NPC Natural Product Communications

2012
Vol. 7
No. 4
451 - 454

Four New Bisabolane-type Sesquiterpenes from *Ligularia lankongensis*

Hiroshi Hirota[a, b, *], Yurie Horiguchi[a, c], Satoru Kawaii[c], Chiaki Kuroda[d], Ryo Hanai[e] and Xun Gong[f]

[a]*RIKEN Advanced Science Institute, Hirosawa, Wako 351-0198, Japan*

[b]*Cooperative Research and Development Center, Yokohama National University, Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan*

[c]*Faculty of Science and Engineering, Tokyo Denki University, Hatoyama, Saitama 350-0394, Japan*

[d]*Department of Chemistry and Research Center for Smart Molecules, Rikkyo University, Nishi-Ikebukuro, Toshima-ku, Tokyo 171-8501, Japan*

[e]*Department of Life Science and Research Center for Life Science, Rikkyo University, Nishi-Ikebukuro, Toshima-ku, Tokyo 171-8501, Japan*

[f]*Kunming Institute of Botany, Chinese Academy of Science, Kunming 654202, China*

hhirota@riken.jp, hirota@gsc.riken.jp

Received: December 15th, 2011; Accepted: December 28th, 2011

The chemical constituents of the roots of two *Ligularia lankongensis* samples collected in Yunnan and Sichuan Provinces, China, were investigated, together with the DNA sequence of the *atpB-rbcL* and ITS regions. Four new highly oxygenated bisabolane-type sesquiterpenes (**1** – **4**) were obtained. Intraspecific diversity in the DNA sequence was found to be limited.

Keywords: *Ligularia lankongensis*, Asteraceae, Bisabolane, Sesquiterpene, Diversity, *atpB-rbcL*, ITS.

Plants of the genus *Ligularia* (Asteraceae) are widely distributed in the Hengduan Mountains of China. We are studying the intraspecific diversity and evolution of *Ligularia* species in this area on the basis of chemical constituents and evolutionarily neutral DNA sequences. So far we have revealed the presence of intraspecific diversity in many species [1-8]. Although most *Ligularia* species in the Hengduan Mountains area produce furanoeremophilanes [9], some species do not, indicating the presence of different lineages.

We previously reported that *L. lankongensis* in northwestern Yunnan Province produced highly oxygenated bisabolane-type sesquiterpenes with an epoxide ring between C-10 and C-11, and intraspecific diversity was recognized in the oxidation pattern of the bisabolane compounds, but not in the DNA sequence of the *atpB-rbcL* region [1]. Several new bisabolane-type sesquiterpenes from *Ligularia* species have been reported in the past five years. [10-12]. Further search in the field provided us two additional *L. lankongensis* samples from Yongsheng County of Yunnan Province (sample 1) and Yanyuan County of Sichuan Province (sample 2) (see Figure 1). In the present study, we obtained four new highly oxygenated bisabolane-type sesquiterpenes (**1** - **4**) from these samples.

The dried roots of the two samples were extracted with EtOAc. The extracts were subjected to silica gel column chromatography followed by ODS HPLC to give oxygenated bisabolane sesquiterpenes. From the Yunnan sample (sample 1), a new compound (**1**) was isolated together with three known compounds (**5** – **7**). From the Sichuan sample (sample 2), three new compounds (**2** – **4**) together with two known compounds (**7** – **8**) were obtained. Compounds **5** - **8** were identified by comparison of NMR data with those reported previously [1].

