Bisabolane Sesquiterpenes from the Roots of *Ligularia cymbulifera*

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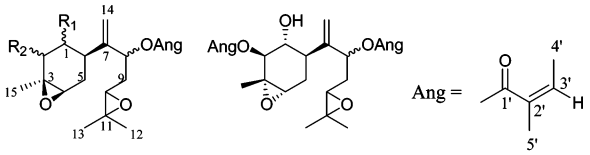
Eight new highly oxygenated bisabolane sesquiterpenes (**1**−**8**), of which one contains a chlorine atom, were obtained in a phytochemical investigation of the roots of *Ligularia cymbulifera*, and their structures were elucidated by interpretation of spectroscopic data. Their relative configurations were clarified by a detailed analysis of ^{1}H NMR coupling constants and by NOE experiments. Compounds **1**−**8** were evaluated for antimicrobial activity against three bacterial cultures and a yeast culture.

Much attention has been focused on *Ligularia* (Compositae) for the long history of use of members of this genus as folk remedies due to their antibiotic, antiphlogistic, and antitumor activities.[1] Selected species of *Ligularia* have been studied by our research group, and several types of sesquiterpenoids were found.[2−5] In the course of our continuing research into this genus, we have made a systematic study of *Ligularia cymbulifera* (W. W. Smith) Hand.-Mazz. From this plant, the isolation of several furanoeremophilane sesquiterpenes has been reported.[6] We have now obtained eight new highly oxygenated bisabolane sesquiterpenes (**1**−**8**) from this species, and their isolation, structure determination, and antimicrobial evaluation are described in this report.

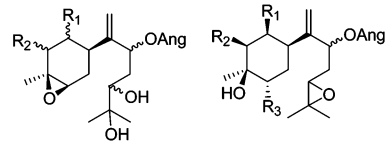


1 R_1 = αOH, R_2 = αOAng

3 R_1 = βOAng, R_2 = βOAng

4 R_1 = βOAng, R_2 = 2H

2



5 R_1 = βOAng, R_2 = βOH

6 R_1 = αOAng, R_2 = O=

7 R_1 = OH, R_2 = OAng, R_3 = OAng

8 R_1 = OAng, R_2 = OH, R_3 = Cl

Compound **1** was obtained as a colorless gum, showing a green spot on TLC when sprayed with 5% sulfuric acid reagent followed by heating on a hot plate. Its molecular formula was determined to be $C_{25}H_{36}O_7$ (eight degrees of unsaturation) by positive HRESIMS (*m*/*z* 471.2347, calcd for $C_{25}H_{36}O_7Na$ $[M + Na]^+$ 471.2353). The IR spectrum showed absorption bands for a hydroxyl group (3489 cm^{-1}), double bonds (1647 cm^{-1}), and ester carbonyl groups (1715 cm^{-1}). Two angeloyloxy groups were present in **1**, as evidenced from the observed ^{1}H NMR signals [δ 6.06 × 2 (1H each, qq, J = 7.6, 1.5 Hz), 1.96 × 2 (3H each, dq, J = 7.6, 1.2 Hz), 1.91(3H, dq, J = 1.5, 1.2 Hz), and 1.84 (3H, dq, J = 1.5,1.2 Hz)] in combination with the ^{13}C NMR signals [δ 167.1 and 166.6; 139.0 and 138.9; 127.3 and 127.3; 20.4 and 20.4; 15.8 and 15.7].[7] In addition, its FABMS also gave a series of characteristic fragment peaks at *m*/*z* 349 [*m*/*z* 449 − AngOH]$^+$ and 249 [*m*/*z* 449 − 2 × AngOH]$^+$. Apart from these two angeloyloxy groups, the ^{13}C NMR and DEPT spectroscopic data of compound **1** revealed 15 skeletal carbon signals: three methyls, three methylenes, six methines, and three quaternary carbons, among which there were seven oxygen-bearing carbon signals (between δ 57.0 and 75.0). The signals at δ 61.2, 60.8, 60.6, and 58.2 were characteristic signals for two epoxy groups.[8] On the basis of these observations, **1** was proposed as having a monocyclic sesquiterpene skeleton with two angeloyloxy units, two epoxy groups, a hydroxyl, and a terminal double bond [δ 146.9 (C) and 114.8 (CH_2)]. The ^{1}H−^{1}H COSY spectrum of **1** showed two main structural sequences, −CH−CH_2−CH−CH−CH− and −CH−CH_2−CH−, which were connected by the following long-range ^{1}H−^{13}C correlations in the HMBC spectrum: H-15/C-2; H-2, H-4, H-15/C-3; H-5α, H-15/C-4; H-5α, H-14a, H-14b/C-6; H-8, H-9a, H-9b, H-14a, H-14b/C-7; H-9a, H-9b, H-14a, H-14b/C-8; H-8, H-10/C-9; H-8, H-9a, H-9b, H-12, H-13/C-10; and H-12, H-13/C-11. Consequently, **1** was determined as being a highly oxygenated diepoxy bisabolane sesquiterpene.[8,9]

