Full Papers

Chemical Constituents and Diversity of *Ligularia lankongensis* in Yunnan Province of China

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The chemical constituents of the roots of *Ligularia lankongensis* collected at seven different places in Yunnan Province, China, were investigated together with the DNA sequence of the *atpB-rbcL* intergenic region. All the samples contained a new, highly oxygenated bisabolane-type sesquiterpene (**1**). Four other oxygenated bisabolanes (**2** and the new **3**, **4**, and **5**) were also obtained. Intraspecific diversity was observed in the composition of the compounds, but not in the DNA sequence.

Ligularia Cass. (Asteraceae, tribe Senecioneae) in the Hengduan Mountains can provide good materials for the study of plant diversity and evolution, since the genus is highly diversified and is considered to be still evolving.[1,2] We have been studying the intraspecific diversity of *Ligularia* species in this area by the combination of chemical and genetic analyses and have demonstrated intraspecific diversity in several species.[3–7] For instance, *L. pleurocaulis* (Franch.) Hand.-Mazz.,[3] *L. tsangchanensis* (Franch.) Hand.-Mazz., and *L. virgaurea* (Maxim.) Mattf. can be divided into two groups on the basis of chemical components, and in each case, the grouping agrees with that based on the DNA sequence. In this series of study, we chose sesquiterpenes, especially furanoeremophilane and related compounds, in the root of *Ligularia* species for chemical analysis. We sequenced the *atpB-rbcL* region (and at times the internal transcribed spacers of the rRNA gene). This region, which is unrelated to the production of sesquiterpenes, can provide a measure of intraspecific diversity because it has little genetic information and tends to accumulate variations.[8]

As a part of the intraspecific diversity research of *Ligularia* species, we focused on *L. lankongensis* (Franch.) Hand.-Mazz., which belongs to the section Ligularia, series Racemiferae,[9] in the present study. This plant is distributed in southwestern Sichuan and northwestern to northeastern Yunnan Provinces and grows in forest understories, slopes, and scrubs at altitudes of 2100–3800 m.[9] The dried root of this plant has been used as a local herbal medicine.[10] Some pyrrolizidine alkaloids have been isolated,[11] and two bisabolanepentaol derivatives have been very recently reported as terpenoid components.[12]

We report here that *L. lankongensis* of northwestern Yunnan produces highly oxygenated bisabolane sesquiterpenes with epoxide ring(s) and that intraspecific diversity is recognized in the composition of the compounds but not in the *atpB-rbcL* sequence.

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Table 1. Location of Collection and Terpenoid Components of *L. lankongensis* Samples

sample[a]	locality	elevation (m)	terpenoid component[b] 1	2	3	4	5	*atpB-rbcL*[c]
A	Ganhaizi	3100	++					G-A12
B	Yulongxueshan	2800	++					G-A12
C	Xiaopingzi	3000	++	++	+			G-A12
D	Yongning	3100	+	+		+	+	G-A12
E	Luguhu	3300	+			++	+	G-A12
F	Geza	2900	++	++				G-A12
G	Nixi	3200	++	++				G-A12

[a] Samples A and C−F were collected in 2004; sample B, in 2005; sample G, in 2006. Sample collection places are also shown in Figure 1. [b] ++ indicates major component (more than 8% w/w of the extract), + indicates minor component (not more than 8% w/w of the extract). [c] See our previous report[5] for the designation.

Results and Discussion

Chemical Constituents. Seven samples of *L. lankongensis* were collected in northwestern Yunnan Province (Table 1, Figure 1, A and B). The roots of each sample were extracted with EtOH or EtOAc.[13] No extract was positive for Ehrlich's test on TLC plates,[14] suggesting that this species produces no furanoeremophilane derivative. The extracts were subjected to Si gel chromatography to give oxygenated bisabolane-type sesquiterpene(s). We obtained five epoxidized bisabolane-type sesquiterpenes (**1**−**5**), four of which were new compounds (**1**, **3**, **4**, and **5**).

