Institute of Organic Chemistry, State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, P.R. China

New sesquiterpenes from *Ligularia songarica*

Bo Fu, Qi-Xiu Zhu, Xiu-Ping Yang and Zhong-Jian Jia

The first phytochemical investigation of *Ligularia songarica* (Compositae) afforded three new sesquiterpenes (**1**, **3**: named ligusongaricone, **4**: named ligusongaricanolide A) which are bisabolane, cadinane and eremophilane type sesquiterpenes, respectively. Their structures were elucidated on the basis of spectroscopic methods, especially 2D-NMR techniques and chemical evidences. Four known compounds, the sesquiterpene derivatives **2** and **5**, β-sitosterol (**6**), and daucosterol (**7**), were found as well.

1. Introduction

Ligularia songarica (Fish.) Ling grows in Xinjiang Province of China. It is a medicinal plant of the genus *Ligularia* (Compositae). *L. songarica* roots are used for invigorating the circulation of blood, as an antiinflammatory and analgesic drug, curing tuberculosis and bronchitis, in relieving coughing up blood [1–3]. However, chemical studies of this plant have not been reported until now. In our preliminary investigation, we isolated five sesquiterpenes from the roots of this plant (**1**–**5**), three of which are new sesquiterpenes (**1**, **3**, **4**) of bisabolane, cadinane and eremophilane type. Recently, the antitumor and antimalarial properties of these types of sesquiterpenes were researched [4–5], and antibacterial activities of compounds **1**, **3** and **4** against *Streptococcus*, *Klebsiella* and other species were found in preliminary bioassays. The details of the isolation and structural determination of these compounds are described herein.

2. Investigations, results and discussion

Compound **1** was obtained as needles (recrystallized from petrol-acetone, 15 : 1). FAB-MS showed the quasi-molecular ion peak $[M + H]^+$ at m/z 491, and together with elemental analysis the molecular formula was suggested to be $C_{27}H_{38}O_8$ with nine centers of unsaturation deduced by ^1H NMR and ^{13}C NMR (Table 1). Its IR spectra showed the presence of two kinds of carbonyl groups (1744 cm^{-1}: OAc; 1707 cm^{-1}: $C=CCO_2R$) and a double bond (1650 cm^{-1}: C=C; 857 cm^{-1}: $C=CH_2$). In the ^1H NMR and the ^{13}C NMR spectra of **1**, there were two angeloyl groups and one acetyl group (Table 1). The FAB-MS also gave main fragments at m/z: 391 $[M + H-AngOH]^+$, 291 $[M + H\text{-}2 \times AngOH]^+$, 431 $[M + H-AcOH]^+$, 331 $[M + H\text{-}AngOH]^+$, 231 $[M + H-AcOH\text{-}2 \times AngOH]^+$, 83 $[C_4H_7CO]^+$, which supported this assumption. Apart from the three ester groups, the ^1H NMR spectra exhibited three methyls [δ 1.26 (6 H, s), 1.28 (3 H, s)], a terminal ethylene [δ 5.23 (1 H, brs), 5.04 (1 H, brs)], two methylenes [δ 1.98–2.17 (2 H, m), 2.02–1.83 (2 H, m)], one methine [δ 2.45, 1 H, ddd], and five oxygenated methines [δ 3.17 (1 H, brd), 5.35 (1 H, d), 5.30 (1 H, br dd), 5.38 (1 H, dd), 2.71 (1 H, dd)]. The ^{13}C NMR and DEPT spectra also showed three quaternary carbons [two oxygenated carbons (δ 56.5, 58.1); one olefinic carbon (δ 145.9)] except for giving relative carbon signals. According to the above information, compound **1** was confirmed as a bisabolane sesquiterpene [6–7]. The position of ester groups was determined by 2D-NMR techniques (^1H, ^1H-COSY, HMQC, HMBC). In the HMBC experiment of **1**, at first, the presence of three ester groups was further confirmed by the correlated peaks of δ_H 2.01 with δ_C 170.1, δ_H 1.98 withδ_c 166.7 and δ_H 1.95 with δ_C 166.6. Furthermore, the correlation of H-5 with δ_C 170.1 (OAc), C-3,

