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New bisabolane sesquiterpenes from *Ligularia songarica*

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Phytochemical investigation of *Ligularia songarica* (Compositae) afforded seven new bisabolane-type sesquiterpenes. Their structures were confirmed on the basis of spectroscopic methods, especially 2D-NMR techniques, and compound **7** showed stronger antibacterial activity against *Escherichia coli*, *Pseudomonas acruginosa* and *Salmonella pullorum*.

1. Introduction

Ligularia songarica (Fish) Ling, growing in Xinjiang, China, is a plant of the genus *Ligularia* (Compositae), traditionally used for tuberculosis and bronchitius, invigorating the circulation of blood, as an antiinflammatory to reduce pain, and to relieve coughing of blood etc [1–3]. Three new sesquiterpenes from this plant were reported earlier [4]. In continuation of our investigation, we now report the isolation, and structural elucidation and the antibacterial activities of seven new bisabolane-type sesquiterpenes.

2. Investigations, results and discussion

Compound **1** was obtained as a colorless gum. Its FAB-MS showed quasi-molecular ion peak $[M+Na]^+$ at m/z 531 and $[M+H]^+$ at m/z 509. HRFAB-MS also gave the quasi-molecular ion peak $[M+H]^+$ at m/z 509.2792 ($C_{27}H_{41}O_9$ requires 509.2749) and an ion peak associated with loss of water at m/z 491.2653 ($C_{27}H_{39}O_8$ requires 491.2645). Taking this together with elemental analysis, the molecular formula was proposed to be $C_{27}H_{40}O_9$ with eight degrees of unsaturation, which were also deduced by ^{1}H NMR (Table 1), ^{13}C NMR and DEPT (Table 2) spectra. Its IR spectrum showed the presence of two kinds of carbonyl groups (1743 cm^{-1}: OAc; 1720 cm^{-1}: C=CCO_2R), hydroxyl groups (br 3448 cm^{-1}) and double bonds (1646 cm^{-1}: C=C; 856 cm^{-1}: C=CH_2). In the ^{1}H NMR and the ^{13}C NMR spectra of **1**, there were signals of an acetyl and two angeloyl groups. FAB-MS also gave significant fragment peaks at m/z 491 $[M+H-H_2O]^+$, 391 $[491-AngOH]^+$, 291 $[491-2\times AngOH]^+$, 231 $[491-2\times AngOH-AcOH]^+$, and 83 $[C_4H_7CO]^+$, which supported this assumption while further confirming the existence of the hydroxyl group. Apart from these groups, the ^{1}H NMR spectrum (in $CDCl_3$) exhibited three methyl signals at δ 1.21 (3 H, s), 1.22 (3 H, s) and 1.30 (3 H, s), a terminal ethylene signal at δ 4.94 (1 H, brs) and 4.80 (1 H, brs), two methylene signals at δ 1.79 (1 H, m), 2.16 (1 H, dd) and 1.94–1.86 (2 H, m), one methine signal at δ 2.58 (1 H, ddd) and five oxygenated methine signals at δ 3.17 (1 H, brd), 5.42 (1 H, d), 5.41 (1 H, brdd), 4.25 (1 H, t) and 4.80 (1 H, dd). The ^{13}C NMR and DEPT spectra (in $CDCl_3$) showed three quaternary carbon signals (two oxy-

Table 1: ^{1}H NMR Spectral data of compounds 1, 2 and 3 (400 MHz, $CDCl_3$, TMS, δ, ppm)[a, b, c]

Proton	1	1[c]	2	3
1α	1.79 (1 H, m)	2.08 (1 H, m)	1.62 (1 H, m)	1.62 (1 H, m)
1β	2.16 (1 H, dd, 16.1, 13.2)	2.13 (1 H, dd, 17.2, 12.4)	2.19 (1 H, dd, 17.0, 14.1)	2.16 (1 H, dd, 17.0, 14.4)
2	3.17 (1 H, d, 5.5)	3.27 (1 H, d, 5.2)	3.21 (1 H, d, 3.5)	3.22 (1 H, d, 3.4)
4	5.42 (1 H, d, 4.6)	5.44 (1 H, d, 4.5)	5.37 (1 H, d, 4.2)	5.29 (1 H, d, 4.6)
5	5.41 (1 H, brdd, 4.6, 2.1)	5.37 (1 H, brdd, 4.5, 2.0)	5.45 (1 H, brdd, 4.2, 2.0)	5.46 (1 H, brdd, 4.6, 1.9)
6	2.58 (1 H, ddd, 13.2, 6.9, 2.1)	2.63 (1 H, ddd, 12.4, 6.5, 2.0)	2.63 (1 H, ddd, 14.1, 6.4, 2.0)	2.59 (1 H, ddd, 14.4, 6.4, 1.9)
8	4.25 (1 H, t, 6.5)	4.07 (1 H, dd, 8.7, 4.0)	5.22 (1 H, dd, 11.2, 1.7)	5.39 (1 H, dd, 10.4, 1.9)
9	1.94 ∼ 1.86 (2 H, m)	2.04 ∼ 1.84 (2 H, m)	2.02 ∼ 1.82 (2 H, m)	1.98 ∼ 1.85 (2 H, m)
10	4.80 (1 H, dd, 10.3, 2.1)	4.84 (1 H, dd, 11.2, 1.6)	3.28 (1 H, dd, 10.3, 3.3)	3.35 (1 H, dd, 11.4, 1.9)
12	1.21 (3 H, s)	1.18 (3 H, s)	1.17 (3 H, s)	1.17 (3 H, s)
13	1.22 (3 H, s)	1.20 (3 H, s)	1.19 (3 H, s)	1.20 (3 H, s)
14	4.94 (1 H, brs)	5.07 (1 H, brs)	5.30 (1 H, brs)	5.12 (1 H, brs)
14′	4.88 (1 H, brs)	5.00 (1 H, brs)	5.12 (1 H, brs)	4.99 (1 H, brs)
15	1.30 (3 H, s)	1.30 (3 H, s)	1.35 (3 H, s)	1.32 (3 H, s)
OAng				
3′	6.10 (1 H, qq, 7.3, 1.5)	6.17 (1 H, qq, 7.3, 1.5)	6.14 (1 H, qq, 7.5, 1.6)	6.14 (1 H, qq, 7.2, 1.6)
	6.07 (1 H, qq, 7.3, 1.5)	6.12 (1 H, qq, 7.3, 1.5)	6.12 (1 H, qq, 7.5, 1.6)	6.13 (1 H, qq, 7.2, 1.6)
4′	1.99 (3 H, dq, 7.3, 1.4)	2.00 (3 H, dq, 7.3, 1.4)	2.00 (3 H, dq, 7.5, 1.3)	2.03 (3 H, dq, 7.2, 1.4)
	1.97 (3 H, dq, 7.3, 1.4)	1.99 (3 H, dq, 7.3, 1.4)	1.99 (3 H, dq, 7.5, 1.3)	2.02 (3 H, dq, 7.2, 1.4)
5′	1.90 (3 H, dq, 1.5, 1.4)	1.92 (3 H, dq, 1.5, 1.4)	1.91 (3 H, dq, 1.6, 1.3)	1.92 (3 H, dq, 1.6, 1.4)
	1.86 (3 H, dq, 1.5, 1.4)	1.87 (3 H, dq, 1.5, 1.4)	1.88 (3 H, dq, 1.6, 1.3)	1.88 (3 H, dq, 1.6, 1.4)
OAc	2.01 (3 H, s)	2.02 (3 H, s)	2.06 (3 H, s)	2.05 (3 H, s)
$OCHMe_2$				
	–	–	3.73 (1 H, m)	–
	–	–	1.26 (6 H, d, 7.3)	–