Compound **1** was found in all seven samples. Its molecular formula was determined to be $C_{30}H_{42}O_9$ on the basis of positive HRFABMS data [*m/z* 547.2922 $(M + H)^+$ Δ 1.5 mmu]. The IR spectrum showed absorptions at 3446 and 1716 cm^{-1} for a hydroxy group and ester carbonyl groups, respectively. Three angeloyloxy (AngO) groups were present based on ^1H and ^{13}C NMR spectra (Tables 2 and 3). Their presence was supported by the appearance of a series of fragment peaks in the FABMS spectrum at *m/z* 447 $(M + H - AngOH)^+$, 347 $(M + H - 2AngOH)^+$, and 247 $(M + H - 3AngOH)^+$. Fifteen of the 30 carbons were assigned to the three angeloyl moieties. The connectivity of the remaining 15 carbons was investigated by analysis of COSY and HMBC spectra, and a highly oxygenated cyclohexane ring was deduced (Figure 2). The ring contained one nonoxygenated methine (H-6, δ_H 2.73, δ_C 43.5), which had HMBC correlations with exomethylene protons

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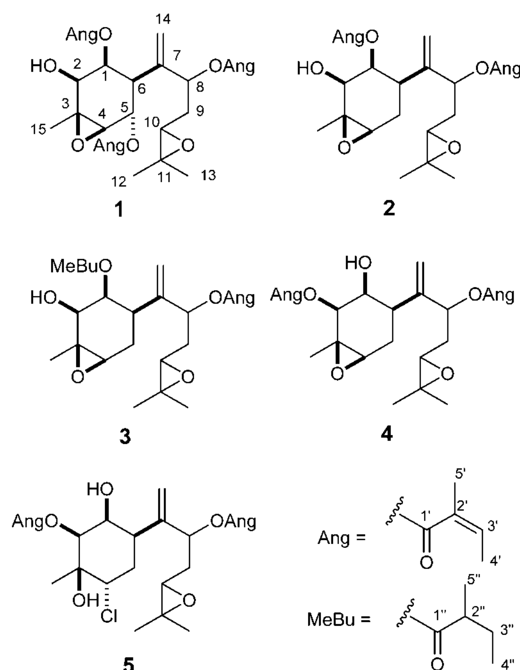
Table 2. ^{1}H NMR Data of Compounds **1**−**5** (500 MHz) δ_H (mult., *J* in Hz)

	1 ($CDCl_3$)	**2** (C_6D_6)	**3** (C_6D_6)	**4** (C_6D_6)	**5** (C_6D_6)
1	5.48 (brd, 4.7)	5.69 (brd, 4.8)	5.56 (d, 4.7)	4.40 (dd, 11.1, 3.7)	4.43 (brdd, 3.5, 2.4)
2	4.12 (br)	3.68 (dd, 8.8, 4.8)	3.64 (dd, 8.3, 4.7)	5.26 (d, 3.7)	5.66 (d, 2.4)
4	3.12 (s)	2.82 (d, 5.7)	2.83 (d, 5.7)	2.71 (d, 5.0)	4.19 (dd, 3.0, 2.9)
5	5.45 (d, 11.1)	2.16 (m)	2.18 (m)	2.00 (m)	2.73 (ddd, 14.6, 14.2, 3.0)
		1.81 (ddd, 13.8, 5.7, 5.6)	1.77 (m)	2.00 (m)	1.79 (ddd, 14.6, 2.9, 2.5)
6	2.73 (brd, 11.1)	2.41 (dd, 12.8, 5.6)	2.43 (dd, 12.8, 5.4)	2.24 (dd, 12.0, 7.0)	3.31 (brd, 13.1)
8	5.52 (dd, 8.6, 4.9)	5.72 (dd, 8.4, 5.3)	5.73 (dd, 8.4, 5.1)	5.67 (dd, 8.0, 5.5)	5.54 (dd, 8.1, 5.0)
9	1.92 (m)	2.10 (m)	2.16 (m)	2.00 (m)	1.91 (m)
					1.81 (m)
10	2.77 (dd, 6.0, 6.0)	2.92 (dd, 6.9, 4.8)	2.94 (dd, 7.0, 4.7)	2.84 (dd, 6.0, 5.8)	2.71 (dd, 6.9, 5.0)
12	1.28 (s)	1.33 (s)	1.35 (s)	1.27 (s)	1.16 (s)
13	1.28 (s)	1.25 (s)	1.26 (s)	1.23 (s)	1.18 (s)
14	5.29 (s)	5.31 (s)	5.36 (s)	5.55 (s)	5.15 (s)
	5.09 (s)	5.05 (s)	5.07 (s)	5.41 (s)	4.86 (s)
15	1.50 (s)	1.32 (s)	1.33 (s)	1.17 (s)	1.64 (s)
OH	2.33 (br)	2.13 (d, 8.8)	2.15 (d, 8.3)	3.54 (d, 11.1)	3.98 (d, 3.5; C-1)
					5.30 (s; C-3)
OAng					
3′	6.12 (brq, 7.3, 1.5)	5.82 (qq, 7.2, 1.4)	5.83 (qq, 7.2, 1.2)	5.84 (qq, 7.2, 1.2)	5.89 (qq, 7.3, 1.4)
	6.12 (qq, 7.3, 1.5)	5.79 (qq, 7.2, 1.4)		5.82 (qq, 7.2, 1.2)	5.83 (qq, 7.3, 1.4)
	6.12 (qq, 7.3, 1.5)				
4′	2.01 (dq, 7.3, 1.4)	2.10 (dq, 7.2, 1.5)	2.10 (dq, 7.2, 1.3)	2.14 (dq, 7.3, 1.5)	2.18 (dq, 7.3, 1.5)
	2.01 (dq, 7.3, 1.4)	2.10 (dq, 7.2, 1.5)		2.12 (dq, 7.3, 1.5)	2.01 (dq, 7.3, 1.5)
	1.97 (dq, 7.3, 1.4)				
5′	1.92 (dq, 1.5, 1.4)	2.00 (dq, 1.5, 1.5)	2.00 (dq, 1.3, 1.2)	2.04 (dq, 1.5, 1.2)	2.07 (dd, 1.5, 1.4)
	1.91 (dq, 1.5, 1.4)	2.00 (dq, 1.5, 1.5)		2.02 (dq, 1.5, 1.2)	1.95 (dd, 1.5, 1.4)
	1.86 (dq, 1.5, 1.4)				
OMeBu					
2″			2.37 (dq, 13.7, 7.0)		
3″			1.79 (m)		
			1.41 (m)		
4″			0.92 (t, 7.4)		
5″			1.16 (d, 7.0)		