Furthermore, the HMBC spectrum of compound **1** showed that the ester carbonyl carbons of two angeloyloxy groups exhibited cross-peaks with the signals of H-2 and H-8, which indicated that these two angeloyloxy groups are attached to C-2 and C-8, respectively. Thus, the positions of the other substituent groups were determined as 1-hydroxy, 3,4-epoxy, and 10,11-expoxy. The configuration of **1** was deduced from the ^{1}H NMR coupling constants: if H-6 is α-oriented, H-1 should be β, because of the large coupling constant observed between H-1 with H-6 ($J_{1,6}$ = 11.1 Hz). Similarly, H-2 should have a β-configuration because of the small coupling constant between H-2 with H-1 ($J_{1,2}$ = 3.6 Hz), and H-4 must be α-oriented because of the small coupling constant ($J_{4\alpha,5\alpha}$ = 4.8 Hz) observed. The coupling constant of H-5β with H-4α was almost zero because their dihedral angle was about 90° due to the 3β,4β-epoxy group (shown by a molecular modeling). The NOESY spectrum of **1** exhibited correlations of H-1 with H-2 and H-5β, and H-4 with H-5α, H-6, and H-15. Accordingly, **1** was determined as 2α,8-diangeloyloxy-3β,4β,10,11-diepoxy-1α-hydroxybisabol-7(14)-ene.

Compound **2** was obtained as a colorless gum, showing also a green spot on TLC when sprayed with 5% sulfuric acid reagent followed by heating on a hot plate. Its molecular formula was also determined to be $C_{25}H_{36}O_7$ on the basis of HRESIMS (*m*/*z* 471.2353, calcd for $C_{25}H_{36}O_7Na$ $[M + Na]^+$ 471.2353). This compound gave the same $[M + H]^+$ and fragment peaks as **1** in the FABMS and also had similar features in the ^{1}H NMR and ^{13}C NMR spectra. Thus, **2** was assigned as an isomer of **1**. A close comparison of the NMR data of **2** with **1** showed that the H-2 proton signal of **2** exhibited a downfield shift from δ 5.12 to δ 5.38 and

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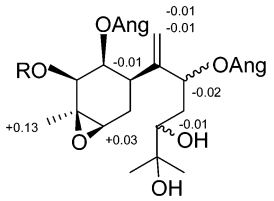
the H-6 proton signal showed an upfield shift from δ 3.17 to δ 2.26. In turn, the signal of C-2 was shifted upfield from δ 72.8 to δ 72.1 and the signal of C-3 was shifted upfield from δ 60.6 to δ 58.5, which could be attributed to a 3α,4α-epoxy group. In a NOE experiment, irradiation of H-15 enhanced the signals of H-1 (3.56%) and H-4 (4.10%), and irradiation of H-6 enhanced the signals of H-2 (2.18%) and H-5α (2.00%). Hence, **2** was proposed as 2*β*,8-diangeloyloxy-3α,4α,10,11-diepoxy-1α-hydroxybisabol-7(14)-ene.

Most of the NMR spectra of **3** [HRESIMS *m*/*z* 548.3223 $[M + NH_4]^+$ (calcd for $C_{30}H_{42}O_8NH_4$, 548.3218)] were very similar to those of **1**, with the exception that signals for an additional angeloyloxy group were apparent. The HMBC spectrum showed cross-peaks for the ester carbonyl carbons of three angeloyloxy groups with the H-1, H-2, and H-8 signals and indicated that these angeloyloxy groups are attached to C-1, C-2, and C-8, respectively. The configuration of **3** was determined from the ^{1}H NMR spectroscopic splitting pattern of H-1 and H-6 ($J_{1,6}$ almost zero) and the NOE difference spectra, in which irradiation of H-6 enhanced the signals of H-1 (3.78%) and H-2 (3.90%), and irradiation of H-15 enhanced the signals of H-2 (1.65%) and H-4 (1.65%). Thus, the angeloyloxy groups at C-1 and C-2 and the epoxy group at C-3 and C-4 were all *β*-oriented. Therefore, the structure of **3** was assigned as 3*β*,4*β*,10,11-diepoxy-1*β*,2*β*,8-triangeloyloxybisabol-7(14)-ene.

Compound **4** was obtained as a colorless gum. Its HRESIMS [*m*/*z* 450.2841 $[M + NH_4]^+$ (calcd for $C_{25}H_{36}O_6NH_4$, 450.2850)] revealed a molecular formula of $C_{25}H_{36}O_6$. This isolate was also found to possess a diepoxy bisabolane skeleton by comparing its NMR spectra with those of **1**−**3**. The NMR spectra of **4** were very similar to those of **3** except for the presence of one less angeloyloxy group. A signal for an oxygen-bearing carbon (δ 71.9) in **3** was absent, and a methylene signal (δ 30.8, CH_2) was apparent in the ^{13}C NMR spectrum of **4**. Two ester carbonyl groups exhibited cross-peaks in the HMBC spectrum at δ 166.7/H-1 and 166.7/H-8, and this information was used to place the two angeloyoxy groups at C-1 and C-8. In the ^{1}H NMR spectrum, H-1 showed a one-proton multiplicity due to two protons at C-2, which was in agreement with the HMBC experiment observed. The configuration of **4** was identical to that of **3** and was confirmed by a NOE difference spectrum as follows: irradiation of H-15 enhanced the signals of H-1 (3.16%), H-4 (2.28%), and H-6 (1.32%). Accordingly, compound **4** was proposed as 1*β*,8-diangeloyloxy-3*β*,4*β*,10,11-diepoxybisabol-7(14)-ene.