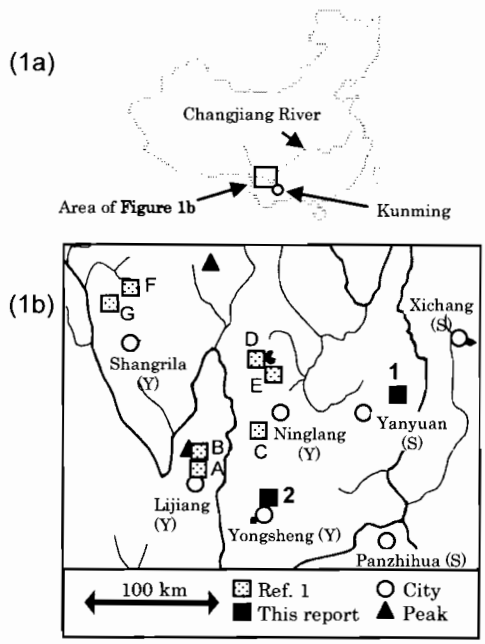
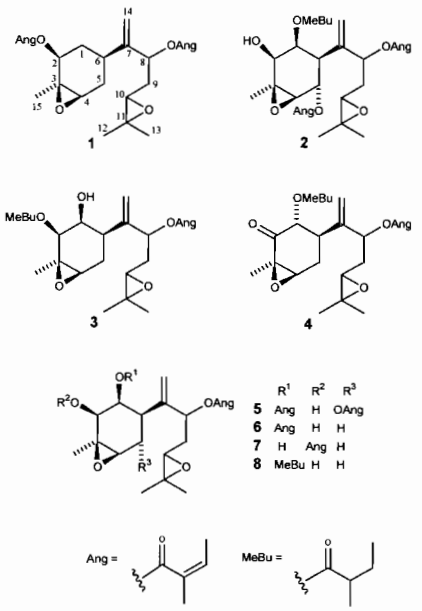


Figure 1: Locations of sample collection of *L. lankongensis*. Solid lines indicate rivers. In **1b**, (S): Sichuan Province; (Y): Yunnan Province

Compound **1** had the molecular formula $C_{25}H_{36}O_6$ from the positive HR-ESIMS data [*m/z* 455.2409 $(M+Na)^+$ Δ 0.7 mmu]. Based on ^{13}C (Table 1), ^1H (Table 2), and various standard 2D NMR spectra, together with comparison with those of compounds **5** - **8**, it became clear that compound **1** had the bisabolane-type sesquiterpene



skeleton with an exomethylene moiety, two epoxide rings, and two angeloyloxy (AngO) groups. Exomethylene protons at C-14 (δ_H 4.98 and 5.14) showed HMBC correlations with C-8 (δ_C 73.2) and C-6 (δ_C 35.3). The COSY spectrum showed that H-6 (δ_H 2.14) was adjacent to two methylene protons, δ_H 1.62 and 1.77 (C-1), and δ_H 1.77 and 2.32 (C-5). The methylene protons at C-1 were also correlated with H-2 (δ_H 5.25). Methyl protons at C-15 (δ_H 1.34) had HMBC correlations with C-3 (δ_C 58.1), C-4 (δ_C 60.9), and C-2 (δ_C 73.6). These data showed that one of the two epoxide functions was attributed to C-3/C-4 and the two angeloyloxy groups were attached to C-2 and C-8. The other epoxide ring was deduced to be located at C-10/C-11 from ^{13}C and ^{1}H chemical shifts (C-10: δ_C 61.0, δ_H 2.80; C-11: δ_C 58.4). The relative configuration of the cyclohexane ring in **1** was deduced from ^{1}H-^{1}H coupling constants and the NOESY spectrum. That is, H-2 (δ_H 5.25) appeared as a doublet of doublets with coupling constants of 10.5 and 5.5 Hz. This indicated that H-2 was located in an axial orientation. The NOESY spectrum showed correlations between H-2 and H-6, between H-2 and methyl protons at C-15, and between methyl protons at C-15 and H-4 (δ_H 3.13). These data showed that compound **1** had the same relative configuration as the previously reported compound **5** [1], even though the relative configuration of C-8 and C-10 could not be determined from the spectroscopic data. Thus the structure of **1** was determined to be 2β,8-bisangeloyloxy-3β,4β,10,11-bisepoxybisabol-7(14)-ene.