[a] Coupling constants in parenthese in Hz
[b] Assignments from ^{1}H, ^{1}HCOSY and HMQC experiments
[c] CD_3OD as solvent

Table 2: ^{13}C NMR spectral data of compounds 1, 2, 3, 4, 5, 6 and 7 (100.6 Mz, $CDCl_3$, TMS, δ, ppm

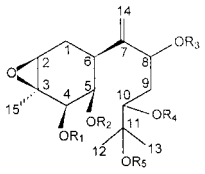
No.	1	1[b]	2	3	4[b]	5	6	7	DEPT
1	25.5	27.2	25.5	25.9	27.0	29.2	29.2	71.4 (CH)	CH_2
2	59.7	61.5	59.6	59.6	61.4	64.3	69.3	76.7	CH
3	56.8	58.2	56.7	56.3	58.2	72.4	72.4	76.1	C
4	71.8	73.4	68.4	72.5	73.4	69.9	69.8	202.3 (C)	CH
5	69.8	71.2	75.4	73.5	70.3	72.6	73.7	74.5	CH
6	36.1	36.6	39.2	39.0	38.7	34.6	33.5	46.6	CH
7	148.4	150.1	146.1	147.3	149.2	147.6	147.5	146.7	C
8	73.5	74.6	75.2	74.6	75.8	75.3	74.7	75.9	CH
9	35.6	36.4	35.3	37.1	37.4	35.3	35.1	36.9	CH_2
10	76.9	77.7	72.1	67.6	75.5	76.7	76.9	61.2	CH
11	72.2	72.6	72.6	72.0	73.4	72.0	74.5	58.5	C
12	25.8	35.0	25.0	25.5	25.0	26.5	25.2	18.9	CH_3
13	26.1	26.8	23.8	23.8	25.7	25.2	26.5	22.4	CH_3
14	114.5	114.9	115.7	113.9	114.7	115.7	115.8	112.9	CH_2
15	19.6	19.9	19.6	19.4	19.8	22.7	24.1	19.8	CH_3
OAng									
1′	167.7	168.9	167.3	167.8	168.6	168.1	167.9	167.5	C
	166.9	168.1	167.0	167.6	168.0	166.1	166.1	166.5	C
2′	127.6	129.3	127.6	127.7	129.1	128.5	127.7	127.3	C
	127.2	128.5	127.1	127.3	128.6	127.0	126.9	127.2	C
3′	139.3	140.0	139.5	139.5	139.9	139.6	139.8	140.8	CH
	138.8	138.9	138.9	139.0	139.5	138.0	138.8	139.5	CH
4′	14.4	16.1	15.8	15.8	16.1	12.1	15.9	16.0	CH_3
	14.1	16.0	15.8	15.9	16.0	15.8	15.9	16.0	CH_3
5′	20.6	20.9	20.7	20.7	20.9	14.4	20.5	20.6	CH_3
	20.5	20.7	20.4	20.7	20.6	20.4	21.1	20.4	CH_3
OAc	21.0	21.0	20.6	20.7	20.9	20.6	20.7	19.9	CH_3
	171.3	172.5	171.0	170.4	172.2	170.5	170.3	170.3	C
OiBu	–	–	–	–	–	24.1	–	–	CH
	–	–	–	–	–	29.7, 29.7	–	–	CH_3
	–	–	–	–	–	171.7	–	–	C
$OCHMe_2$	–	–	77.0	–	–	–	–	–	CH
	–	–	29,7, 29.7	–	–	–	–	–	CH_3

a Assignmennts from ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY experiments
b CD_3OD as solvent