The molecular formula of **5** was determined as $C_{25}H_{38}O_8$ by the HRESIMS at *m*/*z* 489.2455 $[M + Na]^+$ (calcd for $C_{25}H_{38}O_8Na$, 489.2459). The spectroscopic data of **5** showed a close resemblance to those of **3** and exhibited 15 skeletal carbons with seven oxygen-bearing carbon signals between δ 57.0 and 77.0. However, compound **5** exhibited signals for one less angeloyoxy group, and the H-10 proton shifted downfield from δ 2.71 to δ 3.35 in the ^{1}H NMR spectrum. In the ^{13}C NMR spectrum, the corresponding C-10 and C-11 signals shifted downfield from δ 60.7 and 58.2 to δ 74.6 and 72.5, respectively, which indicated that the epoxy ring at C-10 and C-11 in **3** was absent in **5**. The HMBC spectrum of **5** showed cross-peaks between δ 168.0/H-1; 167.8/H-8; 74.6/H-8, H-9, H-12, H-13; and 72.5/H-12, H-13, from which it was concluded that two angeloyloxy groups are attached to C-1 and C-8 and two hydroxyl groups are at C-10 and C-11. Comparison of the coupling constants of **5** and **3** showed they have the same stereochemistry, and the NOE difference spectrum confirmed this deduction: irradiation of H-2 enhanced the signals of H-1 (6.26%), H-6 (4.02%), and H-15 (3.45%). Accordingly, the structure of **5** was elucidated as 1*β*,8-diangeloyloxy-3*β*,4*β*-epoxy-2*β*,10,11-trihydroxybisabol-7(14)-ene. Compound **5** was treated with (*R*)- and (*S*)-MTPA-Cl, and the (*S*)- and (*R*)-MTPA esters at C-2 of **5** (**5a** and **5b**) were obtained, respectively.[10] Comparison of the ^{1}H NMR chemical shifts for **5a** and **5b** (△ values shown in Figure 1) led to the assignment of the *S*-configuration at C-2 in **5**. Therefore, this compound was determined as (1*S*,2*S*,3*R*,4*R*,6*R*)-1,8-diangeloyloxy-3,4-epoxy-2,-10,11-trihydroxybisabol-7(14)-ene.



5a R = (*S*)-MTPA
5b R = (*R*)-MTPA

△δ = δ(*S*)-MTPA - δ(*R*)-MTPA (ppm)

Figure 1. ^{1}H NMR chemical shift differences **[**δ(*S*)-MTPA − δ-(*R*)-MTPA**]** of the MTPA esters for **5**.

A molecular formula of $C_{25}H_{36}O_8$ was established for compound **6** from its HRESIMS {*m*/*z* 487.2306 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_8Na$, 487.2302)}. The NMR spectra showed this compound to be another bisabolane-type sesquiterpene with two angeloyloxy ester units, two hydroxyls, an epoxy group, a terminal double bond, and a ketone carbonyl (δ 201.8).[2] Further analysis of the NMR data of **6** indicated that it has the same side chain as **5**. The HMBC spectrum revealed cross-peaks of two ester carbonyl groups (δ 167.2/H-1 and 167.0/H-8) and the ketone carbonyl (δ 201.8/H-1, H-15) and was used to assign the two angeloyloxy groups at C-1 and C-8 and the ketone carbonyl at C-2. The relative configuration of **6** was elucidated from the ^{1}H NMR coupling constants. If H-6 is α-oriented, H-1 must be *β*-oriented, since a large coupling constant was observed between H-1 and H-6 ($J_{1,6}$ = 12.3 Hz). The 3,4-epoxy group was *β*-oriented because of the NOE correlation observed of H-6 with H-15 and H-4. Consequently, the structure of **6** was elucidated as 1α,8-diangeloyloxy-10,11-dihydroxy-3*β*,4*β*-epoxybisabol-7(14)-en-2-one.