Compound **2** had the molecular formula $C_{30}H_{44}O_9$. This compound had two angeloyloxy groups and a 2-methylbutyryloxy (MeBuO) group, together with a hydroxy group and two epoxide rings. ^{1}H, ^{13}C, and 2D NMR spectra of **2** closely resembled those of **5** ($C_{30}H_{42}O_9$). This implied that **2** had a structure where one of the angeloyloxy groups of **5** was saturated to afford a 2-methylbutyryloxy group. HMBC correlation between carbonyl C-1" (δ_C 176.7) and H-1 (δ_H 5.35) revealed that the 2-methylbutyryloxy group was connected at C-1. Consequently, the structure of **2** was established to be 5α,8-bisangeloyloxy-3β,4β,10,11-bisepoxy-1β-(2-methlybutyryloxy)bisabol-7(14)-en-2β-ol.

Compound **3** ($C_{25}H_{38}O_7$) had one angeloyloxy group and one 2-methylbutyryloxy group, together with two epoxide rings. ^{1}H, ^{13}C, and 2D NMR spectra of **3** resembled those of **7** ($C_{25}H_{36}O_7$). Thus, **3** should have the structure where one of the angeloyloxy groups of **7**

Table 1: ^{13}C NMR data of compounds **1** – **4** (in $CDCl_3$; 125 MHz) δ_C (mult.)

	1	2	3	4
1	30.8 (CH_2)	69.2 (CH)	70.0 (CH)	74.2 (CH)
2	73.6 (CH)	71.5 (CH)	72.8 (CH)	201.4 (C)
3	58.1 (C)	59.4 (C)	60.7 (C)	61.4 (C)
4	60.9 (CH)	63.9 (CH)	61.3 (CH)	64.1 (CH)
5	31.0 (CH_2)	66.9 (CH)	25.6 (CH_2)	31.8 (CH_2)
6	35.3 (CH)	43.5 (CH)	39.8 (CH)	44.1 (CH)
7	151.0 (C)	142.7 (C)	146.9 (C)	147.9 (C)
8	73.2 (CH)	73.3 (CH)	74.5 (CH)	73.3 (CH)
9	33.9 (CH_2)	33.3 (CH_2)	33.7 (CH_2)	33.6 (CH_2)
10	61.0 (CH)	60.9 (CH)	61.0 (CH)	60.9 (CH)
11	58.4 (C)	58.6 (C)	58.4 (C)	58.5 (C)
12	24.7 (CH_3)	24.6 (CH_3)	24.7 (CH_3)	24.7 (CH_3)
13	18.9 (CH_3)	18.8 (CH_3)	18.9 (CH_3)	18.9 (CH_3)
14	110.0 (CH_2)	115.8 (CH_3)	115.3 (CH_2)	112.0 (CH_2)
15	19.2 (CH_3)	19.0 (CH_3)	19.0 (CH_3)	14.9 (CH_3)
OAng				
1'	166.7 (C)	166.6 (C)	166.7 (C)	166.5 (C)
	167.6 (C)	166.8 (C)		
2'	127.5 (C)	127.0 (C)	127.4 (C)	127.4 (C)
	127.5 (C)	127.2 (C)		
3'	138.3 (CH)	139.5 (CH)	139.0 (CH)	139.5 (CH)
	139.0 (CH)	139.7 (CH)		
4'	15.8 (CH_3)	15.9 (CH_3)	15.8 (CH_3)	15.9 (CH_3)
	15.9 (CH_3)	15.9 (CH_3)		
5'	20.6 (CH_3)	20.4 (CH_3)	20.6 (CH_3)	20.6 (CH_3)
	20.6 (CH_3)	20.6 (CH_3)		
OMeBu				
1"		176.7 (C)	176.2 (C)	175.4 (C)
2"		41.3 (CH)	41.1 (CH)	40.9 (CH)
3"		26.4 (CH_2)	26.8 (CH_2)	26.8 (CH_2)
4"		11.6 (CH_3)	11.6 (CH_3)	11.5 (CH_3)
5"		16.9 (CH_3)	16.7 (CH_3)	16.6 (CH_3)

was substituted with a 2-methylbutyryloxy group. HMBC correlations between carbonyl C-1' (δ_C 166.7) and H-8 (δ_H 5.37), and between carbonyl C-1" (δ_C 176.2) and H-2 (δ_H 5.09) revealed that the 2-methylbutyryloxy group was connected at C-2 and the angeloyloxy group was at C-8. Thus the structure of **3** was established to be 8-angeloyloxy-3β,4β,10,11-bisepoxy-2β-(2-methlybutyryloxy)bisabol-7(14)-en-1β-ol.

