, 176, 149.

HRMS-CI: *m/z* 453.1554 [$M+H^+$], calcd for $C_{25}H_{25}O_8$: 453.1549.

^1H and ^{13}C NMR data in Table 3.

Cell Lines and Cell Culture

Human cervical cancer cell line HeLa and human promyelocytic leukemia HL-60 were obtained from the RIKEN BioResource Center (Tokyo, Japan). These cell lines were cultured in the minimum essential medium (MEM) (Gibco) supplemented with 10% fetal bovine serum (FBS) (v/v) (Nichirei Bioscience, Japan) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere of 5% CO_2 (v/v).

Cell Viability Assay

Cells were cultured in 96-well plates for 12 hours. The cells were then treated with compounds **1** to **8** at various concentrations for 48 hours. Then, 10 μL of Cell Counting Kit-8 (CCK-8, DOJINDO, Japan) solution was added to each well and incubated for another 2 hours. The absorbance was measured at 450 nm using a microplate reader (Bio Rad 680, United States). IC_{50} values were calculated from cell survival curves.

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Declaration of Conflicting Interests

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