Compound **7**, a colorless gum {HRESIMS *m*/*z* 566.3324 $[M + NH_4]^+$ (calcd for $C_{30}H_{44}O_9NH_4$, 566.3328)}, was purified from a mixture of **7** and **8** by HPLC. The structure and functional groups present were similar to **3** except that an epoxy group in **3** was replaced by two oxygen functions in **7**. This was supported by the downfield shifts of H-4 (δ 5.11), C-4 (δ 75.7), and C-3 (δ 73.7) in the NMR spectra. In the HMBC spectrum, the positions of the three angeloyloxy groups were fixed at C-2, C-4, and C-8 from the correlated peaks observed (δ 167.8/H-2, 167.4/H-4, and 166.4/H-8). From the ^{1}H NMR coupling constants (Table 1) and NOE difference spectra, in which irradiation of H-15 enhanced the signals of H-1 (2.58%) and H-2 (3.00%), and irradiation of H-6 enhanced the signals of H-1 (2.58%) and H-2 (6.50%), the configurations of H-1, H-2, and H-15 were determined as α-oriented with H-4 being *β*-oriented. Therefore, the structure of **7** was assigned as 10,11-epoxy-1*β*-hydroxy-2*β*,4α,8-triangeloyloxybisabol-7(14)-ene.

The FABMS of **8** gave a protonated molecular ion peak at *m*/*z* 485 $[M + H]^+$, accompanied by an isotopic peak at *m*/*z* 487 (their relative abundance ratio was 3:1), and a series of characteristic isotopic fragment peaks with the ratio of 3:1 at *m*/*z* 467/469, 385/387, 367/369, 285/287, and 267/269, suggesting the presence of a chlorine atom.[11] Its HRESIMS {*m*/*z* 485.2301 $[M + H]^+$ (calcd for $C_{25}H_{38}O_7Cl$, 485.2301)} further revealed a molecular formula of $C_{25}H_{37}O_7Cl$. Its NMR spectra indicated that it was also a bisabolane sesquiterpene and very similar to **7** except for the presence of a chlorine atom and in having one less angeloyloxy group in **8**. The NMR signals were assigned using the HMBC spectrum, in which the signals of the quaternary carbons (δ 167.0 and 166.7) of the two angeloyloxy groups showed cross-peaks with the signals of H-1 (δ 5.57) and H-8 (δ 5.65), and indicated that the angeloyloxy groups were affixed to C-1 and C-8, respectively.

Table 1. ^{1}H NMR Data of Compounds **1**−**8** (300 MHz, $CDCl_3$, δ ppm)

