

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

Natural Product Communications
 July 2019: 1–7
 © The Author(s) 2019
 Article reuse guidelines:
sagepub.com/journals-permissions
 DOI: 10.1177/1934578X19863582
journals.sagepub.com/home/npx



Yoshinori Saito¹ , Mayu Ichihara², Koji Takiguchi², Ayumi Ohsaki³, Yasuko Okamoto², Chiaki Kuroda⁴, Xun Gong⁵, and Motoo Tori²

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

Received: May 31st, 2019; Accepted: June 21st, 2019.

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 belianthus*,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 C₃₀H₄₀O₉ 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 ¹³C nuclear magnetic resonance (NMR) spectra (Table 2). Their presence was supported by the appearance of a series of fragment ion peaks at

¹Graduate School of Biomedical Sciences, Nagasaki University, Bunkyo-machi, Japan

²Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Japan

³Department of Chemistry, College of Humanities and Sciences, Nihon University, Setagaya, Tokyo, Japan

⁴Department of Chemistry, Rikkyo University, Toshima-ku, Tokyo, Japan

⁵Kunming Institute of Botany, Chinese Academy of Science, China

Corresponding Author:

Yoshinori Saito, Graduate School of Biomedical Sciences, Nagasaki University, Bunkyo-machi, Nagasaki 852-8521, Japan.
 Email: saiyoshi@nagasaki-u.ac.jp



Table 1. Localities of 3 Samples and Isolated Compounds.

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

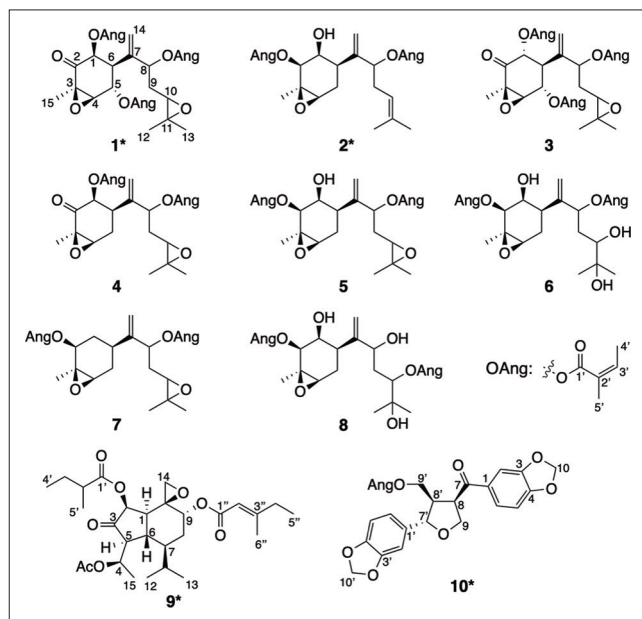
Asterisks denote new compounds and underlines the major constituents.

^aBorder between Jiulong and Kangding counties, Sichuan.

^bXiangcheng County, Sichuan.

^cShangrila County, Yunnan.

m/z 445 ($M-\text{AngOH}+\text{H}^+$), 345 ($M-2\times\text{AngOH}+\text{H}^+$), and 245 ($M-3\times\text{AngOH}+\text{H}^+$) 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. ¹H and ¹³C NMR Data of Compounds **1** and **2** (in C₆D₆).

Position	1		2
	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)

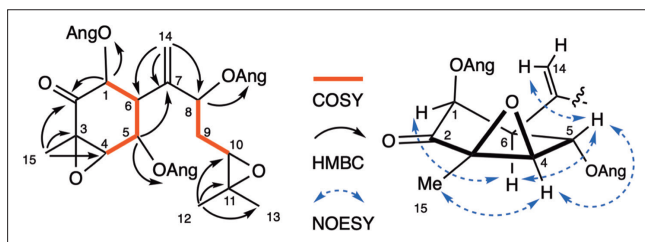


Figure 2. Major 2D correlations detected for compound 1.

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 ^1H 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 1 β ,5 α ,8-trisangeloyloxy-3 β ,4 β ,10,11-bisepoxybisabol-7(14)-en-2-one.