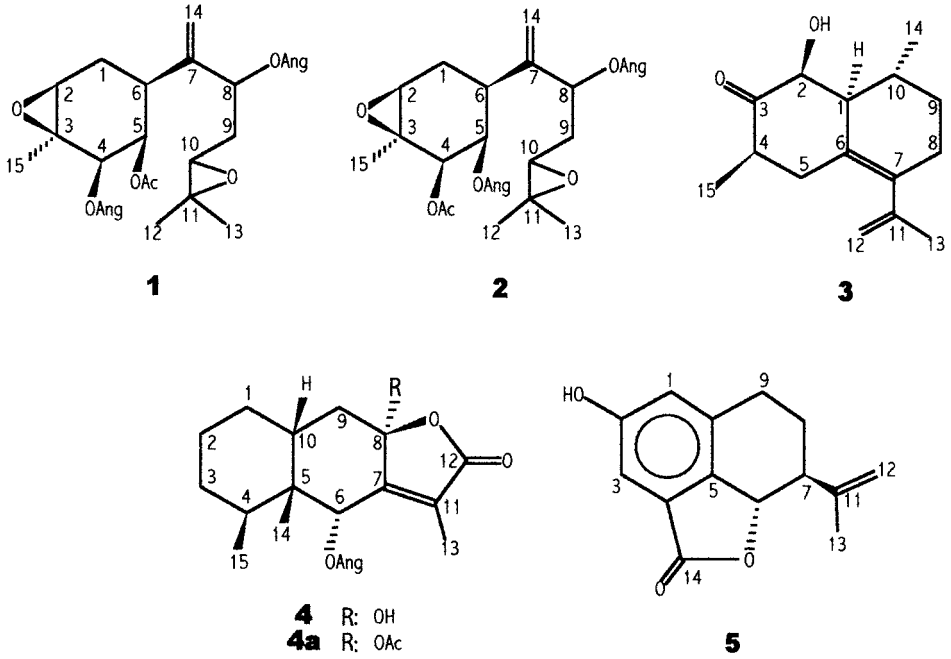

Table 1: NMR data of compounds 1 and 2

No.	^{1}H NMR[a]		^{13}C NMR[b]	
	1	**2**	**1**	**2**
1α	1.98 (1 H, m)	2.16 (1 H, m)		
1β	2.17 (1 H, dd, 15.2, 12.5)	2.22 (1 H, dd, 15.1, 11.7)	25.7	25.9 (CH_2)
2	3.17 (1 H, brd, 5.2)	3.22 (1 H, brd, 5.0)	59.5	59.7 (CH)
3	–	–	56.5	56.2 (C)
4	5.35 (1 H, d, 4.8)	5.28 (1 H, d, 4.2)	71.8	72.4 (CH)
5	5.30 (1 H, brdd, 4.8, 2.4)	5.35 (1 H, brdd, 4.2, 1.8)	68.3	67.7 (CH)
6	2.45 (1 H, ddd, 12.5, 4.0, 2.4)	2.48 (1 H, ddd, 11.7, 5.1, 1.8)	37.9	38.0 (CH)
7	–	–	145.9	145.9 (C)
8	5.38 (1 H, dd, 11.6, 2.2)	5.40 (1 H, dd, 8.6, 2.6)	74.6	74.6 (CH)
9	2.02 (1 H, m)	2.09 (1 H, m)		
9′	1.83 (1 H, m)	1.86 (1 H, m)	33.4	33.5 (CH^2)
10	2.71 (1 H, dd, 6.6, 5.2)	2.76 (1 H, dd, 6.7, 5.4)	60.7	60.8 (CH)
11	–	–	58.1	58.2 (C)
12	1.26 (3 H, s)	1.29 (3 H, s)	24.6	24.6 (CH_3)
13	1.26 (3 H, s)	1.29 (3 H, s)	24.6	24.6 (CH_3)
14	5.23 (1 H, brs)	5.25 (1 H, brs)		
14′	5.04 (1 H, brs)	5.04 (1 H, brs)	115.4	115.4 (CH_2)
15	1.28 (3 H, s)	1.32 (3 H, s)	18.9	18.9 (CH_3)
OAng	6.10, 6.06 (3′-H, qq, 7.6, 1.2), 1.98, 1.95 (4′-H, dq, 7.6, 1.5), 1.88, 1.83 (5′-H, dq, 1.5, 1.2)	6.12, 6.06 (3′-H, qq, 7.6, 1.2), 2.00, 2.01 (4′-H, dq, 7.6, 1.5), 1.91, 1.88 (5′-H, dq, 1.5, 1.2)	166.6, 166.7, 127.2, 127.4, 139.1, 139.2, 15.7, 19.5, 20.8, 20.5	166.7, 167.1, 127.4, 127.8, 138.1, 139.2, 15.7, 15.8, 20.5, 20.6
OAc	2.01 (3H, s)	2.06 (3 H, s)	170.1, 20.4	170.3, 19.9