Compound **4** had the molecular formula $C_{25}H_{36}O_7$. This compound had a ketone (δ_C 201.4) together with one angeloyloxy group, one 2-methylbutyryloxy group and two epoxide rings. From the comparison with other compounds (**1** - **3** and **5** - **8**), the two epoxide rings could be assigned at C-3/C-4 and C-10/C-11 since these carbons resonated at a higher field than other oxygenated carbons in the ^{13}C NMR spectrum. HMBC correlation between the ketone and methyl protons at C-15 (δ_H 1.46) revealed that the carbonyl group was located at C-2. Exomethylene protons at C-14 (δ_H 5.10 and 5.28) showed HMBC correlations with C-8 (δ_C 73.3) and C-6 (δ_C 44.1). The epoxide proton at C-4 (δ_H 3.44) showed HMBC correlations with C-5 (δ_C 31.8) and C-6 (δ_C 44.1), as well as with C-3 (δ_C 61.4) and C-15 (δ_C 14.9). H-6 (δ_H 2.73) and H-1 (δ_H 5.74) showed a COSY correlation each other. These implied that the cyclohexane ring of **4** was substituted by 1-acyloxy, 2-oxo, 3,4-epoxy, 3-methyl, and 6-alkyl groups. HMBC correlations between carbonyl C-1' (δ_C 166.5) and H-8 (δ_H 5.27), and between carbonyl C-1" (δ_C 175.4) and H-1 (δ_H 5.74) revealed that the angeloyloxy group was connected at C-8 and the 2-methylbutyryloxy group was at C-1. H-1 (δ_H 5.74) appeared as a doublet with J = 12.0 Hz, and had an NOE correlation not with H-6 (δ_H 2.73) but with one of the H-5 protons (δ_H 2.20), indicating that H-1 and one of the H-5 protons (δ_H 2.20) were β-axial and the H-6 proton was α-axial. H-4 (δ_H 3.44) appeared as a doublet signal, and this ^{1}H-^{1}H coupling (J = 4.5 Hz) was assigned, from the COSY spectrum, to between H-4 and one of the H-5 protons (δ_H 2.67). H-4 had NOESY correlations with methyl protons at C-15 and both protons at C-5 {relatively

Table 2: ^{1}H NMR spectroscopic data of compounds **1** – **4** (in $CDCl_3$; 500 MHz) δ_H (multi, *J* in Hz).