The molecular formula of compound **2** was determined to be $\text{C}_{25}\text{H}_{36}\text{O}_6$ by HRMS. The molecular and fragment ion peaks were detected at m/z 432 (M^+), 333 ($\text{M}-\text{AngOH}+\text{H}^+$), and 233 ($\text{M}-2\times\text{AngOH}+\text{H}^+$), indicating the presence of 2 angeloyloxy groups. The ^1H 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

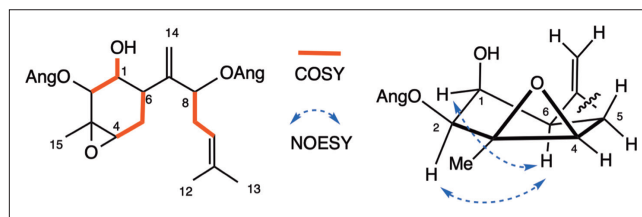


Figure 3. Major 2D correlations detected for compound 2.

angle H-4/C-4/C-5/H-5 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 2 β ,8-bisangeloyloxy-3 β ,4 β -epoxybisabol-7(14),10-dien-1 β -ol.

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.

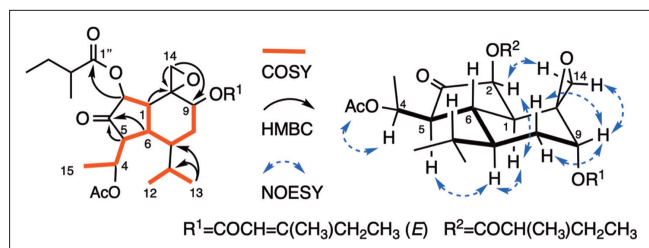
The molecular formula of compound **9** was determined to be $\text{C}_{28}\text{H}_{42}\text{O}_8$ using HRMS and ^{13}C NMR data (Table 3). The IR spectrum showed carbonyl absorptions at 1756 and 1730 cm^{-1} , which was supported by ^{13}C NMR signals at δ 170.4, 174.8, and 207.8. The ^1H NMR spectrum 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 H₃-15/H-4/H-5/H-6/H-1/H-2, H-6/H-7/H₂-8/H-9, and H₃-12/H-11/H₃-13 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 H₃-12/H₃-13 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-8 α and H-8 β) and H-9/H-14 (δ_{H} 2.75)

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

Position	9		Position	10	
	δ_{H}	δ_{C}		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 (dq, 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-enyloxy 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

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

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_{H} 2.75)/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}

The spectroscopic data of compound **10**, $\text{C}_{25}\text{H}_{24}\text{O}_8$ (by HRMS), were very different from those of compounds **1** to **8**. The ^1H NMR spectrum suggested the presence of an

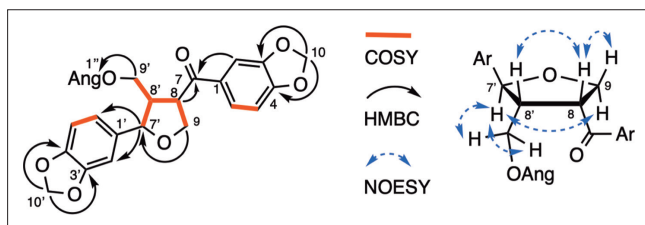


Figure 5. Major 2D correlations for compound **10**.

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-9 β , H-7'/H-9 α , 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 sesquiterpenoids 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 data²⁹ 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 μ M for HeLa and 1.51 μ M for HL-60).

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

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; ¹H and ¹³C 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 \times 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 F₂₅₄ 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.

1 β ,5 α ,8-Trisangeloyloxy-3 β ,4 β ,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 $[\text{M}+\text{H}^+]$, 445, 375, 345, 245, 83 (100).

HRMS-CI: m/z 545.2744 $[\text{M}+\text{H}^+]$, calcd for $\text{C}_{30}\text{H}_{41}\text{O}_9$: 545.2750.

CD $[\theta]$ (nm) (EtOH): +7400 (245), -9800 (228), +360 (220).

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

2 β ,8-Bisangeloyloxy-3 β ,4 β ,10,11-bisepoxybisabol-7(14)-en-1 β -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 $[\text{M}^+]$, 363, 333, 263, 233, 83 (100).

HRMS-CI: m/z 432.2502 $[\text{M}^+]$, calcd for $\text{C}_{25}\text{H}_{36}\text{O}_6$: 432.2511.

^1H NMR data in Table 2.

4-Acetoxy-10,14-epoxy-2 β -(2-methylbutanoyl)oxy-9 α -(*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 $[\text{M}+\text{H}^+]$ (100), 447, 333, 231, 97.

HRMS-CI: m/z 507.2966 $[\text{M}+\text{H}^+]$, calcd for $\text{C}_{28}\text{H}_{43}\text{O}_8$: 507.2958.

CD $[\theta]$ (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'-epoxy lignan-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 $[\text{M}+\text{H}^+]$, 435, 353 (100), 335, 203, 176, 149.