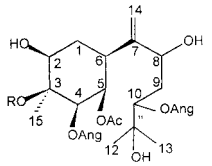
genated carbon signals at δ 56.8 and 72.2; one olefinic carbon signal at δ 148.4) apart from relative carbon signals. An epoxy signal was also observed in the ^{1}H NMR and ^{13}C NMR spectra (δ_H 3.17, 1 H; δ_C 59.7, CH, 56.8, C). On the basis of the above information, compound **1** was proposed to be a bisabolane sesquiterpene skeleton [5–7], which was confirmed by the correlation peaks of ^{1}H, ^{1}H COSY, HMQC and HMBC spectra. The position of functional groups was determined by an HMBC experiment on **1** in CD_3OD as solvent (H-4 and H-5 overlapped so severely that they could not show which correlated with angeloyl or acetyl carbonyl carbon in $CDCl_3$ as solvent). At first, the presence of three ester groups was confirmed by the correlated peaks of the proton at δ_H 2.02 (CH_3) with the ester carbonyl at δ_C 172.5 and H-5′ at δ_H 1.92 and δ_H 1.87 with the ester carbonyl at δ_C 168.9 and δ_C 168.1, respectively. Furthermore, the correlations of H-5 with the carbonyl at δ_C 172.5 (OAc), C-3, C-4, C-6 and C-1; H-4 with the carbonyl at δ_C 168.1 (OAng), C-5, C-2 and C-6; H-10 with carbonyl at δ_C 168.9 (OAng), C-8, C-9, C-11, C-12 and C-13, indicated the acetyl group at C-5 and the two angeloyl groups at C-4 and C-10, respectively. The correlation of H-2 (δ_H 3.27) with C-3, C-6, C-1 and C-15 indicated the epoxy group at C-2, and C-3; H-8 (δ_H 4.07) with C-6, C-7, C-9, C-10 and C-14 implied a hydroxy at C-8. And the correlation of C-11 (δ_C 72.6) with H-9, H-10, H-12, and H-13 showed another hydroxy at C-11. The relative stereochemistry of **1** was determined by the coupling constants of H-1, H-2, H-4, H-5 and H-6. If H-6 were α-oriented, H-5 must be α-oriented because the coupling constant between H-5 and H-6 was small ($J_{5\alpha,6\alpha} = 2.0$ Hz), and H-4 and H-2 must likewise be α-oriented because of the small coupling constants of H-4 with H-5, H-1 with H-2 and H-1 with H-6 ($J_{4\alpha,5\alpha} = 4.5$, $J_{1\alpha,6\alpha} = 6.5$, $J_{1\alpha,2\alpha} = 5.2$ Hz). The coupling constant of H-1β with H-2α was almost zero because their dihedral angle is about 90° for the existence of 2β, 3β-epoxy (shown by the molecular model). The configuration was further ascertained by the ^{1}H, ^{1}H NOESY information as follows: There were the obvious correlated peaks of H-2 with H-1α and H-15; H-4 with H-5 and H-15; H-5 with H-6. Therefore, the ester groups at C-4/C-5, and the 2,3-epoxy group must all be β-configuration. Consequently, the structure of **1** was elucidated as 5β-acetoxy-4β, 10-diangeloyloxy-8, 11-dihydroxy-2β, 3β-epoxy-bisabol-7(14)-ene.

For compound **2**, FAB-MS gave quasi-molecular ion peaks $[M+H]^+$ at m/z 551, and taking this together with elemental analysis, the molecular formula was established as $C_{30}H_{46}O_9$. Its IR, ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectra were similar to those of **1** apart from the appearances of an oxygenated methine proton (δ_H 3.73, 1 H, m) and two methyl protons (δ_H 1.26, 6 H, brd). In the spectrum of its ^{1}H, ^{1}H COSY, the obvious correlated peak of the methine proton with the two methyl protons indicated the existence of an isopropoxy, which should be at a quaternary carbon because of the absence of the other correlations about the oxygenated methine and the main fragments $[OCHMe_2]^+$ at m/z 59 and $[M-C(OCHMe_2)Me_2]^+$ at m/z 449 showed by FAB-MS

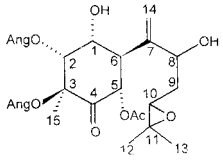
and EI-MS. Thus compound **2** also had the bisabolane skeleton. By comparing its ^{1}H NMR spectrum with that of **1**, the downfield chemical shift of H-8 at δ_H 5.22 (1 H, dd) and the upfield chemical shift of H-10 at δ_H 3.28 (1 H, dd) revealed an ester group (OAng) at C-8 and the hydroxy at C-10, and this was supported by the crossed peaks of H-8 with the ester carbonyl at δ_C 167.3 (OAng) C-6, C-7, C-9, C-10 and C-14; H-10 with C-11, C-12 and C-13, in the HMBC spectrum. In addition, the spectrum showed the correlated peaks of H-4 with another ester carbonyl at δ_C 167.0 (OAng), H-5 with δ_C 171.4 (OAc) and C-11 with the methine of the isopropoxy at δ_H 3.73, H-9, H-10, H-12 and H-13. Therefore, the acetyl group should be at C-5 and the other angeloyl groups at C-4 like **1**, while the hydroxy at C-11 of **1** is replaced by isopropoxy in **2**. A comparison of coupling constants, of **2** with **1** suggested that they both had similar stereochemistry. Thus, the compound **2** was determined to be 5β-acetoxy-4β, 8-diangeloyloxy-2β, 3β-epoxy-10-hydroxy-11-isopropoxy-bisabol-7(14)-ene.



	R_1	R_2	R_3	R_4	R_5
1	Ang	Ac	H	Ang	H
2	Ang	Ac	Ang	H	$CHMe_2$
3	Ac	Ang	Ang	H	H
4	Ang	Ac	H	H	Ang



	R
5	iBu
6	H



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Compound **3** was isolated from the mixture of **1** and **3** by repeated preparative TLC as a colorless gum. FAB-MS showed the same information as that of **1** such as quasi-molecular ion peak $[M+H]^+$ at m/z 509 and main fragments at m/z 491 $[M+H-H_2O]^+$, 409 $[M+H-AngOH]^+$, 349 $[M+H-AngOH-AcOH]^+$, 249 $[M+H-2\times AngOH-AcOH]^+$ and 83 $[C_4H_7CO]^+$ etc. Its IR spectra revealed the presence of similar groups as in **1** such as hydroxyl (3434 cm^{-1}), angeloyl (1717 cm^{-1}) and acetyl groups (1744 cm^{-1}). So compound **3** is an isomer of **1**, and the molecular formula should be $C_{27}H_{40}O_9$ by elemental analysis combined with the ^{1}H NMR and ^{13}C NMR data (Tables 1 and 2). The spectral data of **3** were similar to those of **1**, but in the ^{1}H NMR, the H-8 signal of **3** was shifted downfield to δ 5.39 from 4.25, while the H-10 was shifted upfield to 3.35 from 4.80, respectively, which indicated that angeloyl group was located at C-8 and a hydroxyl group at C-10 respectively, and these were also confirmed by the HMBC study. In addition, the HMBC spectra gave the correlation of H-4 with the ester at δ_C 170.4 (OAc) and H-5 with δ_C 167.6 (OAng), showing the acetyl group at C-4 and another angeloyl group at C-5. The position of another hydroxy at C-11, which was confirmed by comparing the chemical shift of the C-11 of **3** with that of **1** and the ^{13}C NMR data of 10,11-dihydroxy substituent type agreed with that of the reported compounds [8, 9]. This was also supported by HMBC studies. According to the similar information given by ^{1}H, ^{1}H NOESY studies and coupling constants as for **1**, **3** and **1** had the same relative stereochemistry. Consequently, the structure of **3** was assigned as 4β-acetoxy-5β, 8-diangeloyloxy-2β, 3β-epoxy-10, 11-dihydroxy-bisabol-7(14)-ene.