	1	2	3	4	5	6	7	8
1	3.92 (dd, 11.1, 3.6)	3.99 (dd, 9.0, 4.8)	5.31 (brd, 4.8)	5.26 (m)	5.40 (brd, 3.0)	5.89 (d, 12.3)	4.22(brd 2.1)	5.57 (brd, 3.3)
2	5.12 (d, 3.6)	5.38 (d, 4.8)	5.33 (d, 4.8)	1.66 (m) 1.66 (m)	4.04 (d, 3.0)		5.05 (d, 2.1)	3.99 (d, 3.3)
4	3.24 (brd,4.8)	3.22 (brd,6.0)	3.14 (brd, 4.2)	3.13 (brd, 3.9)	3.19 (brd 5.4)	3.45(brd,4.2)	5.11(brd,2.4)	4.24 (t, 2.1)
5α	2.26 (brdd, 14.6, 4.8)	2.41 (brdd, 12.3, 6.0)	1.92 (overlapped)	2.15 (brd, 12.0)	1.97 (overlapped)	2.75 (m)	1.72 (brdd, 14.1, 3.0)	1.82 (overlapped)
5β	2.12 (brd, 14.6)	2.11(brd, 12.3)	2.11 (ddd 15.3,11.1,4.2)	2.32 (ddd, 12.0,11.1,3.9)	1.81 (overlapped)	2.19 (brdd, 14.7,12.7)	2.48 (brtd, 14.1, 2.4)	2.61 (brtd, 14.7, 2.1)
6	3.17 (brd, 11.1)	2.26 (brd, 9.0)	2.47 (brdd 11.1, 4.5)	1.80 (brdd, 11.1, 4.8)	2.48 (brdd 12.3, 6.0)	2.78 (m)	2.82 (brdd, 14.1, 3.0)	3.08 (brd, 14.7)
8	5.33 (m)	5.38 (m)	5.41 (m)	5.38 (m)	5.37 (m)	5.28 (m)	5.42 (m)	5.65 (t,7.5)
9a	1.95 (overlapped)	2.01 (overlapped)	2.03 (m)	1.98 (overlapped)	2.01 (m)	1.82 (overlapped)	2.01 (overlapped)	2.02 (overlapped)
9b	1.82 (overlapped)	1.82 (m)	1.90 (overlapped)	1.86 (m)	1.88 (overlapped)	1.74 (m)	1.81 (m)	1.90 (overlapped)
10	2.73 (t, 5.1)	2.78 (t, 6.0)	2.71 (t, 5.4)	2.80 (t, 4.5)	3.35 (brd, 10.8)	3.58 (brd, 10.8)	2.84 (t, 6.0)	2.81 (t, 5.7)
12	1.23 (s)	1.28 (s)	1.19 (s)	1.30 (s)	1.13 (s)	1.52 (s)	1.30(s)	1.28 (s)
13	1.23 (s)	1.28 (s)	1.19 (s)	1.30 (s)	1.19 (s)	1.57 (s)	1.26 (s)	1.28 (s)
14a	5.30 (brs)	5.23 (brs)	5.18 (brs)	5.14 (brs)	5.17 (brs)	5.22 (brs)	5.30 (brs)	5.30 (brs)
14b	5.25 (brs)	4.99 (brs)	4.91 (brs)	4.98 (brs)	4.77 (brs)	5.10 (brs)	5.18 (brs)	5.03 (brs)
15	1.30 (s)	1.43 (s)	1.25 (s)	1.36 (s)	1.40 (s)	1.51 (s)	1.16 (s)	1.45 (s)
OAng								
3′	6.06 (qq, 7.6, 1.5) 6.06 (qq, 7.6, 1.5)	6.08 (qq, 7.2, 1.5) 6.08 (qq, 7.2, 1.5)	6.03 (qq, 7.5, 1.2) 6.03 (qq, 7.5, 1.2) 6.03 (qq, 7.5, 1.2)	6.11 (qq, 7.3, 1.5) 6.11 (qq, 7.3, 1.5)	6.08 (qq, 6.9, 1.5) 6.08 (qq, 6.9, 1.5)	6.07 (qq, 7.3, 1.5) 6.14 (qq, 7.3, 1.5)	6.15 (qq, 6.9, 1.5) 6.15 (qq, 6.9, 1.5) 6.15 (qq, 6.9, 1.5)	6.12 (qq, 7.2, 1.5) 6.12 (qq, 7.2, 1.5)
4′	1.96 (dq, 7.6, 1.2) 1.96 (dq, 7.6, 1.2)	2.02 (dq, 7.2, 1.2) 1.97 (dq, 7.2, 1.2)	1.90 (dq, 7.5, 0.9) 1.90 (dq, 7.5, 0.9) 1.90 (dq, 7.5, 0.9)	2.02 (dq, 7.3, 1.2) 2.00 (dq, 7.3, 1.2)	1.97 (dq, 6.9, 1.2) 1.97 (dq, 6.9, 1.2)	2.02 (dq, 7.3, 1.2) 2.02 (dq, 7.3, 1.2)	2.05 (dq, 6.9, 1.2) 2.05 (dq, 6.9, 1.2) 2.05 (dq, 6.9, 1.2)	2.02 (dq, 7.2, 1.2) 2.02 (dq, 7.2, 1.2)
5′	1.91 (dq, 1.5, 1.2) 1.84 (dq, 1.5, 1.2)	1.90 (dq, 1.5, 1.2) 1.90 (dq, 1.5, 1.2)	1.80 (dq, 1.2, 0.9) 1.80 (dq, 1.2, 0.9) 1.78 (dq, 1.2, 0.9)	1.91 (dq, 1.5, 1.2) 1.91 (dq, 1.5, 1.2)	1.82 (dq, 1.5, 1.2) 1.82 (dq, 1.5, 1.2)	1.92 (dq, 1.5, 1.2) 1.86 (dq, 1.5, 1.2)	1.92 (dq, 1.5, 1.2) 1.89 (dq, 1.5, 1.2) 1.88 (dq, 1.5, 1.2)	1.93 (dq, 1.5, 1.2) 1.84 (dq, 1.5, 1.2)

Similarly, the oxygen-bearing carbons showed cross-peaks between δ 70.3/H-4, H-1, and H-6 and δ 74.8/H-1, H-2, H-4, and H-15, suggesting that an OH was located at both C-2 and C-3. Because **8** had the same partial structure as **7** from C-6 to C-13, the chlorine atom must be located at the C-4 position. The configuration of **8** was assigned as identical to that of **7** due to their similar ^{1}H NMR spectra (Table 1). Therefore, the structure of compound **8** was assigned as 4α-chloro-1β,8-diangeloyloxy-10,11-epoxy-2β-hydroxybisabol-7(14)-ene.

The antimicrobial activities of compounds **1**−**8** were tested against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), clinically isolated methicillin-resistant *Staphylococcus aureus* (MRSA), and *Candida albicans.* However, none of these compounds showed appreciable antimicrobial activity (MIC $>$ 62.5 μg/mL for all organisms tested).

Experimental Section

General Experimental Procedure. Optical rotations were measured using a Perkin-Elmer model 341 polarimeter. IR spectra were obtained on a Nicolet NEXUS 670 FT-IR spectrometer. NMR spectra were recorded on a Varian Mercury-300BB NMR instrument with TMS as internal standard and $CDCl_3$ as solvent. EIMS were measured on a VG ZABHS mass spectrometer at 70 eV. FABMS were recorded on a ZAB-HS mass spectrometer, and HRESIMS were carried out on a Bruker APEX II mass spectrometer with glycerol as the matrix. Normal-phase HPLC was conducted on a Nova-Pak silica column (7.8 mm i.d. × 300 mm; Waters). Silica gel (200−300 mesh) was used for column chromatography and silica GF_{254} (10−40 μm) for TLC, both supplied by the Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. TLC was detected at 254 nm or by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v).