^{1}H NMR, 400 MHz, ^{13}C NMR, 100 MHz, $CDCl_3$, TMS, δ, ppm
[a] Coupling constants in parentheses in Hz; [b] DEPT data in parentheses (**1** and **2** are same)

C-4, C-6, and C-1; H-4 with δ_C 166.6 (OAng), C-5, C-2, and C-6; H-8 with δ_C 166.7 (OAng), C-6, C-7, C-9, C-10 and C-14, pointed to the acetyl group at C-5 and the two angeloyl groups at C-4 and C-8, respectively. The correlation of H-2 with C-3, C-6, C-1 and C-15; H-10 with C-8, C-9, C-12 and C-13, indicated two epoxy groups at C-2, C-3 and C-10, C-11, respectively. The relative stereochemistry of **1** was determined on the basis of the coupling constants of H-1, H-2, H-4, H-5, H-6. If H-6 were α-oriented, H-5 must have been α-oriented because the coupling constant between H-5 and H-6 was small ($J_{5\alpha,6\alpha} = 2.4$ Hz), and H-4 and H-2 must have been likewise α-oriented because of the small coupling constants of H-4 with H-5, H-1 with H-2 andH-1 with H-6 ($J_{4\alpha,5\alpha} = 4.8$, $J_{1\alpha,6\alpha} = 4.0$, $J_{1\alpha,2\alpha} = 5.2$ Hz). It was mentioned that the coupling constant of H-1β with H-2α was almost zero for their dihedral angle being about 90° which resulted from the 2β, 3β-epoxy (it was shown by the molecular model). The stereochemistry 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, H-15; H-4 with H-5 and H-15; H-5 with H-6. Therefore, the ester groups at C-4 and C-5, and the 2,3-epoxy group must be all β-configuration.

Consequently, the structure of **1** was elucidated as 5β-acetoxy-4β,8-diangeloyloxy-2β,3β; 10,11-diepoxy-bisabola-7(14)-ene.

Compound **2**, a colorless gum, was isolated from the mixture of **1** and **2** by HPLC. Its FAB-MS gave the quasi-molecular ion peaks at m/z 491 $[M + H]^+$ and at m/z 513 $[M + Na]^+$. Other fragments were the same as those of **1**. The IR, ^{1}H NMR and ^{13}C NMR (Table 1) spectra of **2** also displayed very similar information to that of **1**. So compound **2** was an isomer of **1** and its molecular formula was also $C_{27}H_{38}O_8$. However, the HMBC experiment showed the correlated peaks of H-5 with δ_C 166.7 (OAng), H-4 with δ_C 170.3 (OAc) and H-8 with δ_C 167.1. Therefore, the acetyl group must be at C-4, and the two angeloyl groups at C-5 and C-8. The stereochemistry of **2** was confirmed by a ^{1}H,^{1}H-NOESY experiment. If H-6 was set as α-configuration, the cross peaks of H-4 with H-5 and H-6, H-2 with H-1α and H-15, H-1α with H-6, indicated that H-2, H-4, H-5 and H-6 must have been close in space, i.e. they were all α-configuration. Finally, compound **2** was determined to be 4β-acetoxy-5β,8-diangeloyloxy-2β,3β;10,11-diepoxy-bisabola-7(14)-ene. The compound had been reported [8], its highfield ^{1}H NMR, ^{13}C NMR, DEPT data were first reported there the signals were assigned by the ^{1}H, ^{1}H-COSY, HMQC, HMBC and ^{1}H, ^{1}H-NOESY experiment).