Compound **4**, was obtained as a colorless gum. The information shown by its IR spectra was similar to that of **1** and **3**. FAB-MS gave the quasi-molecular ion peak $[M+1]^+$ at m/z 509 and a strong water loss fragment peak at m/z 491 which were same as those of **1** and **3**; and their other main fragments were almost identical. Its ^{1}H NMR and ^{13}C NMR spectra (Table 2 and 3) were analogous with those of **1** and **3**. Therefore, compound **4** was an isomer of **1** and **3**; its molecular formula should also be $C_{27}H_{40}O_9$, coupled with elemental analysis. The position of two esters of **4** was exactly the same as that of **1** because of the obvious correlated peaks of H-4 (δ_H 5.37) with the carbonyl of an angeloyl at δ_C 168.6 as well as H-5 (δ_H 5.39) with the carbonyl of acetyl group (δ_C 171.2) in HMBC of **4**. However, in the HMBC spectra of **4**, the absence of a crossed peak of the carbonyl of another angeloyl group (δ_C 168.0) with any oxygenated methine proton suggested that the angeloyl group was possibly at a quaternary carbon. Due to the existence of the 2,3-epoxy (confirmed by ^{1}H NMR, ^{13}C NMR and HBMC spectra), the quaternary carbon should be C-11 (The HBMC spectra exhibited the correlation of the oxygenated quaternary carbon with H-10, H-12 and H-13), thus, the angeloyl group was designated to C-11. Furthermore, comparing the ^{13}C NMR spectrum of **4**, with that of **1** and **3**, the C-11 signal of **4** was shifted downfield to 73.4 (δ_C of **1** and **3** less than 72.6). And a fragment peak at m/z 141 $[Me_2COAng]^+$ was shown by FAB-MS, further confirming an angeloyl group at C-11. The coupling constants of methines in the ring of **4** were all small (see Table 3). This indicated that **4** had the same stereochemistry as that of **1** and **3**. Its ^{1}H, ^{1}H NOESY spectra given in the correlated peaks (H-2/H-1α, H-15; H-4/H-5, H-6; H-6/H-1α, H-2, H-5) also supported this conclusion. Finally, compound **4** was confirmed as 5β-acetoxy-4β, 11-diangeloyloxy-8, 10-dihydroxy-2β, 3β-epoxy-bisabol-7(14)-ene.

Compound **5**, was a colorless gum. FAB-MS gave a quasi-molecular ion peak at m/z 597 $[M+1]^+$. Its molecular formula was established as $C_{31}H_{48}O_{11}$ by elemental analysis together with ^{13}C NMR and DEPT spectra (Table 2). The IR absorption spectrum exhibited signals at 3382, 1744, 1719, 1648 and 850 cm^{-1}. The ^{1}H and ^{13}C NMR of **5** showed its skeleton was also of bisabolane-type, with only one difference with **1**, that an epoxy was replaced by an isobutyryl and a hydroxy in the structure of **5**. This was showed by the shifting to downfield of H-2 (δ_H 4.22, t), C-2 (δ_C 64.3) and C-3 (δ_C 72.4), FAB-MS presented an ion fragment at m/z 71 $[C_3H_7CO]^+$ and the signals at δ_H 2.34 (1 H, m) and 1.26 (6 H, d) as well as δ_C 24.1, 29.7, 29.7 and 171.7 given by the ^{1}H NMR and ^{13}C NMR spectra. In the HMBC study, the position of the isobutyryl at C-3 was ascertained by the correlations of the methine proton at δ_H 2.34 in the isobutyryl group and H-1 with C-3. The correlated peaks of H-2 with C-15 and C-4 showed that a hydroxy must be at C-2. The relative stereochemistry studied by ^{1}H, ^{1}H NOESY was exactly the

Table 3: ^{1}H NMR spectral data of compounds 4, 5, 6 and 7 (400 MHz, $CDCl_3$, TMS, δ, ppm)[a, b, c]