Plant Material. The roots of *Ligularia cymbulifera* were collected in Muli, Sichuan Province, People's Republic of China, in August 2004. The plant was identified by Prof. Guo-Liang Zhang, Department of Biology, Lanzhou University. A voucher specimen (No. 2004814) was deposited in the College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation. Powdered, dried rhizomes of *L. cymbulifera* (1.6 kg) were extracted three times (each for 7 days) successively with petroleum ether (60−90 °C)−diethyl ether−methanol (1:1:1) (5 L each) at room temperature. The resultant extract was concentrated under reduced pressure to yield a residue (140 g), which was chromatographed on a silica gel (200−300 mesh, 1660 g, 8.0 × 140 cm) column, eluting successively with a gradient of petroleum ether (60−90 °C)−EtOAc (50:1, 25:1, 10:1, 2:1, and 0:1). According to the differences in composition indicated by TLC, five crude fractions (A−E) were collected. Fractions C (10:1) and D (2:1) were pooled (31 g) and further purified, and then subjected to column chromatography on silica gel (250 g), with elution by petroleum ether−EtOAc (10:1−0:1) to afford three fractions (D1−D3). From fraction D1 (1 g), compounds **3** (62 mg, R_f 0.75, petroleum ether−EtOAc, 2:1) and **4** (10 mg, R_f 0.55, petroleum ether−EtOAc, 3:1) were obtained by repeated column chromatography on silica gel with petroleum ether−EtOAc (8:1). Fraction D2 (2.5 g) was separated by column chromatography on a silica gel column (48 g), eluting with petroleum ether−acetone (7:1), to yield **1** (150 mg, R_f 0.60, petroleum ether−EtOAc, 2:1). Fraction D3 (2 g) was further separated by column chromatography with petroleum ether−EtOAc (4:1) and petroleum ether−acetone (3:1), then

Table 2. ^{13}C NMR Data of Compounds **1**−**8** (75 MHz $CDCl_3$, δ ppm)

	1	2	3	4	5	6	7	8
1	69.8 d	70.1 d	67.5 d	73.6 d	70.2 d	73.9 d	72.9 d	72.6 d
2	72.8 d	72.1 d	71.9 d	30.8 t	71.7 d	201.8 s	73.3 d	70.3 d
3	60.6 s	58.5 s	56.3 s	58.1 s	58.3 s	61.4 s	73.7 s	74.8 s
4	61.2 d	61.0 d	59.5 d	61.0 d	60.5 d	64.1 d	75.7 d	64.5 d
5	25.5 t	25.1 t	25.7 t	31.0 t	24.7 t	31.4 t	24.6 t	29.4 t
6	39.7 d	39.0 d	38.1 d	35.3 d	40.0 d	45.0 d	40.4 d	35.4 d
7	146.9 s	146.5 s	145.8 s	151.0 s	147.9 s	148.7 s	148.1 s	145.4 s
8	74.1 d	74.0 d	74.3 d	73.2 d	73.0 d	72.0 d	69.0 d	73.2 d
9	33.6 t	33.4 t	33.3 t	33.9 t	36.8 t	36.8 t	34.8 t	32.7 t
10	60.8 d	61.0 d	60.7 d	60.8 d	74.6 d	75.0 d	60.7 d	61.0 d
11	58.2 s	58.5 s	58.2 s	58.4 s	72.5 s	74.4 s	58.5 s	58.3 s
12	18.8 q	18.9 q	19.5 q	18.9 q	23.7 q	27.3 q	19.0 q	18.9 q
13	24.5 q	24.6 q	24.5 q	24.6 q	25.9 q	29.6 q	25.7 q	24.2 q
14	114.8 t	114.7 t	115.1 t	111.0 t	113.5 t	110.6 t	115.9 t	115.5 t
15	19.0 q	19.7 q	18.8 q	19.1 q	19.6 q	14.9 q	22.3 q	24.6 q
OAng								
1′	167.1 s	167.8 s	166.7 s	166.7 s	168.0 s	167.2 s	167.8 s	167.0 s
	166.6 s	166.9 s	166.6 s	166.7 s	167.8 s	167.0 s	167.4 s	166.7 s
			166.6 s				166.4 s	
2′	127.3 s	127.4 s	127.6 s	127.6 s	127.4 s	127.4 s	127.6 s	127.5 s
	127.3 s	127.4 s	127.3 s	127.6 s	127.4 s	127.4 s	127.6 s	126.4 s
			127.1 s				127.1 s	
3′	139.0 d	139.4 d	139.1 d	138.8 d	139.7 d	139.4 d	140.1 d	140.8 d
	138.9 d	139.1 d	139.1 d	138.2 d	139.0 d	138.7 d	139.1 d	139.0 d
			138.0 d				139.1 d	
4′	15.8 q	15.9 q	15.7 q	15.8 q	15.9 q	15.9 q	15.9 q	15.8 q
	15.7 q	15.8 q	15.7 q	15.8 q	15.8 q	15.9 q	15.9 q	15.8 q
			15.5 q				15.9 q	
5′	20.4 q	20.7 q	20.6 q	20.6 q	20.7 q	20.5 q	20.7 q	20.7 q
	20.4 q	20.6 q	20.5 q	20.6 q	20.6 q	20.5 q	20.5 q	20.6 q
			20.2 q				20.5 q	