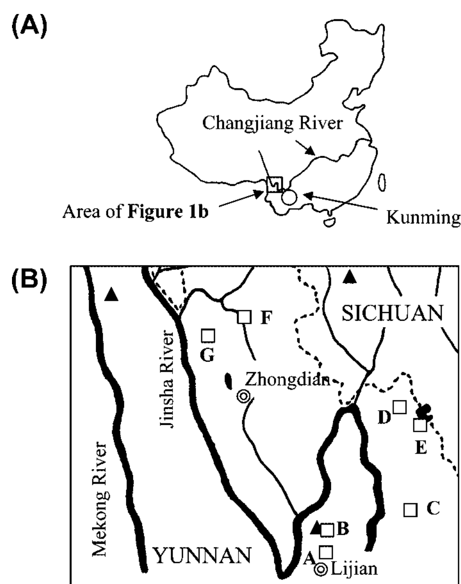


Figure 1. (A and B) Locations where samples of *L. lankongensis* were collected. Open squares, filled triangles, and open circles in Part B indicate sample collection places, major peaks, and major cities, respectively. Letters A−G (sample collection places) correspond to those in Tabel 1.

(H-14, δ_H 5.29 and 5.09). These exomethylene protons correlated with an oxymethine (H-8, δ_H 5.52), which was in turn attached to a methylene followed by an oxymethine (H-10, δ_H 2.77, δ_C 60.8) moiety. The oxymethine proton (H-10) correlated with a carbon (C-11, δ_C 58.5), which was a part of a 2-methylpropylene oxirane moiety. On the basis of these observations, **1** had a highly oxygenated bisabolane-type sesquiterpene skeleton, including an exomethylene moiety as well as three angeloyl groups.

Compound **1** had 10 degrees of unsaturation on the basis of its molecular formula. Eight degrees of unsaturation were assigned to three angeloyl groups, the bisabolane skeleton, and one exomethylene group. The remaining two degrees of unsaturation were attributed to two epoxide functionalities, 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. The chemical shifts of the protons in the oxirane rings were also observed at a higher field (H-4, δ_H 3.12, H-10, δ_H 2.77). The three angeloyloxy groups were attached to C-1, C-5, and C-8, since the protons attached to these carbons

Table 3. ^{13}C NMR Data of Compounds **1**−**5** (125 MHz) δ_C (mult.)