Proton	4	4[c]	5	6	7
1α	1.69 (1 H, m)		1.70 (1 H, m)	1.53 (1 H, ddd, 14.7, 2.7, 2.6)	
1β	2.18 (1 H, dd, 16.8, 12.8)	2.15–2.12 (2 H, m)	2.68 (1 H, ddd, 15.7, 14.8, 2.7)	2.67 (1 H, ddd, 14.7, 14.2, 2.7)	4.65 (1 H, dd, 11.2, 2.6)
2	3.20 (1 H, d, 5.2)	3.28 (1 H, t, 3.0)	4.22 (1 H, t, 3.0)	4.21 (1 H, t, 2.7)	5.55 (1 H, d, 2.6)
4	5.37 (1 H, d, 4.4)	5.42 (1 H, d, 4.4)	5.46 (1 H, d, 2.0)	5.44 (1 H, d, 3.5)	–
5	5.39 (1 H, brdd, 4.4, 1.8)	5.30 (1 H, brdd, 4.4, 1.3)	5.59 (1 H, brdd, 2.2, 2.0)	5.58 (1 H, brdd, 3.5, 1.9	5.93 (1 H, d, 13.2)
6	2.54 (1H, ddd, 12.8, 6.1, 1.8)	2.66 (1 H, ddd, 11.5, 6.3, 1.3)	3.24 (1 H, ddd, 14.8, 6.2, 2.2)	3.12 (1 H, ddd, 14.2, 2.6, 1.9)	2.81 (1 H, dd, 13.2, 11.2)
8	4.21 (1 H, t, 6.5)	4.38 (1 H, dd, 8.8, 2.2)	4.29 (1 H, t, 6.7)	4.29 (1 H, t, 7.2)	5.19 (1 H, dd, 8.0, 2.8
9	2.09 ∼ 1.62 (2 H, m)	2.02 ∼ 1.58 (2 H, m)	2.00 ∼ 1.91 (2 H, m)	1.98 ∼ 1.86 (2 H, m)	2.05 ∼ 1.92 (2 H, m)
10	3.35 (1 H, dd, 10.7, 2.3)	3.37 (1 H, dd, 10.2, 1.3)	4.72 (1 H, dd, 8.4, 3.0)	4.76 (1 H, dd, 8.4, 3.4)	2.86 (1 H, dd, 7.6, 3.5)
12	1.17 (3 H, s)	1.16 (3 H, s)	1.20 (3 H, s)	1.22 (3 H, s)	1.28 (3 H, s)
13	1.21 (3 H, s)	1.18 (3 H, s)	1.22 (3 H, s)	1.23 (3 H, s)	1.31 (3 H, s)
14	5.24 (1 H, brs)	5.22 (1 H, brs)	5.10 (1 H, brs)	5.24 (1 H, brs)	5.40 (1 H, brs)
14′	5.02 (1 H, brs)	5.03 (1 H, brs)	4.97 (1 H, brs)	5.10 (1 H, brs)	5.39 (1 H, brs)
15	1.32 (3 H, s)	1.30 (3 H, s)	1.34 (3 H, s)	1.34 (3 H, s)	1.32 (3 H, s)
OAng					
3′	6.13 (1 H, qq, 7.3, 1.6)	6.17 (1 H, qq, 7.3, 1.5)	6.91 (1 H, qq, 7.4, 1.8)	6.13 (1 H, qq, 7.2, 1.4)	6.19 (1 H, qq, 7.2, 1.3)
	6.12 (1 H, qq, 7.3, 1.6)	6.13 (1 H, qq, 7.3, 1.5)	6.15 (1 H, qq, 7.4, 1.8)	6.12 (1 H, qq, 7.2, 1.4)	6.11 (1 H, qq, 7.2, 1.3)
4′	2.15 (3 H, dq, 7.3, 1.4)	1.98 (3 H, dq, 7.3, 1.3)	2.00 (3 H, dq, 7.4, 1.3)	2.02 (3 H, dq, 7.2, 1.3)	2.03 (3 H, dq, 7.2, 1.0)
	1.91 (3 H, dq, 7.3, 1.4)	1.97 (3 H, dq, 7.3, 1.3)	1.99 (3 H, dq, 7.4, 1.3)	1.99 (3 H, dq, 7.2, 1.3)	1.99 (3 H, dq, 7.2, 1.0)
5′	1.88 (3 H, dq, 1.6, 1.4)	1.92 (3 H, dq, 1.5, 1.3)	1.93 (3 H, dq, 1.8, 1.3)	1.92 (3 H, dq, 1.4, 1.3)	1.91 (3 H, dq, 1.3, 1.0)
	1.84 (3 H, dq, 1.6, 1.4)	1.86 (3 H, dq, 1.5, 1.3)	1.89 (3 H, dq, 1.8, 1.3)	1.88 (3 H, dq, 1.4, 1.3)	1.86 (3 H, dq, 1.3, 1.0)
OAc	2.03 (3 H, s)	2.00 (3 H, s)	2.06 (3 H, s)	2.06 (3 H, s)	2.08 (3 H, s)
OiBu					
	–	–	2.34 (1 H, m)	–	–
	–	–	1.28 (6 H, brd, 7.4)	–	–

[a] Coupling constants in parenthese in Hz
[b] Assignments from ^{1}H, ^{1}H, COSY and HMQC experiments
[c] CD_3OD as solvent

same as for compound **1**. Thus, compund **5** was elucidated as 5β-acetoxy-4β, 10-diangeloyloxy-3β-isobutyryloxy-2β, 8,11-trihydroxy-bisasbol-7(14)-ene.

Compound **6** was also obtained as a colorless gum. FAB-MS revealed a molecular ion at m/z 527 $[M+H]^+$ and other fragments such as 427 $[M+H-AngOH]^+$, 367 $[M+H-AngOH-AcOH]^+$, 267 $[M+H-2\times AngOH-AcOH]^+$, 213 $[M+H-2\times AngOH-AcOH-3\times H_2O]^+$, 195 $[M+H-2\times AngOH-AcOH-4\times H_2O]^+$. The molecular formula was determined as $C_{27}H_{42}O_{10}$ by elemental analysis combined with ^{1}H NMR, ^{13}C NMR and DEPT spectra (Tables 2 and 3). Its ^{1}H NMR spectrum was almost the same as that of **5**. But there was no isobutyryl signal consistent with the lack of a corresponding isobutyryl signal in ^{13}C NMR. According to its molecular formula, this isobutyryl was substituted for a hydroxy group. Its substitutent positions and stereochemistry were completely homologous with **5** supported by HMBC and ^{1}H, ^{1}H NOESY spectra. So the compound **6** was identified as 5β-acetoxy-4β, 10-diangeloyloxy-2β, 3β, 8,11-terahydroxy-bisabol-7(14)-ene.