	1	**2**	**3**	**4**
1	1.62 (1H, m)	5.35 (1H, dd, 5.0, 2.0)	3.92 (1H, brdd, 10.5, 3.7)	5.74 (1H, d, 12.0))
	1.77 (1H, m)			
2	5.25 (1H, dd, 10.5, 5.5)	4.08 (1H, dd, 8.5, 5.0)	5.09 (1H, d, 3.7)	
4	3.13 (1H, d, 5.0)	3.10 (1H, s)	3.28 (1H, d, 5.0)	3.44 (1H, d, 4.5)
5	1.77 (1H, m)	5.44 (1H, d, 11.0)	2.15 (1H, m)	2.20 (1H, dd, 15.0, 10.5)
	2.32 (1H, m)		2.28 (1H, m)	2.67 (1H, m)
6	2.14 (1H, m)	2.70 (1H, brd, 11.0)	2.30 (1H, m)	2.73 (1H, m)
8	5.37 (1H, dd, 8.5, 4.5)	5.50 (1H, dd, 8.5, 4.5)	5.37 (1H, dd, 8.0, 6.0)	5.27 (1H, dd, 9.0, 5.0)
9	1.88 (1H, m)	1.91 (1H, m)	1.83 (1H, m)	1.87 (1H, m)
	1.92 (1H, m)	1.96 (1H, m)	2.00 (1H, m)	1.87 (1H, m)
10	2.80 (1H, dd, 6.0, 6.0)	2.75 (1H, dd, 6.5, 6.0)	2.78 (1H, dd, 6.0, 5.2)	2.81 (1H, dd, 9.0, 5.0)
12	1.29 (3H, s)	1.26 (3H, s)	1.29 (3H, s)	1.30 (3H, s)
13	1.28 (3H, s)	1.27 (3H, s)	1.28 (3H, s)	1.27 (3H, s)
14	4.98 (1H, s)	5.11 (1H, s)	5.31 (1H, s)	5.10 (1H, brs)
	5.14 (1H, s)	5.31 (1H, s)	5.35 (1H, s)	5.28 (1H, brs)
15	1.34 (3H, s)	1.48 (3H, s)	1.35 (3H, s)	1.46 (3H, s)
OH		2.18 (1H, d, 8.5)	3.14 (1H, d, 10.5)	
OAng				
3'	6.11 (1H, qq, 7.1, 1.4)	6.09 (1H, qq, 7.5, 1.5)	6.10 (1H, qq, 7.5, 2.0)	6.15 (1H, qq, 7.3, 1.5)
	6.11 (1H, qq, 7.1, 1.4)	6.12 (1H, qq, 7.5, 1.5)		
4'	2.01 (3H, brd, 7.1)	1.98 (3H, brd, 7.5)	2.00 (3H, dq, 7.5, 2.0)	2.02 (3H, dq, 7.3, 1.0)
	2.01 (3H, brd, 7.1)	1.98 (3H, brd, 7.5)		
5'	1.93 (3H, brd, 1.4)	1.84 (3H, quin, 1.5)	1.89 (3H, quin, 2.0)	1.93 (3H, dq, 1.5, 1.0)
	1.93 (3H, brd, 1.4)	1.89 (3H, quin, 1.5)		
OMeBu				
2"		2.40 (1H, sext, 7.0)	2.54 (1H, sext, 7.0)	2.34 (1H, sext, 7.0)
3"		1.43 (1H, dquin, 14.0, 7.0)	1.53 (1H, m)	1.45 (1H, m)
		1.68 (1H, dquin, 14.0, 7.0)	1.78 (1H, m)	1.68 (1H, m)
4"		0.88 (3H, t, 7.0)	0.95 (3H, t, 7.5)	0.93 (3H, t, 7.5)
5"		1.16 (3H, d, 7.0)	1.22 (3H, d, 7.0)	1.10 (3H, d, 7.0)

stronger with H-5 α (δ_H 2.67) than with H-5 β (δ_H 2.20)}. The methyl protons at C-15 correlated only with H-4 in the NOESY spectrum. These implied that the 3,4-epoxide was β–oriented and both methyl protons at C-15 and H-4 had an α-equatorial orientation. Consequently, the structure of **4** was established to be 8-angeloyloxy-3β,4β,10,11-bisepoxy-1α-(2-methlybutyryloxy)-bisabol-7(14)-en-2-one. This structure (**4**) is a little different from the other seven compounds (**1** - **3** and **5** - **8**) in terms of the carbonyl moiety and the C-1 stereochemistry of the cyclohexane ring.

The DNA sequences of the *atpB-rbcL* intergenic region of the plastid genome and the ITS1-5.8S-ITS2 region of the nuclear rRNA gene were determined. The *atpB-rbcL* sequence was the same for the two samples and for our previous seven samples: G-A12 under our designation [1,13]. The ITS1-5.8S-ITS2 region was analyzed for our previous seven samples as well. The results are summarized in Table 3. When the nine sequences were subjected to a standard neighbor-joining cladistic analysis using MEGA5 [14], they were not separated into clades. Thus, both DNA regions indicate that the samples are genetically more or less uniform.