	1 ($CDCl_3$)	**2** (C_6D_6)	**3** (C_6D_6)	**4** (C_6D_6)	**5** (C_6D_6)
1	69.1 d	70.3 d	70.3 d	70.3 d	73.6 d
2	71.3 d	71.9 d	72.3 d	73.5 d	71.8 d
3	59.4 s	58.0 s	58.2 s	60.7 s	75.2 s
4	63.9 d	60.1 d	60.2 d	61.2 d	66.0 s
5	67.1 d	25.2 t	25.0 t	25.9 t	28.4 t
6	43.5 d	39.7 d	40.1 d	40.3 d	39.7 d
7	142.8 s	147.4 s	148.1 s	148.0 s	147.9 s
8	73.2 d	74.3 d	74.4 d	74.7 d	74.7 d
9	32.2 t	33.9 t	34.2 t	34.3 t	34.9 t
10	60.8 d	60.4 d	60.6 d	60.6 d	60.3 d
11	58.5 s	57.5 s	58.0 s	57.7 s	57.6 s
12	18.7 q	18.8 q	19.0 q	19.0 q	19.0 q
13	24.6 q	24.6 q	24.8 q	24.7 q	24.6 q
14	115.6 t	114.0 t	114.2 t	114.9 t	115.9 t
15	18.9 q	19.6 q	19.8 q	19.1 q	24.2 q
OAng					
1′	167.4 s	167.6 s	166.8 s	167.2 s	167.5 s
	166.7 s	166.6 s		166.5 s	167.0 s
	166.5 s				
2′	127.1 s	128.0 s	128.0 s	128.0 s	128.0 s
	127.0 s	128.0 s		128.0 s	128.0 s
	127.0 s				
3′	139.8 d	139.0 d	139.0 d	139.0 d	140.3 d
	139.7 d	138.8 d		138.9 d	139.1 d
	139.4 d				
4′	15.9 q	16.0 q	15.9 q	16.0 q	16.1 q
	15.8 q	15.9 q		15.9 q	16.0 q
	15.8 q				
5′	20.7 q	21.0 q	20.8 q	20.8 q	20.9 q
	20.6 q	20.8 q		20.7 q	20.6 q
	20.3 q				
OMeBu					
1″			176.6 s		
2″			41.8 d		
3″			26.8 t		
4″			11.8 q		
5″			17.2 q		

resonated at a lower field than other oxymethine protons in the ^1H NMR. Thus, the planar structure of **1** was established.

The relative configuration of the cyclohexane ring in **1** was deduced from the ^1H NMR coupling constants and the NOESY spectrum (Figure 2). When H-6 is assumed to be in an α-orientation, H-5 should be *β*-axial due to a large $^3J_{5,6}$ value (11.1 Hz). The $^3J_{1,6}$ value was very small (∼1.5 Hz), so H-1 had an α-equatorial orientation. The orientation of H-2 was determined to be α-axial because of a strong NOESY correlation between H-2 and H-6 and the $^3J_{1,2}$ value of 4.7 Hz. Considering that the cyclohexane ring should have a twist-boat conformation due to the oxirane group, H-4 had an α-equatorial-like disposition because the coupling constant between H-4 and H-5 was 0 Hz and there was only one NOESY correlation for H-4, that is, H-4/H-15. This NOESY correlation of H-4/H-15 together with that of H-2/H-15 deduced that H-15 was in α-equatorial disposition. Thus the relative configuration within the cyclohexane ring was established. The relative configuration at C-8 and C-10 could not, however, be

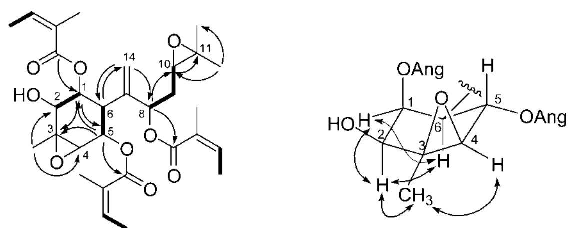


Figure 2. (Left) Selected HMBC correlations (arrows) and COSY connectivities (bold lines) for **1**. (Right) Observed NOESY correlations in the cyclohexane ring of **1**.

determined from the spectroscopic data. Thus, the structure of **1** was established to be 1*β*,5α,8-trisangeloyloxy-3*β*,4*β*,10,11-bisepoxybisabol-7(14)-en-2*β*-ol.

Compound **2** was found in samples C, F, and G as a major component and in sample D as a minor component (Table 1). NMR analysis revealed that **2** had a bisabolane skeleton with two oxirane rings, as in **1**. The structure, including the relative configuration of the stereocenters of the cyclohexane ring, was identical to the known 1*β*,8-bisangeloyloxy-3*β*,4*β*,10,11-bisepoxybisabol-7(14)-en-2*β*-ol.[15] Our NMR analysis based on COSY, HMQC, and HMBC spectra revealed that the assignment of the ^1H NMR signals concerning H-5 and H-10 needed revision; the correct assignments of ^1H NMR are given in Table 2 together with ^{13}C NMR data in Table 3.