purified by preparative TLC (30 g) with petroleum ether−acetone (1:1, two developments), to afford **2** (15 mg, R_f 0.55, petroleum ether−EtOAc, 1:1), **5** (30 mg, R_f 0.35, petroleum ether−acetone, 3:1), and **6** (3 mg, R_f 0.50, petroleum ether−EtOAc, 2:1). Compounds **7** (5 mg, t_R 25.7 min) and **8** (5 mg, t_R 29.1 min) were obtained by preparative normal-phase HPLC with *n*-hexane−EtOAc (83:17).

2α,8-Diangeloyloxy-3*β*,4*β*,10,11-diepoxy-1α-hydroxybisabol-7(14)-ene (1): colorless gum (acetone); $[\alpha]_D^{21}$ −50 (*c* 0.90, acetone); IR (KBr) ν_{max} 3489, 1715, 1647 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 449 $[M + H]^+$, 431, 349, 331, 249; HRESIMS *m*/*z* 471.2347 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_7Na$, 471.2353).

2*β*,8-Diangeloyloxy-3α,4α,10,11-diepoxy-1α-hydroxybisabol-7(14)-ene (2): colorless gum (acetone); $[\alpha]_D^{21}$ −8 (*c* 0.90, acetone); IR (KBr) ν_{max} 3489, 1715, 1647 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 449 $[M + H]^+$, 431, 349, 331, 249; HRESIMS *m*/*z* 471.2353 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_7Na$, 471.2353).

3*β*,4*β*,10,11-Diepoxy-1*β*,2*β*,8-triangeloyloxybisabol-7(14)-ene (3): colorless gum (acetone); $[\alpha]_D^{28}$ −27 (*c* 1.23, acetone); IR (KBr) ν_{max} 1715, 1647 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 548.3223 $[M + NH_4]^+$ (calcd for $C_{30}H_{42}O_8NH_4$ 548.3218).

1*β*,8-Diangeloyloxy-3*β*,4*β*,10,11-diepoxybisabol-7(14)-ene (4): colorless gum (acetone); $[\alpha]_D^{21}$ −75 (*c* 0.90, acetone); IR (KBr) ν_{max} 1715, 1647 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 433 $[M + H]^+$, 333, 233; HRESIMS *m*/*z* 450.2841 $[M + NH_4]^+$ (calcd for $C_{25}H_{36}O_6NH_4$, 450.2850).

1*β*,8-Diangeloyloxy-3*β*,4*β*-epoxy-2*β*,10,11-trihydroxybisabol-7(14)-ene (5): colorless gum (acetone); $[\alpha]_D^{21}$ −24 (*c* 0.40, acetone); IR (KBr) ν_{max} 3489, 1715, 1647 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 489.2455 $[M + Na]^+$ (calcd for $C_{25}H_{38}O_8Na$, 489.2459).

1α,8-Diangeloyloxy-10,11-dihydroxy-3*β*,4*β*-epoxybisabol-7(14)-en-2-one (6): colorless gum (acetone); $[\alpha]_D^{21}$ −34 (*c* 0.30, acetone); IR (KBr) ν_{max} 3489, 1715, 1647 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 487.2306 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_8Na$, 487.2302).

10,11-Epoxy-1*β*-hydroxy-2*β*,4α,8-triangeloyloxybisabol-7(14)-ene (7): colorless gum (acetone); $[\alpha]_D^{21}$ −24 (*c* 0.40, acetone); IR (KBr) ν_{max} 3489, 1715, 1647 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 566.3324 $[M + NH_4]^+$ (calcd for $C_{30}H_{44}O_9NH_4$, 566.3328).

4α-Chloro-1*β*,8-diangeloyloxy-10,11-epoxy-2*β*-hydroxybisabol-7(14)-ene (8): colorless gum (acetone); $[\alpha]_D^{21}$ −50 (*c* 0.90, acetone); IR (KBr) ν_{max} 3396, 1647, 1714 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 485, 467, 385, 285; HRESIMS *m*/*z* 485.2301 $[M + H]^+$ (calcd for $C_{25}H_{38}O_7Cl$, 485.2301).

(*R*)- and (*S*)-MTPA Derivatives of 5. To a solution of compound **5** (10.0 mg, 21.5×10^{-3} mmol) in (dimethylamino)pyridine (10.7 mg, 87.3×10^{-3} mmol) and triethylamine (4.5 μL, 31.5×10^{-3} mmol) at room temperature was added (*S*)-MTPA-Cl (7.8 μL, 42.9×10^{-3} mmol), and the resultant mixture was stirred for 24 h at room temperature. The reaction mixture was worked up by adding 2 mL of water. Further purification was performed with preparative TLC (20 g) with petroleum ether−EtOAc, 4:1, to give the (*R*)-MTPA ester **5b** (6 mg) as a colorless gum. The (*S*)-MTPA ester **5a** (7 mg) was prepared in a similar manner.