HRMS-CI: m/z 453.1554 $[\text{M}+\text{H}^+]$, calcd for $\text{C}_{25}\text{H}_{25}\text{O}_8$: 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.

Acknowledgments

We thank Prof Ryo Hanai, Rikkyo University, and Dr Takayuki Kawahara, Forestry and Forest Products Research Institute, for their helpful suggestions.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was partly supported by a Grant-in-Aid for Scientific Research from JSPS (21404009, 25303010 and No. 16K18897), the Japan-China Scientific Cooperation Program from JSPS and NSFC, and the Strategic Research Foundation Grant-aided Projects for Private Universities from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

ORCID ID

Yoshinori Saito  <https://orcid.org/0000-0002-3587-9743>

References

1. Kuroda C, Hanai R, Nagano H, Tori M, Gong X. Diversity of furanoeremophilanes in major *Ligularia* species in the Hengduan mountains. *Nat Prod Commun.* 2012;7(4):539-548.
2. Kuroda C, Hanai R, Tori M, et al. Diversity in furanoeremophilane composition produced by *Ligularia* species (Asteraceae) in the Hengduan Mountains area of China. *J Syn Org Chem Jpn.* 2014;72(6):717-725.
3. Liu S, Illarionova ID. Cremanthodium. In: WuZY, Raven RH, Hong DY, eds. *Flora of China* Beijing and St. Louis: Science Press and Missouri Botanical Garden Press; 2011:Vol. 20-21. 415-435.
4. Liu J, Liu S, Ho T, Lu A. Karyological studies on the Sino-Himalayan genus, *Cremanthodium* (Asteraceae: Senecioneae). *Bot J Linn Soc.* 2001;135(2):107-112.
5. Liu J-Q, Wang Y-J, Wang A-L, Ohba H, Abbott RJ. Radiation and diversification within the *Ligularia-Cremanthodium-Parasenecio* complex (Asteraceae) triggered by uplift of the Qinghai-Tibetan Plateau. *Mol Phylogenet Evol.* 2006;38(1):31-49.