Compound **3** was found in sample C as a minor component. It ($C_{25}H_{38}O_7$) contained one angeloyloxy group and one 2-methylbutyryloxy group in the bisabolane-type skeleton (Tables 2 and 3). HMBC correlation between carbonyl C-1′ (δ_C 166.8) and H-8 (δ_H 5.73) showed that the angeloyloxy group was connected at C-8. Correlation between carbonyl C-1″ (δ_C 176.6) and H-1 (δ_H 5.56) revealed that the 2-methylbutyryloxy group was connected at C-1. The COSY spectrum showed correlation between H-2 and a hydroxy proton (δ_H 2.15), which showed no correlation in the HMQC spectrum. Therefore, a hydroxy group was connected at C-2. NOE correlation peaks were observed between H-6/H-1, H-1/H-2, H-2/H-15, and H-15/H-4, respectively. All of these protons were considered to be α-oriented, when H-6 (axial) was assumed to be in an α-orientation. The relative configuration of the stereocenter in the 2-methylbutyryloxy group has not yet been determined. Thus, the structure of **3** was determined to be 8-angeloyloxy-3*β*,4*β*,10,11-bisepoxy-1*β*-(2-methylbutyryloxy)bisabol-7(14)-en-2*β*-ol.

Compound **4** was found in sample D as a minor component and in sample E as a major component. It ($C_{25}H_{36}O_7$) was an isomer of **2** with two angeloyloxy groups. From the chemical shifts of H-1 (δ_H 4.40) and H-2 (δ_H 5.26), and from the presence of COSY correlation between a hydroxy proton (δ_H 3.54) and H-1, one angeloyloxy group was deduced to be attached at C-2. The relative configuration of the stereogenic centers of the cyclohexane ring was the same as those in **2** and **3** because the NOESY spectrum showed correlations of H-6/H-1, H-1/H-2, H-2/H-15, and H-15/H-4. Consequently, the structure of **4** was determined to be 2*β*,8-bisangeloyloxy-3*β*,4*β*,10,11-bisepoxybisabol-7(14)-en-1*β*-ol.

Compound **5** was found in samples D and E as a minor component. It contained a chlorine atom with the molecular formula $C_{25}H_{37}O_7Cl$. Two angeloyloxy groups were attached at C-2 and C-8 from the NMR chemical shifts. Compared with **1**, **3**, and **4**, the chemical shifts of C-3, C-4, and H-4 of **5** were observed at a lower field (C-3 δ_C 75.2, C-4 δ_C 66.0, H-4 δ_H 4.19). This observation indicated that the oxirane ring between C-3 and C-4 was cleaved. Two hydroxy protons were observed (δ_H 5.30, 3.98). The proton at δ_H 5.30 was assigned to be C-3-OH from the correlation with C-3, C-4, and C-15 in the HMBC spectrum. Similarly, the other signal (δ_H 3.98) was assigned to be C-1-OH based on correlations with H-1 in the COSY spectrum and C-1 and C-2 in the HMBC spectrum. ^1H and ^{13}C NMR chemical shifts revealed that the chlorine atom was located at C-4. The relative configuration of the stereogenic centers of the cyclohexane ring was deduced from ^1H NMR coupling constants and NOE. When H-6 (axial) is assumed to be in an α-orientation, H-2 should α-axial according to the NOESY correlation. The α-orientation of the methyl group at C-3 was deduced from the NOE correlation of H-2/H-15. The orientation of H-4 was *β*-equatorial because the $^3J_{4,5}$ values were small (dd, J = 3.0, 2.9 Hz). Since the two hydroxy protons and one of the H-5 protons (δ_H 2.73) were correlated in the NOESY spectrum, they were all *β*-axial. Therefore, the structure

of **5** was determined to be 2β,8-bisangeloyloxy-4α-chloro-10,11-epoxybisabol-7(14)-ene-1β,3β-diol.

DNA Sequencing. The base sequence of the *atpB-rbcL* intergenic region[5] on the plastid DNA in each sample was determined. As shown in Table 1, the sequence turned out to be the same in all of the samples. This lack of variation is unusual in *Ligularia*, compared with the intraspecific variations that we have observed in *Ligularia* species except for *L. cymbulifera*.[3–7,16,17]

Antimicrobial Activity. Compound **1** isolated from sample B was subjected to an antimicrobial assay. It showed a partial inhibitory zone against *Bacillus subtilis* PCI219[18] on an agar plate, whereas a one-tenth amount of erythromycin showed a clear 31 mm zone of inhibition.

We investigated the chemical constituents of *L. lankongensis* collected at seven different locations. All of the samples contained compound **1**, a highly oxygenated bisepoxybisabolene derivative. Other constituents were also bisabolane-type sesquiterpenoids. To date, several bisabolane-type sesquiterpenes have been reported from the genus *Ligularia*: *L. songarnica*,[19] *L. thyrsoidea*,[20] and *L. cymbulifera*.[21] Recently, Tan et al. isolated two bisabolanepentaol derivatives from *L. lankongensis* collected in Lijiang,[12] which is in roughly the same area as where we obtained our samples A and B. Their compounds showed the same relative configuration for the cyclohexane ring. We suppose that their compounds were formed from **1** by hydrolysis of the oxirane ring.