Compound **7** was obtained as a colorless gum and IR, ^{1}H NMR (Table 3), ^{13}C NMR (Table 2) and FAB-MS indicated the existence of one acetyl, two angeloyl and two hydroxyl groups in its structure. Apart from these groups, the ^{13}C NMR and DEPT spectra of **7** exhibited 15 carbons including three methyls, two methylenes, six methines and four quaternary carbons. Comparison of the ^{13}C NMR and DEPT spectra of **7** with those of **1** showed that a carbonyl carbon occured at δ_C 202.3 in **7**. Moreover, ^{1}H NMR and ^{13}C NMR spectra of **7** showed a characteristic signal of epoxy (δ_H 2.86, 1 H; δ_C 61.2, CH, 58.5, C). HRFAB-MS gave a quasi-molecular ion peak $[M+1]^+$ at m/z 523.2554 ($C_{27}H_{39}O_{10}$ reqires 523.2543) and a water loss fragment at m/z 505.2457 $[M+H-H_2O]^+$ ($C_{27}H_{39}O_9$ requires 505.2437), so the molecular formula should be $C_{27}H_{38}O_{10}$ with 9 degrees of unsaturation. Thus, **7** was proposed to be a monocycle sesquiterpene and there were two hydroxyl groups in its structure. The ^{1}H, ^{1}H COSY and HMQC spectra of **7** exhibited two main fragments: –CH(OR)–CH(OH)–CH(C=CH_2)–CH(OH)– and CH(OH)–CH_2–CH(O)–, which were connected by the correlated peaks of the HMBC spectrum (C-3/H-2, H-15; C-4/H-2, H-5, H-15; C-7/H-5, H-6, H-8, H-14; C-10/H-9, H-12, H-13). Thus compound **7** was further confirmed as a bisabolane-type susquitepene. The HMBC spectrum showed the obvious correlations of ester carbonyl at δ_C 166.5 (OAng) with H-2 (δ_H 5.55), ester carbonyl at δ_C 170.3 (OAc) with H-5 (δ_H 5.93) and C-11 at δ_C 58.5 with H-10 (δ_H 2.86), H-12 and H-13. This indicated an angeloyl group at C-2, the acetyl group at C-5 and the epoxy at C-10 and C-11. Similar to **4** was the lack of the correlation of the oxygenated methine proton with another ester carbonyl at δ_C 167.5 (OAng). Because of the existence of 10,11-epoxy, the angeloy group should be at an oxygenated quaternary carbon, and this was attributed to C-3. The obvious correlated peaks of carbonyl at δ_C 202.3 with H-2, H-5 and H-15 confirmed the carbonyl was at C-4. Two hydroxyl groups were arranged at C-1 and C-8, respectively, considering the determined positions of above substituted groups, and the ^{1}H, ^{1}H COSY, HMQC and HMBC studies supported this conclusion. The relative stereochemistry of **7** was elucidated by the coupling constants. If H-6 were α-oriented,

Table 4: Antibacterial activity

	1&**3** (Mixture)	**2**	**4**	**5**	**6**	**7**	nor-floxacin
S aureus	+	++	++	+	+	+	++
E. coli (human being)	−	−	−	−	−	+	+++
E. coli (cow)	++	++	++	++	++	++	+++
E. coli (pig)	−	−	−	−	−	+++	+++
Strep. agalactiae	−	−	−	−	−	−	++
Strep. dysgalactiae	−	−	−	−	−	−	++
Proteus vulgaris	−	−	−	−	−	−	+++
Ps. multocida	−	−	−	−	−	++	+++
Klebsiella pneumon	−	−	−	−	−	−	+++
P. aeruginosa	−	−	−	−	−	+++	+++
E. rhusiopathiae	−	−	−	−	−	++	++
Salm. pullorum	−	−	−	−	−	+++	+++

"−": Antibacteria circle less than 9 mm. "+" less than 12 mm, "++" equal to 13–16 mm, "+++" more than 17 mm

H-1 and H-5 should be β-configuration for the large coupling constants between H-1 with H-6 (11.2 Hz) and H-5 with H-6 (13.2 Hz). And because of the small coupling constant (2.6 Hz) between H-1 with H-2, H-2 should be β-configuration. 3β-Angeloyloxy was determined according to the biogenic rule: the compounds mentioned above from this plant were all 3α-methyl bisabolane derivatives, and furthermore, its ^{13}C NMR data were identical with those in the literature [9, 10]. Therefore, the compound **7** was finally ascertained as 5α-acetoxy-2α, 3β-diangeloyloxy-12, 8-dihydroxy-10, 11-epoxy-bisabol-7(14)-en-4-one.

In antibacterial testing, the highly-oxygenated bisabolane sesquiterpenes were found to have some antibacterial effect on *Staphylococcus aureus* and *Escherichia coli*, but the effect did not depend on the epoxy and the numbers of hydroxyl groups. And bisabolane sesquiterpenes with carbonyl group such as compound **7** were observed to have stronger antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella pullorum* than that of others containing epoxy or hydroxyl groups (Table 4).

3. Experimental

3.1. Apparatus

Optical rotations were determined on a JASCO-20 auto recording polarimeter. IR spectra were measured on a Nicolet 170SX FT-IR instrument. ^{1}H NMR, ^{13}C NMR and 2D-NMR spectra were recorded on a Bruker AM-400 FT-NMR spectrometer using tetramethylsilane (TMS) as internal standard. EIMS and FABMS were obtained on a VG-ZAB-HS mass spectrometer. HRFABMS were recorded on a Finnigan-4510 mass spectrometer. Silica gel (200–300 mesh) for column chromatography and silica GF254 for TLC were supplied by the Qingdao Marine Chemical Factory of China. MOR-Norfloxacin Drug Sensitive Paper disks were offered by Shanghai Yihua Medical Science and Technology CO. Ltd. All the elemental analysis were in an acceptable range.

3.2. Plant material

Ligularia songarica (Fish) Ling was collected in August 1997, in the South Suburb of Urumchi, Xinjiang People's Republic of China. The plant was identified by Prof. Guan-mian Shen from the Xinjiang Institute of Biology and Pedology of Chinese Academy of Science. A voucher specimen has been preserved in the Herbarium of our institute.