Compound **3**, was recrystallized from petrol/acetone/ethyl acetate (3 : 1 : 1) as colorless grains. Its IR spectra revealed the presence of a hydroxy group (3434 cm^{-1}), a carbonyl group (1704 cm^{-1}), double bond and terminal ethylene (1640, 1452, 888 cm^{-1}). FAB-MS gave a quasi-molecular ion peak at m/z 235 $[M + H]^+$ and lossing water peak at m/z 217 $[M + H-H_2O]^+$. EI-MS also gave a $[M]^+$ at m/z 234 and other main fragments at m/z 216 $[M\text{-}H_2O]^+$, 206 $[M\text{-}CO]^+$, 191 $[M\text{-}C_3H_5-2H]^+$, 149 $[191\text{-}C_3H_6]^+$. The ^{13}C NMR (Table 2) of **3** showed 15 carbon signals including three methyls, four methylenes (one terminal ethylene at δ 109.6), four methines (one oxygenated at δ 74.7) and four quaternary carbons (one carbonyl carbon at δ 208.5, three olefinic carbons at δ 149.5, 133.8, 125.1). Combining ^{1}H NMR data analysis (Table 2) and information displayed by MS, the molecular formula of **3** was suggested to be $C_{15}H_{22}O_2$ with five degrees of unsaturation. The ^{1}H NMR of **3** showed three methyl groups [at δ1.77 (brs) connected to a double bond at δ 1.75 (d) adjacent to a carbonyl group and δ 1.18 (d)], a terminal ethylene at δ 4.75 (brs), three methylenes (at δ 2.30–1.12,m), four methines [at δ 2.82 (brdd), δ 2.77 (m), δ 2.21 (m) and an oxygenated methine at δ 3.92 (d, connected to a hydroxy group)]. From the above results, **3** must be a bicyclic sesquiterpene. Together with two main fragments [separated by quaternary carbon atoms: CH(O)–CH–CH

Table 2: NMR data of compounds 3

No.	^{1}H NMR[a]	^{13}C NMR[b]
1	2.82 (1 H, brdd, 8.6, 3.4)	54.8 (CH)
2	3.92 (1 H, d, 3.4)	74.7 (CH)
3	–	208.5 (C)
4	2.77 (1 H, m)	34.1 (CH)
5β	2.30 (1 H, dd, 12.0, 8.6)	
5α	1.94 (1 H, m)	34.3 (CH_2)
6	–	133.8 (C)
7	–	125.1 (C)
8α	1.86 (1 H, m)	
8β	1.51 (H, m)	35.8 (CH_2)
9α	1.45 (1 H, m)	
9β	1.12 (1 H, m)	31.1 (CH_2)
10	2.21 (1 H, m)	48.7 (CH)
11	–	149.5 (C)
12	4.75 (1 H, brs)	109.6 (CH_2)
13	1.77 (3 H, brs)	20.8 (CH_3)
14	1.18 (3 H, d, 7.2)	20.4 (CH_3)
15	1.75 (3 H, d, 2.4)	7.7 (CH_3)

^{1}H NMR, 400 MHz, ^{13}C NMR, 100 MHz, $CDCl_3$, TMS, δ ppm
[a] Coupling constants in parentheses in Hz; [b] DEPT data in parentheses

($-CH_3)-CH_2-CH_2$ and $CH_3-CH-CH_2$] showed by the ^{1}H, ^{1}H-COSY and HMQC experiments [in the ^{1}H, ^{1}H-COSY spectrum, there were cross peaks of δ 3.92 (H-2) with δ 2.82 (H-1), H-1 with δ 2.21 (H-10), H-10 with δ 1.45, 1.12 (H-9) and δ 1.18 (H-14), H-9 with δ 1.86, 1.51 (H-8); δ 2.77 (H-4) with δ 2.30, 1.94 (H-5) and δ 4.75 (H-12) with δ 1.77 (H-13)], it should have a cadinene sesquiterpene skeleton [4, 9–10]. The carbon signals and the position of the substitutents were assigned with the aid of 2D-NMR experiments. Its HMBC spectrum gave the correlation of δ_C 208.5 (carbonyl group) with δ_H 3.92 (H-2), δ_H 2.30 (H-5β), and δ_H 1.75 (H-15); δ_C 74.7 (C-2) with δ_H 1.45 (H-4); δ_C 54.8 (C-1) with δ_H 3.92 (H-2), δ_H 2.30 (H-5β) and δ_H 1.18 (H-14). So the hydroxy group was at C-2 and the carbonyl group was at C-3. And the position of one double bond was pointed to be at C-6 and C-7, the other one was at C-11 and C-12 on the basis of the correlated peaks of δ_C 133.8 (C-6) with δ_H 2.82 (H-1), δ_H 2.30 (H-5β) and δ_H 1.75 (H-15), δ_C 125.1 (C-7) with δ_H 2.82 (H-1) and δ_C 149.5 (C-11) with δ_H 4.75 (H-12) and δ_H 1.77 (H-13). In addition, the characteristic UV absorption (λ_{nm}^{EtOH} 225) of **3** also indicated the presence of a conjugated diene. Its relative stereochemistry was mainly confirmed by the ^{1}H, ^{1}H-NOESY experiment. If H-4 was set as α-orientation, H-1, H-2 and H-14 should be all α-oriented due to the correlated peaks H-4α with H-1 and H-14 with H-1, H-2. The obvious correlated peaks of H-5α with H-1, H-8α, H-14 with H-8α further supported the conclusion.