The isolated bisabolane compounds **1**−**5** were structurally related. No difference in the *atpB-rbcL* sequence was observed among the samples. However, the chemical composition showed intraspecific diversity (Table 1). There appears to be some correlation between the composition and the geographic distribution. The two samples collected in the Lijiang area (A and B) contained compound **1** as the sole major constituent; the two Luguhu samples (D and E) contained **1**, **4**, and **5**; the samples from northern Zhongdian (F and G) contained **1** and **2** as the major constituents; and sample C, collected at a separate location, had a slightly different composition.

Most of the other *Ligularia* species we have studied showed greater intraspecific diversity. The only exception was *L. cymbulifera*, all samples of which showed the same chemical composition and the same *atpB-rbcL* sequence. *L. cymbulifera* is very abundant in the Zhongdian area and often forms large exclusive colonies in an open field.[5] In our field observation, *L. lankongensis* was only sporadically found in forests and scrubs. Therefore, the observed diversity in the chemical constituents may reflect an accumulation of genetic variations that may be starting due to geographical separation, but which have yet to become apparent in the *atpB-rbcL* region. Further studies are necessary to clarify genetic and environmental influences.

Five bisabolane-type sesquiterpenes, four of which were new compounds, were obtained from *L. lankongensis* collected in seven different locations. Intraspecific diversity was found in the chemical composition of the bisabolane-type sesquiterpenes. In contrast, no variation was found in the nucleotide sequence of the *atpB-rbcL* region.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Horiba SEPA-300 digital polarimeter. IR spectra were recorded on a Horiba FT-720 infrared spectrometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) at 298 K. Mass spectra were obtained on a JEOL JMS-GCmateII (JMS-BU25) mass spectrometer under positive FAB mode. Column chromatography was performed on Merck Si gel 60 (60−230 mesh). Analytical TLC was carried out on Merck Si gel plates 60 F_{254} at a thickness of 0.25 mm with phosphomolybdic acid as a visualizing agent. Si gel HPLC was carried out on YMC Pack SIL A-023 (10 × 250 mm) at a flow rate of 2 mL/min. Ehrlich's test on TLC was carried out as described.[3–5]

Plant Materials. Samples of *L. lankongensis* were collected in August 2004, 2005, and 2006 in northwestern Yunnan Province (Table 1, Figure 1, A and B). Each plant was identified by X.G., one of the authors.

Extraction, Purification, and Identification of Chemical Components. The dried root of sample A (33 g) was extracted with EtOH (200 mL). A portion of the EtOH extract (100 mL) was concentrated under reduced pressure to give a residue (410 mg), which was chromatographed on Si gel. The fractions eluted by *n*-hexane−EtOAc (3:1 to 1:1) gave **1** (77.9 mg).

The dried root of sample B (22 g) was extracted with EtOAc (50 mL). A residue (208 mg) obtained from half of the extract (25 mL) was chromatographed on Si gel. A fraction eluted with *n*-hexane−EtOAc (1:1) gave crude **1** (22 mg, ~90% purity by ^{1}H NMR).

The dried root of sample C (31 g) was extracted with EtOH (100 mL). An oil (380 mg) from half of the extract was chromatographed on Si gel. Fractions eluted with *n*-hexane−EtOAc (1:2) (63 mg) were further purified by Si gel chromatography with *n*-hexane−EtOAc (2:1) to give **1** (34 mg) and a mixture of **2** and **3** (7.0 mg). The mixture was purified by Si gel HPLC with *n*-hexane−EtOAc (3:1) to give **3** (0.4 mg).

The dried root of sample D (13 g) was extracted with EtOH (70 mL). An oil (380 mg) from half of the extract was chromatographed on Si gel. A fraction eluted with *n*-hexane−EtOAc (1:2) (27 mg) was purified by Si gel HPLC with *n*-hexane−EtOAc (3:1) to give **4** (2.1 mg). Another fraction eluted with *n*-hexane−EtOAc (1:2) (50 mg) was purified by open column chromatography on Si gel followed by HPLC with *n*-hexane−EtOAc (3:1) to give **2** (2.6 mg, t_R 19 min), **5** (0.8 mg, t_R 22 min), and **1** (1.0 mg, t_R 29 min).