Table 3: The ITS1-5.8S-ITS2 sequence of *L. lankongensis* samples[a]

Sample number[b]	ITS1				ITS2							
	132	196	199	239	4	27	28	72	84	131	146	159
A	C	G	C	G	R	C	G	A G		A	C	G
B	C	G	Y G		R	C	G	R	G	A	M	S
C	C	G	C	G	R	C	G	A G		A	C	S
D	Y	R	C	G	G	C	G	A R		A	C	S
E	Y	R	C	K	G	M	K	A R		A	C	S
F	C	G	Y G		R	C	G	A G		C	M	S
G	C	G	Y G		R	C	G	A G		A	M	G
1	C	G	C	G	R	C	G	A G		A	M	S
2[c]	C	G	C	G	R	C	G	A G		A	C	S

a Only the differences between the samples are shown. K = G+T; M = A+C; R = A+G; S = C+G ; Y = C+T.

b Samples A-G are those in our previous report [1]. c The entire sequence has been deposited in the data-base with an accession number of AB684267.

In conclusion, eight bisabolane-type sesquiterpenes (**1** – **8**), four of which were new compounds (**1** – **4**), were obtained from the two additional *L. lankongensis* samples collected in Yunnan and Sichuan Provinces, China. Intraspecific diversity in the chemical composition was observed in the oxidation pattern of the bisabolane compounds, as previously reported [1]. The results of the DNA sequencing also indicated that the intraspecific diversity was limited.

Experimental

General: Optical rotations were measured on a Horiba SEPA-300 digital polarimeter. IR spectra were recorded on a Horiba FT-720 infrared spectrometer with a DuraSampl IR II ATR instrument. NMR data were obtained on a JEOL ECA-500 spectrometer at 298 K and a Bruker DRX-500 spectrometer at 303 K in $CDCl_3$ solution. Chemical shifts were referenced to either the residual $CHCl_3$ (δ_H 7.26) or $CDCl_3$ (δ_C 77.0). Mass spectra were measured on a JEOL JMS-T100LC spectrometer under positive ESI mode. Column chromatography was performed on Merck silica gel 60 (60 – 230 mesh). Preparative HPLC was performed on a Waters 600E pump system with a SenshuPak Pegasil OSD column (10 mm i.d. x 250 mm).

Plant materials: Samples of *L. lankongensis* were collected in August 2007 near Yongsheng City (Yongsheng County), Yunnan Province (sample 1), and at Weixiang, Yanyuan County, Sichuan Province (sample 2). Each plant was identified by X. G., one of the authors. Voucher specimens were deposited in the Herbarium of Kunming Institute of Botany (No. 2007-01 and 2007-27 for samples 1 and 2, respectively).

Extraction and isolation: The dried roots of sample 1 were extracted with EtOAc. The EtOAc extract was concentrated under reduced pressure to give a residue (310 mg), which was chromatographed on silica gel by the use of a gradient of *n*-hexane-EtOAc (from 100:0 to 0:100). Fractions eluted with *n*-hexane/EtOAc (2:1) were subsequently subjected to HPLC with acetonitrile/water (from 50:50 to 100: 0 with gradation) to give **1** (4.8 mg). Fractions eluted with *n*-hexane/EtOAc (1:1) were also purified by HPLC with acetonitrile/water to give **5** (2.4 mg), **6** (2.5 mg), and **7** (0.8 mg).

The EtOAc extract of the dried roots of sample 2 was concentrated under reduced pressure to give a residue (290 mg). This was chromatographed on silica gel by the use of a gradient of *n*-hexane-EtOAc (from 100:0 to 0:100). Fractions eluted with *n*-hexane/EtOAc (5:1) were subsequently purified by HPLC with acetonitrile/water (from 50:50 to 100:0 with gradation) to give **8** (0.8 mg). Fractions eluted with *n*-hexane/EtOAc (2:1) were subjected to HPLC to give **2** (4.8 mg), **3** (1.7 mg), and **4** (1.2 mg). Fractions eluted with *n*-hexane/EtOAc (1:1) were also purified by HPLC to give **7** (3.3 mg). Compounds **5** - **8** were identified by comparison of NMR spectroscopic data with those reported earlier [1].