(*S*)-MTPA ester of 5 (5a): ^1H NMR ($CDCl_3$, 400 MHz) δ 1.17 (3H, s, H-12), 1.19 (3H, s, H-13), 1.35 (3H, s, H-15), 1.95 (1H, overlapped, H-5*β*), 1.97 (1H, overlapped, H-9b), 2.05 (1H, overlapped, H-9a), 2.09 (1H, overlapped, H-5α), 2.68 (1H, brdd, J = 12.8, 4.0 Hz, H-6), 3.23 (1H, brd, J = 5.2 Hz, H-4), 3.36 (1H, brd, J = 10.8 Hz, H-10), 4.95 (1H, brs, H-14b), 5.21 (1H, brs, H-14a), 5.47 (1H, brd, J = 10.0 Hz, H-8), 5.53 (1H, brd, J = 4.4 Hz, H-1), 5.64 (1H, d, J = 4.4 Hz, H-2).

(*R*)-MTPA ester of 5 (5b): ^1H NMR ($CDCl_3$, 400 MHz) δ 1.18 (3H, s, H-12), 1.19 (3H, s, H-13), 1.22 (3H, s, H-15), 1.95 (1H, overlapped, H-5*β*), 1.98 (1H, overlapped, H-9b), 2.05 (1H, overlapped, H-9a), 2.09 (1H, overlapped, H-5α), 2.69 (1H, brdd, J = 12.8, 4.4 Hz, H-6), 3.20 (1H, d, J = 5.2 Hz, H-4), 3.37 (1H, d, J = 10.0 Hz, H-10), 4.96 (1H, brs, H-14b), 5.22 (1H, brs, H-14a), 5.49 (1H, d, J = 10.0 Hz, H-8), 5.54 (1H, brd, J = 4.4 Hz, H-1), 5.67 (1H, d, J = 4.4 Hz, H-2).

Antimicrobial Assays. The compounds were tested against four microbial strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), a clinically isolated strain of methicillin-resistant *Staphylococcus* aureus (MRSA), and *Candida albicans.* The broth microdilution method[12,13] was applied to assay the minimum inhibitory concentration (MIC), the lowest concentration of a sample at which the microorganism did not demonstrate visible growth, as indicated by the presence of turbidity. All tests were performed in Mueller-Hinton broth. A serial doubling dilution of each compound was prepared in a 96-well microtiter plate over the range 0.0024−1.25 mg/mL. Overnight

broth cultures of each strain were prepared, and the final concentration in each well was adjusted to 2×10^6 cfu/mL. Plates were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeast.[12,13] Each test was performed in duplicate and repeated twice. Levofloxacin was used as positive control for the bacteria and fluconazole for the yeast.

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References and Notes

(1) Jiangsu College of New Medicine. *A Dictionary of Traditional Chinese Medicines*; Shanghai Science and Technology Press: Shanghai, 1977; pp 7, 154, 549, 1152, 2349.
(2) Gao, K.; Yang, L.; Jia, Z. J. *Planta Med.* **1997**, *63*, 461−463.
(3) Wu, Q. H.; Wang, C. M.; Chen, S. G.; Gao, K. *Tetrahedron Lett.* **2004**, *45*, 8855−8858.
(4) Li, X. Q.; Gao, K.; Jia, Z. J. *Planta Med.* **2003**, *69*, 356−360.
(5) Han, Y. F.; Pan, J.; Gao, K.; Jia, Z. J. *Chem. Pharm. Bull.* **2005**, *53*, 1338−1341.
(6) Ryo H.; Xun G.; Motoo T.; Satomi K.; Kanako O. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 1302−1308.
(7) Joseph-Nathan, P.; Wesener, J. R.; Günther, H. *Org. Magn. Reson.* **1984**, *22*, 190−192.
(8) Fu, B.; Zhu, Q. X.; Yang, X. P.; Jia, Z. J. *Pharmazie* **1999**, *54*, 620−624.
(9) Zhu, Y.; Yang, L.; Jia, Z. J. *J. Nat. Prod.* **1999**, *62*, 1479−1483.
(10) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc*. **1991**, *113*, 4092−4096.
(11) Liao, J. C.; Zhu, Q. X.; Yang, H.; Jia, Z. J. *J. Chin. Chem. Soc.* **1999**, *46*, 185−190.
(12) Oluwatuyi, M.; Kaatz, G. W.; Gibbons, S. *Phytochemistry* **2004**, *65*, 3249−3254.
(13) Yu, J. Q.; Lei, J. C.; Yu, H. D.; Cai, X.; Zou, G. L. *Phytochemistry* **2004**, *65*, 881−884.

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