The dried root of sample E (10 g) was extracted with EtOAc (70 mL). Half of the extract (35 mL) was concentrated to give a residue (180 mg), which was chromatographed on Si gel. A fraction eluted with *n*-hexane−EtOAc (2:1) gave crude **4** (19 mg, ~90% purity by ^{1}H NMR). Fractions eluted with *n*-hexane−EtOAc (1:1) gave crude **5** (13 mg, ~30% purity by ^{1}H NMR) and crude **1** (10 mg, ~70% purity by ^{1}H NMR).

The dried root of sample F (12 g) was extracted with EtOAc (60 mL). A residue (180 mg) obtained from half the extract (30 mL) was chromatographed on Si gel. Fractions eluted with *n*-hexane−EtOAc (1:2) gave a 1:1 mixture (48 mg) of **1** and **2**, based on the ^{1}H NMR spectrum.

The dried root of sample G (10 g) was extracted with EtOAc (80 mL), and half of the extract was concentrated to give a residue (112 mg). After chromatography on Si gel, a fraction eluted with *n*-hexane−EtOAc (1:1) gave **1** (18 mg). A fraction eluted with EtOAc gave **2** (15 mg).

1β,5α,8-Trisangeloyloxy-3β,4β,10,11-bisepoxybisabol-7(14)-en-2β-ol (1): colorless oil; $[\alpha]^{27}_D$ 4.3 (*c* 1.00, $CHCl_3$); IR (film) ν_{max} 3446, 1716, 1647, 1227, 1144 cm^{-1}; ^{1}H and ^{13}C NMR (Tables 2, 3); FABMS *m/z* 547, 447, 347, 247 $(M + H)^+$; HRFABMS calcd for $C_{30}H_{43}O_9$ $(M + H)^+$ 547.2907, found *m/z* 547.2922.

1β,8-Bisangeloyloxy-3β,4β,10,11-bisepoxybisabol-7(14)-en-2β-ol (2): colorless oil; $[\alpha]^{28}_D$ −6.8 (*c* 0.21, acetone); IR (film) ν_{max} 3448, 1716, 1647, 1228, 1153 cm^{-1}; ^{1}H and ^{13}C NMR (Tables 2, 3); FABMS *m/z* 449 $(M + H)^+$, 349, 249; HRFABMS calcd for $C_{25}H_{37}O_7$ $(M + H)^+$ 449.2539, found *m/z* 449.2524.

8-Angeloyloxy-3β,4β,10,11-bisepoxy-1β-(2-methylbutyryloxy)bisabol-7(14)-en-2β-ol (3): colorless oil; $[\alpha]^{28}_D$ −15 (*c* 0.04, acetone); IR (film) ν_{max} 3462, 1716, 1647, 1228, 1151 cm^{-1}; ^{1}H and ^{13}C NMR (Tables 2, 3); FABMS *m/z* 451 $(M + H)^+$, 351; HRFABMS calcd for $C_{25}H_{39}O_7$ $(M + H)^+$ 451.2696, found *m/z* 451.2693.

2β,8-Bisangeloyloxy-3β,4β,10,11-bisepoxybisabol-7(14)-en-1β-ol (4): colorless oil; $[\alpha]^{28}_D$ −52 (*c* 0.26, acetone); IR (film) ν_{max} 3470, 1716, 1647, 1228, 1153 cm^{-1}; ^{1}H and ^{13}C NMR (Tables 2, 3); FABMS *m/z* 449 $(M + H)^+$, 349, 249; HRFABMS calcd for $C_{25}H_{37}O_7$ $(M + H)^+$ 449.2539, found *m/z* 449.2553.

2β,8-Bisangeloyloxy-4α-chloro-10,11-epoxybisabol-7(14)-ene-1β,3β-diol (5): colorless oil; $[\alpha]^{28}_D$ −66 (*c* 0.08, acetone); IR (film) ν_{max} 3410, 1716, 1647, 1228, 1151 cm^{-1}; ^{1}H and ^{13}C NMR (Tables 2, 3); FABMS *m/z* 485, 487 (3:1; $(M + H)^+$), 385, 387, 285, 287; HRFABMS calcd for $C_{25}H_{38}O_7Cl$ $(M + H)^+$ 485.2306, found *m/z* 485.2312.

DNA Sequencing. The base sequence of the *atpB-rbcL* intergenic region was determined as described.[5]

Antimicrobial Assay. *Bacillus subtilis* PCI 219 was used for the assay. A preculture (18–22 h at 37 °C) was spread onto an agar plate

containing bouillon (2.3%). A paper disk (8 mm in diameter) was treated with a DMSO solution (20 μL) of the test compound (10 mg/mL) and placed on the agar plate. The diameter of the inhibition zone was observed after incubation at 37 °C for 24 h.