3.3. Extraction and isolation

The air-dried roots of the plant (2.5 kg) were pulverized and extracted four times (each for 7 days) at room temperature with petroleum ether (60–90 °C)-Et_2O–MeOH (1 : 1 : 1). The solvent was then removed under reduced pressure to obtain a residue (100 g), which was subjected to cc over Si gel (100–200 mesh, 950 g), eluted with a gradient of petroleum ether-Me_2CO (40 : 1-1 : 3, 500 ml each eluent) to afford five fractions. On the basis of the results of antibacterial activity testing, fractions C and D were further separated. The fraction C (petroleum ether-Me_2CO: 10 : 1-8 : 1, 12.5 g) eluted with $CHCl_3$–Me_2CO (40 : 1-5 : 1) was subjected to cc on Si gel (200–300 mesh, 110 g) to yield 3 fractions. Fraction 2 (5.8 g) was separated by cc on a Si gel (200–300 mesh, 60 g), eluted with petroleum ether-EtOAc (5 : 1) to yield **2** (22 mg) and a mixture of **1** and **3** (90 mg). The mixture was further separated by preparative TLC developed with $CHCl_3$-petroleum ether-Me_2CO (5 : 1 : 0.5, two developments) to gave **1** (20 mg) and **3** (25 mg). Fraction 3 (2.5 g) eluted with petroleum ether-EtOAc (4 : 1-2 : 1) was subjected to cc over Si gel (200–300 mesh, 25 g) to give a yellowish oil (200 mg). The oil (60 mg) was purified by repeated preparative TLC ($CHCl_3$–Me_2CO 5 : 1, three developments) to yield **5** (28 mg) and **6** (27 mg). The fraction D (3 g) eluted with $CHCl_3$–Me_2CO (10 : 1-5 : 1) was subjected to cc over Si gel (200–300 mesh, 25 g) to obtain **7** (30 mg) and another fraction (50 mg). The latter was purified by repeated preparative TLC ($CHCl_3$–Me_2CO 7 : 1 three developments) to afford **4** (28 mg).

3.4. 5β-Acetoxy-4β, 10-diangeloyloxy-8, 11-dihydoxy-2β, 3β-epoxy-bisabol-7(14)-ene (**1**)

Colorless gum, $[\alpha]_D^{25}$ −83.5 (c = 0.33, CH_3OH), −27.83 (c = 0.12, $CHCl_3$), R_f = 0.30 (chloroform-petroleum ether-acetone, 5 : 1 : 0.5), IR ν_{max} 3448 (OH), 1743 (OAc), 1720 (C=CCO_2R), 1646 (C=C), 1459, 1378, 1255, 1232, 1154, 1043, 856 (C=CH_2) cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; FABMS m/z 531 $[M+Na]^+$ (45), 509 $[M+H]^+$ (11), 491 $[M+H-H_2O]^+$ (15), 471 $[M+Na-AcOH]^+$ (5), 431 $[M+Na-AngOH]^+$ (20) 331 $[M+Na-2\times AngOH]^+$ (16), 83 $[C_4H_7CO]^+$ (100), 55 $[C_4H_7]^+$ (69); HRFAB-MS m/z 509.2792 $[M+Ha]^+$ ($C_{27}H_{41}O_9$ requires 509.2749), 491.2653 $[M+H-H_2O]^+$ ($C_{27}H_{39}O_8$ requires 491.2645).
$C_{27}H_{40}O_9$

3.5. 5β-Acetoxy-4β, 8-diangeloyloxy-2β, 3β-epoxy-10-hydoxy-11-isopropoxy-bisabol-7(14)-ene (**2**)

Colorless gum, $[\alpha]_D^{25}$ −65.7 (c = 0.2, $CHCl_3$); R_f = 0.38 (chloroform-petroleum ether-acetone, 5 : 1 : 0.5); IR ν_{max} 3447 (OH), 1743 (OAc), 1718 (C=CCO_2R), 1647 (C=C), 1457, 1437, 1379, 1233, 1156, 1079, 1042, 853 (C=CH_2) cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; FAB-MS m/z 551 $[M+H]^+$ (1), 509 $[M+H-CH_3CH=CH_2]^+$ (7), 491 $[M+H-H_2O]^+$ (8), 409 $[M+H-H_2O-AngOH]^+$ (3), 309 $[M+H-H_2O-2\times AngOH]^+$ (1), 249 $[M+H-H_20-2\times AngOH-AcOH]^+$ (3), 83 $[C_4H_7CO]^+$ (100); EI-MS m/z 449 $[M-C(OCHMe_2)Me_2]^+$ (1.2), 390 $[449-OCOCH_3]^+$ (5), 350 $[449-OAng]^+$ (25), 349 $[449-AngOH]^+$ (8), 321 $[349-C_2H_4]^+$ (13), 249 $[449-2\times AngOH]^+$ (25), 231 $[249-H_2O]^+$ (24), 207 $[249-C_3H_6]^+$ (26), 179 $[207-C_2H_4]^+$ (25), 161 $[179-H_2O]^+$ (41), 83 $[C_4H_7CO]^+$ (100), 59 $[OCH(CH_3)_2]^+$ (12), 43 $[C_3H_7]^+$ (12), 43 $[C_3H_7]^+$ (5)
$C_{30}H_{46}O_9$

3.6. 4β-Acetoxy-5β, 8-diangeloyloxy-10, 11-dihydoxy-2β, 3β-epoxy-bisabol-7(14)-ene (**3**)

Colorless gum; $[\alpha]_D^{25}$ −44.6 (c = 0.19, $CHCl_3$); R_f = 0.29 (chloroform-petroleum ether-acetone, 5 : 1 : 0.5); IR ν_{max} 3448 (OH), 1743 (OAc), 1720 (C=CCO_2R), 1646 (C=C), 1458, 1377, 1255, 1232, 1155, 1043, 849 (C=CH_2) cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; FAB-MS m/z 509 $[M+H]^+$ (2), 491 $[M+H-H_2O]^+$ (3), 409 $[M+H-AngOH]^+$ (6), 349 $[M+H-AngOH-AcOH]^+$ (1), 249 $[M+H-2\times AngOH-AcOH]^+$ (3), 83 $[C_4H_7CO]^+$ (100), 59 $[OCOCH_3]^+$ (94).
$C_{27}H_{40}O_9$

3.7. 5β-Acetoxy-4β, 11-diangeloyloxy-8, 10-dihydoxy-2β, 3β-epoxy-bisabol-7(14)-ene (**4**)