DNA analysis: Purification of DNA from dried leaves, polymerase chain region (PCR), and DNA sequencing were carried out as described earlier [4], except that the *atpB-rbcL* region was amplified with a new pair of primers, La2 (5' GAACTGAAAGAGTAGGATTCAT 3') and Lr (5' TAGTCTCTGTTTGTGGTGACAT 3') and that sequencing of the region was carried out with the two primers, LtR (5' TTCCATAGATAATATAGATGGGA 3') and LrF (5' GAATAGGGTTGCGCCATATATA3').

2β,8-Bisangeloyloxy-3β,4β,10,11-bisepoxybisabol-7(14)-ene (1)
Colorless oil.
$[\alpha]_D$: -108.4 (*c* 0.042, $CHCl_3$).
IR (film): 1714, 1646, 1228, 1153, 1041, 848 cm^{-1}.
^1H NMR (500 MHz, $CDCl_3$): Table 2.
^{13}C NMR (125 MHz, $CDCl_3$): Table 1.
HR-ESIMS: *m/z* $[M + Na]^+$ calcd for $C_{25}H_{36}O_6Na$: 455.2409; found: 455.2402.

5α,8-Bisangeloyloxy-3β,4β,10,11-bisepoxy-1β-(2-methlybutyryl-oxy)bisabol-7(14)-en-2β-ol (2)
Colorless oil.
$[\alpha]_D$: -12.6 (*c* 0.90, $CHCl_3$).
IR (film): 3550, 1733, 1716, 1228, 1145, 1041, 848 cm^{-1}.
^1H NMR (500 MHz, $CDCl_3$): Table 2.
^{13}C NMR (125 MHz, $CDCl_3$): Table 1.
HR-ESIMS: *m/z* $[M + Na]^+$ calcd for $C_{30}H_{44}O_9Na$: 571.2836; found: 571.2883.

8-Angeloyloxy-3β,4β,10,11-bisepoxy-2β-(2-methlybutyryloxy) biasbol-7(14)-en-1β-ol (3)
Colorless oil.
$[\alpha]_D$: -7.3 (*c* 0.083, $CHCl_3$).
IR (film): 3500, 1716, 1646, 1230, 1153, 1039, 848 cm^{-1}.
^1H NMR (500 MHz, $CDCl_3$): Table 2.
^{13}C NMR (125 MHz, $CDCl_3$): Table 1.
HR-ESIMS: *m/z* $[M + Na]^+$ calcd for $C_{25}H_{38}O_7Na$: 473.2515; found: 473.2471.

8-Angeloyloxy-3β,4β,10,11-bisepoxy-1α-(2-methlybutyryloxy) bisabol-7(14)-en-2-one (4)
Colorless oil.
$[\alpha]_D$: -81.6 (*c* 0.10, $CHCl_3$).
IR (film): 1725, 1714, 1228, 1149, 1039, 1027, 829 cm^{-1}.
^1H NMR (500 MHz, $CDCl_3$): Table 2.
^{13}C NMR (125 MHz, $CDCl_3$): Table 1.
HR-ESIMS: *m/z* $[M + Na]^+$ calcd for $C_{25}H_{36}O_7Na$: 471.2358; found: 471.2353.

Acknowledgments – This paper is dedicated to the memory of Dr Takeyoshi Takahashi (1926-2010), Professor Emeritus of the University of Tokyo. The authors wish to thank Dr Hiroyuki Osada of RIKEN Advanced Science Institute for his encouragement and helpful discussions. This work was supported in part by JSPS (Grant-in-Aid for Scientific Research No. 21404009).

References

[1] Onuki H, Yamazaki M, Nakamura A, Hanai R, Kuroda C, Gong X, Shen Y, Hirota H. (**2008**) Chemical constituents and diversity of *Ligularia lankongensis* in Yunnan Province of China. *Journal of Natural Products*, ***71***, 520-524.