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References and Notes

(1) Jeffrey, C.; Chen, Y. L. *Kew Bull.* **1984**, *39*, 205–446.
(2) Liu, S.-W.; Deng, D.-S.; Liu, J.-Q. *Acta Phytotaxonom. Sin.* **1994**, *32*, 514–524.
(3) Nagano, H.; Iwazaki, Y.; Gong, X.; Shen, Y.; Kuroda, C.; Hanai, R. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 300–304.
(4) Tori, M.; Honda, K.; Nakamizo, H.; Okamoto, Y.; Sakaoku, M.; Takaoka, S.; Gong, X.; Shen, Y.; Kuroda, C.; Hanai, R. *Tetrahedron* **2006**, *62*, 4988–4995.
(5) Hanai, R.; Gong, X.; Tori, M.; Kondo, S.; Otose, K.; Okamoto, Y.; Nishihama, T.; Murota, A.; Shen, Y.; Wu, S.; Kuroda, C. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 1302–1308.
(6) Torihata, A.; Hanai, R.; Gong, X.; Shen, Y.; Kuroda, C. *Chem. Biodiversity* **2007**, *4*, 500–507.
(7) Kuroda, C.; Kiuchi, K.; Torihata, A.; Takeshita, K.; Gong, X.; Shen, Y.; Hirota, H.; Onuki, H.; Hanai, R. *Chem. Biodiversity* **2007**, *4*, 2210–2217.
(8) Soltis, D. E.; Soltis, P. S. In *Molecular Systematics of Plants. II DNA Sequencing*; Soltis, P. S., Soltis, D. E., Doyle, J. J., Eds.; Kluwer Academic: Boston, 1998; pp 1–41..
(9) Liu, S.-W. *Flora Reipublicae Popularis Sinicae*; Science: Beijing 1989; Vol. 77, pp 4–115..
(10) Min, T.-L. *Flora Yunnanica*; Science: Beijing, 2004; Vol. 13, pp 406–515..
(11) Tan, A.-M.; Li, Y.-S.; Yang, H.; Wang, Z.-T.; He, H.-P.; Zhang, M.; Hao, X.-J. *Chin. Chem. Lett.* **2004**, *15*, 68–70.
(12) Tan, A.-M.; He, H.-P.; Zhang, M.; Wang, Z.-T.; Hao, X.-J. *Helv. Chim. Acta* **2007**, *90*, 101–104.
(13) We checked the TLC and column chromatography patterns of both EtOH and EtOAc extracts of samples B and C, respectively, and we could not detect any componential difference between the EtOH extract and the EtOAc extract.
(14) (a) Kuroda, C.; Ueshino, T.; Nagano, H. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 1737–1740. (b) Kuroda, C.; Nishio, E. *Nat. Prod. Commun.*, in press.
(15) Bohlmann, F.; Zdero, C. *Phytochemistry* **1978**, *17*, 1669–1671.
(16) Nagano, H.; Iwazaki, Y.; Matsushima, M.; Sato, M.; Gong, X.; Shen, Y.; Hirota, H.; Kuroda, C.; Hanai, R. *Chem. Biodiversity* **2007**, *4*, 2874–2888.
(17) Kuroda, C.; Hanai, R.; Gong, X.; Shen, Y.; Tori, M. *J. Fudan Univ.* **2005**, *44*, 742–743.
(18) Fujiwara, T.; Kondo, E. *J. Antibiot.* **1981**, *34*, 13–15.
(19) (a) Fu, B.; Yang, X.-P.; Wang, J.; Jia, Z.-J. *Chin. Chem. Lett.* **1999**, *10*, 29–30. (b) Fu, B.; Zhu, Q.-X.; Yang, X.-P.; Jia, Z.-J. *Pharmazie* **1999**, *54*, 620–624. (c) Fu, B.; Li, Y.; Yang, X.-P.; Li, X.-P.; Jia, Z.-J. *Pharmazie* **2000**, *55*, 947–952.
(20) (a) Liao, J.-C.; Zhu, Q.-X.; Yang, H.; Jia, Z.-J. *J. Chin. Chem. Soc.* **1999**, *46*, 185–190. (b) Liao, J.-C.; Yang, L.; Jia, Z.-J. *Chem. Res. Chin. Univ.* **1999**, *15*, 67–69.
(21) Liu, C.-M.; Fei, D.-Q.; Wu, Q.-H.; Gao, K. *J. Nat. Prod.* **2006**, *69*, 695–699.

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