Consequently, the structure of **3** was ascertained as 2β-hydroxy-cadina-6(7),11(12-dien-3-one (named as liguson-garicone).

Compound **4** was obtained as colorless needles (recrystallized from petrol/EtOAc, 5 : 1). The IR bands at 3332, 1746, 1719 and 1692 cm^{-1} indicated the presence of hydroxy groups, an unsaturated ester group and an α,β-unsaturated-γ-lactone. This was supported by the EI-MS which displayed a $[M^+]$ at m/z 348 and main fragments at m/z 330 $[M-H_2O]^+$, 248 $[M-AngOH]^+$, 231 $[M-OH-AngOH]^+$, 83 $[C_4H_7CO]^+$, and showed that the ester group should be an angloyl group. Together with ^{1}H NMR and ^{13} C NMR data (Table 3), the molecular formula was confirmed as $C_{20}H_{28}O_5$ with seven degrees of unsaturation. Expect for the angeloyl group, its ^{13}C NMR spectra also showed that there are 15 carbon atoms which were assigned with data for similar eremophilanolides [11–12]. The ^{1}H NMR data were consistent with the presence of a tertiary methyl (δ 1.06, s), a secondary methyl (δ 0.83, d) and an olefinic methyl (δ 1.97, s), further establishing the lactone as a sesquiterpene of the eremophilane-type [13]. In fact, the skeleton of **4** could be regarded as either eudesmane or eremophilane only by the ^{1}H NMR and ^{13}C NMR data. However, the most important correlations H-14 with C-4 and C-5 found in the HMQC and HMBC firmly indicated that **4** is of eremophilane-

Table 3: NMR data of compounds 4 and 4a

No.	^{1}H NMR[a]		^{13}C NMR[b]	
	4	**4a**	**4**	**4a**
1	1.35 (2 H, m)	1.36 (2 H, m)	25.4	22.1 (CH_2)
2	1.39 (2 H, m)	1.39 (2 H, m)	30.3	30.3 (CH_2)
3	1.76 (2 H, m)	1.78 (2 H, m)	19.7	19.7 (CH_2)
4	1.45 (1 H, m)	1.46 (1 H, m)	29.0	28.7 (CH)
5	–	–	42.3	44.2 (C)
6	5.71 (1 H, s)	5.81 (1 H, s)	71.3	70.7 (CH)
7	–	–	152.6	150.0 (C)
8	–	–	104.2	103.7 (C)
9α	2.09 (1 H, dd, 12.0, 10.0)	2.29 (1 H, dd, 13.6, 12)		
9β	2.27 (1 H, dd, 12.0, 5.7)	2.19 (1 H, dd, 13.6 5.9)	38.7	38.2 (CH_2)
10	1.81 (1 H, m)	1.90 (1 H, m)	35.2	35.0 (CH)
11	–	–	128.2	129.5 (C)
12	–	–	171.2	168.3 (C)
13	1.97 (3 H, s)	1.92 (3 H, s)	9.7	8.7(CH_3)
14	1.06 (3 H, s)	1.07 (3 H, s)	16.4	16.4 (CH_3)
15	0.83 (3 H, d, 6.0)	0.86 (3 H, d, 6.4)	16.5	16.6 (CH_3)
OAng	6.15 (3′-H, qq, 7.6, 1.2)	6.11 (3′-H, qq, 7.2, 1.3)	167.1, 127.2	166.5 (1′, C),
	2.02 (4′-H, dq, 7.6, 1.5)	2.00 (4′-H, dq, 7.2, 1.6)	140.3	127.3 (2′, C)
	1.97 (5′-H, dq, 1.5, 1.2)	1.91 (5′-H, dq, 1.6, 1.3)	15.9, 20.6	139.2 (3′, CH)
				15.8, 20.5 (4′, 5′-CH_3)
OH	3.70 (1 H, brs)	–	–	–
OAc	–	2.04 (3 H, s)	–	22.1