Colorless gum; $[\alpha]_D^{25}$ −54.3 (c = 0.42, MeOH); R_f = 0.33 (chloroform-petroleum ether-acetone, 5 : 1 : 0.5); IR ν_{max} 3448 (OH), 3355 (OH), 2978, 2934, 1743 (OAc), 1720 (C=CCO_2R), 1647 (C=C), 1456, 1437, 1380, 1232, 1152, 1047, 849 (C=CH_2) cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 2 and 3; FAB-MS m/z 509 $[M+H]^+$ (1.5), 491 $[M+H-H_2O]^+$ (4.2), 431 $[M+H-H_2O-AcOH]^+$ (0.7), 391 $[M+H-H_2O-AngOH]^+$ (2.4), 331 $[M+H-AcOH-AngOH]^+$ (2), 313 $[331-H_2O]^+$ (0.5), 291 $[M+H-2\times AngOH]^+$ (1.4), 249 $[291-C_3H_6]^+$ (1.5), 231 $[M+H-H_2O-AcOH-2\times AngOH]^+$ (3.5), 83 $[C_4H_7CO]^+$ (100), 55 $[C_4H_7]^+$ (34), 43 $[COCH_3]^+$ (12)
$C_{27}H_{40}O_9$

3.8. 5β-Acetoxy-4β, 10-diangeloyloxy-3β-isobutyryloxy-2β, 8,11-trihydoxy-bisabol-7(14)-ene (**5**)

Colorless gum; $[\alpha]_D^{25}$ −49.3 (c = 0.41, $CHCl_3$); R_f = 0.45 (chloroform-acetone, 5 : 1); IR ν_{max} 3482 (OH), 1744 (OAc), 1719 (C=CCO_2R), 1648 (C=C), 1455, 1379, 1255, 1231, 1152, 1045, 850 (C=CH_2) cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 2 and 3; FAB-MS m/z 597 $[M+H]^+$ (1), 580 $[M+H-OH]^+$ (4), 579 $[M+H-H_2O]^+$ (1), 537 $[M+H-AcOH]^+$ (4), 437 $[M+H-H_2O-AcOH-AngOH]^+$ (2), 337 $[M+H-H_2O-AcOH-2\times AngOH]^+$ (1), 99 $[AngO]^+$ (23), 83 $[C_4H_7CO]^+$ (97), 71 $[C_3H_7CO]^+$ (58), 59 $[OCOCH_3]^+$ (100).
$C_{31}H_{48}O_{11}$

3.9. 5β-Acetoxy-4β, 10-diangeloyloxy-2β, 3β, 8,11-tetrahydoxy-bisabol-7(14)-ene (**6**)

Colorless gum; $[\alpha]_D^{25}$ −34.6 (c = 0.38, $CHCl_3$); $R_f = 0.32$ ($CHCl_3$–CH_3COCH_3, 5 : 1); IR ν_{max} 3448 (OH), 1744 (OAc), 1720 (C=CCO_2R), 1647 (C=C), 1451, 1380, 1255, 1232, 1152, 1048, 849 (C=CH_2) cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Table 2 and 3; FAB-MS m/z 527 $[M+H]^+$ (9), 427 $[M+H-AngOH]^+$ (2), 367 $[M+H-AngOH-AcOH]^+$ (2), 349 $[M+H-AngOH-AcOH-H_2O]^+$ (1), 267 $[M+H-2\times AngOH-AcOH]^+$ (4), 249 $[M+H-2\times AngOH-AcOH-H_2O]^+$ (4), 231 $[M+H-2\times AngOH-AcOH-2\times H_2O]^+$ (3), 213 $[M+H-2\times AngOH-AcOH-3\times H_2O]^+$ (2), 195 $[M+H-2\times AngOH-AcOH-4\times H_2O]^+$ (2), 83 $[C_4H_7CO]^+$ (100), 43 $[COCH_3]^+$ (16). $C_{27}H_{42}O_{10}$

3.10. 5α-Acetoxy-2α, 3β-diangeloyloxy-12, 8-dihydoxy-10, 11-epoxy-bisabol-7(14)-ene-4-one (**7**)

Colorless gum; $[\alpha]_D^{25}$ +15.2 (c = 0.36, $CHCl_3$); $R_f = 0.58$ (chloroform-acetone, 8 : 1); IR ν_{max} 3418 (OH), 2928, 1743 (OAc), 1707 (C=O), 1718 (C=CCO_2R), 1646 (C=CH_2), 1443, 1381, 1220, 1143, 1039, 990, 850 (C=CH_2) cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 2 and 3; FAB-MS m/z 523 $[M+H]^+$ (5), 505 $[M+H-H_2O]^+$ (7), 423 $[M+H-AngOH]^+$ (4), 323 $[M+H-2\times AngOH]^+$ (5), 363 $[M+H-AngOH-AcOH]^+$ (2), 83 $[C_4H_7CO]^+$ (100), HRFAB-MS m/z 523.2554 $[M+H]^+$ ($C_{27}H_{39}O_{10}$ requires 523.2543), 505.2457 $[M+H-H_2O]^+$ ($C_{27}H_{37}O_9$ requires 505.2437). $C_{27}H_{38}O_{10}$

4. Antimicrobial assays

The plate antibacterial test (paper-disk method) with norfloxacin as a positive control was adopted for the study of bisabolane sesquiterpenes [10]. Ten strains of bacteria, *Erysipelothrix rhusiopathiae, Streptococcus dysgalactiae, Streptococcus agalactiae, Staphylococcus aureus, Salmonella pullorum, Pasteurella multocida, Escherichia coli, Klebsiella pneumonae, Pseudomonas aeruginosa* and *Proteus vulgaris* were cultured in beef soup and incubated at 37 °C for 24 h. After dilution with the beef soup, the bacteria were inoculated in agar medium dishes 0.1 ml of 100 μg/ml of compounds **2**, **4**, **5**, **6**, and **7**, and the mixture of **1** and **3** were respectively added to 6 mm diameter paper disks under aseptic condition. After 1 h, the dried paper disks were placed on the medium dish and were cultured at 37 °C for 24 h. The antibacterial activity was calculated by the diameter (in mm) of the antibacterial circle. Each test was performed in duplicate.

Acknowledgement: This work was supported by the National Natural Science Foundation of China No. 29972017, the Education Ministry of China for Doctoral Program Foundation and the National key basic Research Development Plan No. G 1998051113.

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Received January 11, 2000
Accepted April 4, 2000

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