[2] Nagano H, Hanai R, Yamada H, Matsushima M, Miura Y, Hoya T, Ozawa M, Fujiwara M, Kodama H, Torihata A, Onuki H, Nezu Y, Kawai S, Yamazaki M, Hirota H, Saito Y, Tori M, Ohsaki A, Gong X, Kuroda C. (**2011**) Chemical and genetic study of *Ligularia duciformis* and related species in Sichuan and Yunnan Provinces of China, *Chemistry and Biodiversity, in press* (DOI: 10.1002/cbdv.201100240); and references cited therein.

[3] Nagano H, Matsushima M, Yamada H, Hanai R, Gong X, Kuroda C. (**2010**) Two new furanoeremophilane sesquiterpenoids from *Ligularia oligonema*. *Natural Product Communications*, ***5***, 1-4.

[4] Saito Y, Hattori M, Iwamoto Y, Takashima Y, Mihara K, Sasaki Y, Fujiwara M, Sakaoku M, Shimizu A, Chao X, Kuroda C, Gong X, Hanai R, Tori M. (**2011**) Overlapping chemical and genetic diversity in *Ligularia lamarum* and *Ligularia subspicata*. Isolation of ten new eremophilanes and a new seco-bakkane compound. *Tetrahedron*, ***67***, 2220-2231; and references cited therein.

[5] Nagano H, Kanda M, Yamada H, Hanai R, Gong X, Kuroda C. (**2010**) Chemical and genetic study of *Ligularia anoleuca* and *L. veitchiana* in Yunnan and Sichuan Provinces of China. *Helvetica Chimica Acta*, ***93***, 1945-1952; and references cited therein.

[6] Torihata A, Chao X, Kuroda C, Hanai R, Hirota H, Gong X. (**2009**) Chemical and genetic differentiation of *Ligularia hodgsonii* in Japan and China, *Chemistry and Biodiversity*, ***6***, 2184-2191.

[7] Nagano H, Torihata A, Matsushima M, Hanai R, Saito Y, Baba M, Tanio M, Okamoto Y, Takashima Y, Ichihara M, Gong X, Kuroda C, Tori M. (**2009**) Chemical and genetic study of *Ligularia cyathiceps* in Yunnan Province of China. *Helvetica Chimica Acta*, ***92***, 2071-2081.

[8] Tori M, Tanio Y, Okamoto Y, Saito Y, Gong X, Kuroda C, Hanai R. (**2008**) Chemical constituents of *Ligularia franchetiana* collected in Yunnan Province of China. *Heterocycles*, ***75***, 2029-2034.

[9] Kuroda C, Hanai R, Nagano H, Tori M, Gong X. (**2012**) Diversity of furanoeremophilanes in major *Ligularia* species in the Hengduan Mountains. *Natural Product Communications*, ***7***, 7, 539-548.

[10] Tan AM, He HP, Zhang M, Wang ZT, Hao XJ. (**2007**) Two new sesquiterpenes from *Ligularia lankongensis*. *Helvetica Chimica Acta*, ***90***, 101-104; and references cited therein.

[11] Wang Q, Chen T-H, Bastow KF, Lee K-H, Chen D-F. (**2010**) Altaicalarins A-D, cytotoxic bisabolane sesquiterpenes from *Ligularia altaica*. *Journal of Natural Products*, ***73***, 139-142.

[12] (a) Baba H, Yaoita Y, Kikuchi M. (**2007**) Sesquiterpenoids and lactone derivatives from *Ligularia dentata*. *Helvetica Chimica Acta*, ***90***, 1028-1037; (b) Baba H, Yaoita Y, Kikuchi M. (**2007**) Sesquiterpenes from *Ligularia dentata*. *Helvetica Chimica Acta*, ***90***, 1302-1312.

[13] Hanai R, Gong X, Tori M, Kondo S, Otose K, Okamoto Y, Nishihama T, Murota A, Shen Y, Wu S, Kuroda C. (**2005**) Chemical and genetic study of *Ligularia tongolensis*, *Ligularia cymbulifera*, and *Ligularia atroviolacea* in the Hengduan Mountains of China. *Bulletin of Chemical Society of Japan*, ***78***, 1302-1308.

[14] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. (**2011**) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, ***28***, 2731-2739.