6. Yang L, Chen H, Jia ZJ. Lignan and a coumarin from *Cremanthodium ellisii*. *Indian J Chem*. 1995;34B(11):975-977.
7. Chen H, Zhu Y, Shen X-M, Jia Z-J. Four New Sesquiterpene Polyol Esters from *Cremanthodium ellisii*. *J Nat Prod*. 1996;59(12):1117-1120.
8. Chen H, Jia ZJ, Tan RX. Two new Oplopanol Esters from *Cremanthodium ellisii*. *Planta Med*. 1997;63(3):245-247.
9. Su BN, Zhu QX, Jia ZJ. Nor-lignan and sesquiterpenes from *Cremanthodium ellisii*. *Phytochemistry*. 2000;53(8):1103-1108.
10. Wang A-X, Zhang Q, Jia Z-J. Phenylpropanosids, lignans and other constituents from *Cremanthodium ellisii*. *Pharmazie*. 2004;59(11):889-892.
11. Zhu Y, Yang L, Jia ZJ. Novel highly oxygenated bisabolane sesquiterpenes from *Cremanthodium discoideum*. *J Nat Prod*. 1999;62(11):1479-1483.
12. Zhu Y, Zhu QX, Jia ZJ. Epoxide Sesquiterpenes and Steroids from *Cremanthodium discoideum*. *Aust J Chem*. 2000;53(10):831-834.
13. Yang A, Zeng Y, Yang L, Yang Z. Isolation and structural elucidation of triterpenes from *Cremanthodium potaninii* C. Winkl. *Zhongchengyao*. 2010;32(4):636-639.
14. Tu Y, Yang R, Shou Q, Wang B, Peng T. Studies on chemical constituents of essential oils from *Cremanthodium pleurocaule*. *Zhongguo Zhongyao Zazhi*. 2006;31(6):522-524.
15. Saito Y, Ichihara M, Okamoto Y, Gong X, Kuroda C, Tori M. Four new eremophilane-type alcohols from *Cremanthodium belianthus* collected in China. *Nat Prod Commun*. 2012;7(4):423-426.
16. Saito Y, Ichihara M, Okamoto Y, Gong X, Kuroda C, Tori M. Four eremophil-9-en-8-one derivatives from *Cremanthodium stenactinium* samples collected in China. *Molecules*. 2011;16(12):10645-10652.
17. Saito Y, Takiguchi K, Gong X, Kuroda C, Tori M. Three new bisabolane-type sesquiterpenoids from *Cremanthodium rhodocephalum* (Asteraceae). *Heterocycles*. 2012;86(1):497-503.
18. Saito Y, Ichihara M, Takiguchi K, et al. Chemical and genetic diversity of *Cremanthodium lineare*. *Phytochemistry*. 2013;96:184-190.
19. Kupchan SM, Schubert RM. Selective alkylation: a biomimetic reaction of the antileukemic triptolides? *Science*. 1974;185(4153):791-793.
20. Onuki H, Yamazaki M, Nakamura A, et al. Chemical constituents and diversity of *Ligularia lankongensis* in Yunnan Province of China. *J Nat Prod*. 2008;71(4):520-524.
21. Yaoita Y, Suzuki N, Kikuchi M. Structures of new sesquiterpenoids from Farfarae Flos. *Chem Pharm Bull*. 2001;49(5):645-648.
22. Bohlmann F, Zdero C, King RM, Robinson H. Oplopane derivatives from *Arnoglossum atriplicifolium*. *Rev Latinoam Quím*. 1984;15(1):11-13.
23. Clement JA, Bleich RM, Campbell HE, et al. Cytotoxic oplopane sesquiterpenoids from *Arnoglossum atriplicifolium*. *Nat Prod Res*. 2016;30(18):2028-2033.
24. Maldonado RJI, Arciniegas A, Perez-Castorena AL, Villaseñor JL, de Vivar AR. Furanoeremophilanes and other constituents of *Pittocaulon bombycophole*. *Heterocycles*. 2008;75(12):3035-3042.
25. Wang Q, Chen T-H, Bastow KF, Morris-Natschke SL, Lee K-H, Chen D-F. Songaricalarins A-E, cytotoxic oplopane sesquiterpenes from *Ligularia songarica*. *J Nat Prod*. 2013;76(3):305-310.
26. Arciniegas A, González K, Pérez-Castorena A-L, Nieto-Camacho A, Villaseñor J-L, Romo de Vivar A. Sesquiterpenoids from *Pittocaulon filare*. *J Nat Prod*. 2014;77(6):1304-1310.
27. Dai W, Lu C-Q, Wang Y, Lu Y, Chen D-F, Wang Q. Oplopane sesquiterpenes from *Ligularia knorringiana* and their anti-complementary activity. *Chem Biodivers*. 2018;15(3):e1700515.
28. Hirota H, Horiguchi Y, Kawai S, Kuroda C, Hanai R, Gong X. Four new bisabolane-type sesquiterpenes from *Ligularia lankongensis*. *Nat Prod Commun*. 2012;7(4):451-454.
29. Qin Z-B, Zhang J, Wu X-D, et al. Sesquiterpenoids from *Tussilago farfara* and their inhibitory effects on nitric oxide production. *Planta Med*. 2014;80(8-9):703-709.
30. Jin A, Wu W-M, Yu H-Y, et al. Bisabolane-type sesquiterpenoids from the whole plant of *Parasenecio rubescens*. *J Nat Prod*. 2015;78(8):2057-2066.
31. Hirai M, Miyazaki R, Mitsui K, et al. Synthesis and NMR spectroscopic elucidation of four diastereoisomers of oxygenated bisabolane side chain. *Helv Chim Acta*. 2015;98(8):1035-1060.
32. Kobayashi K, Kunimura R, Takagi H, et al. Total synthesis of highly oxygenated bisabolane sesquiterpene isolated from *Ligularia lankongensis*: relative and absolute configurations of the natural product. *J Org Chem*. 2018;83(2):703-715.
33. Bohlmann F, Suwita A. Über die Inhaltsstoffe von *Senecio abrotanifolius* L. *Chem Ber*. 1976;109(6):2014-2020.
34. Bohlmann F, Ziesche J. Sesquiterpenes from three *Senecio* species. *Phytochemistry*. 1981;20(3):469-472.
35. Kuroda C, Inagaki K, Chao X, et al. Chemical and genetic diversity of *Ligularia bodgsonii* in China. *Nat Prod Commun*. 2015;10(6):823-826.
36. Wu YX, Chen Y-J, Liu C-M, Gao K. Four new sesquiterpenoids from *Ligularia cymbulifera*. *J Asian Nat Prod Res*. 2012;14(12):1130-1136.
37. Liu C-M, Wang H-X, Wei S-L, Gao K. Pyrrolizidine alkaloids and bisabolane sesquiterpenes from the roots of *Ligularia cymbulifera*. *Helv Chim Acta*. 2008;91(2):308-316.