^{1}H NMR, 400 MHz, ^{13}C NMR, 100 MHz, $CDCl_3$, TMS, δ ppm
[a] Coupling constants in parentheses in Hz; [b] DEPT data in parentheses (**4** and **4a** are same)

type [14]. The hydroxy group should be located at C-8 as the signal at δ_C 104.3 in the ^{13}C NMR and a lack of a carbinolic hydrogen in the ^1H NMR. Furthermore, the comparison of ^1H NMR spectral data of **4** with that of the acetylated product (**4a**) indicated that the H-9α of **4a** was shifted downfield from δ 2.09 to δ 2.29, supported again the hydroxy group at C-8. The angeloyl group was confirmed at C-6 by an important correlation of H-6 (a sharp singlet, δ 5.71, 1 H) with δ167.1. The structure of **4** was *cis*-decaline due to the chemical shift of H-14 (δ 1.06 in $CDCl_3$), which resonate at lower field (δ 0.90–1.20 in $CDCl_3$) than those of the *trans*-isomer (δ 0.50–0.90 in $CDCl_3$) [14–16]. Then, in the biogenetic consideration of a eremophilane-type, the methyls at C-4 and C-5 are generally β-configurated, so H-10 should have β-orientation. The lactone attached to C-8 was set as β-oriented according to the negative optical rotation of **4** [17] and in the acetylated product (**4a**), the quartet assigned to H-9α was shifted to down field (δ_H 2.09 to 2.29) because the H-9α was deshielded for its closing to the acetate group at C-8α [11]. And by the comparison of **4** with the reported compounds [18], the presence of 8α-OH was clearly established for the absence of the corresponding long-range spin-coupling between the olefinic methyl (H-13) and H-6β (the dihedral angle of them lies around 30° showed by Dreiding model). The relative stereochemistry of **4** was further determined by the ^1H, ^1H-NOESY. Firstly the obvious correlated peak of H-6 with H-15β, H-14β, H-9β and H-10 indicated H-6 and H-10 should be β-oriented. Next, the presence of the correlated peaks of H-14 with H-9β and H-10, and H-9α with H-4α supported the above conclusion.

Thus, **4** was elucidated as 6α-angeloyloxy-8α-hydroxy eremophila-7(11)-en-12,8β-olide (named as ligusongaricanolide A).

Compound **5** was obtained as colorless needles (petrol/EtOAc, 3 : 1), its reaction with $FeCl_3$–EtOH solution was positive. Its ^1H NMR, IR data was completely the same to those of the reported compound, 2-hydroxyplatyphyllide [19–20]. So, compound **5** was indentified as 2-hydroxyplatyphyllide.

Compound **6** was recrystallized from petrol/acetone (20 : 1) as colorless needles and was indentified as β-sitosterol by comparison with an authentic sample and data references [21–22].

Compound **7** was obtained as a colorless wax. Its Molish reaction (α-naphthol-H_2SO_4) was positive. It was indentified as daucosterol by comparing its properties (m.p., mass, IR, ^1H and ^{13}C NMR) either with literature values or an authentic sample [21, 23].

3. Experimental

3.1. Apparatus

Optical rotations: Perkin-Elmer 241 polarimeter; IR: Nicolet 170SX FT-IR instrument; EI-MS or FAB-MS: VG-ZAB-HS spectrometer (at 70 ev); ^1H NMR (400.13 MHz, $CDCl_3$), ^{13}C NMR (100.16 MHz, $CDCl_3$), 2D-NMR (^1H, ^1H-COSY, HMQC, HMBC, ^1H, ^1H-NOESY): Bruker AM-400 FT-NMR spectrometer using tetramethylsilane (TMS) as the internal standard; UV: Shimadzu UV-260 visible recording spectrometer; Elemental analysis: Carlo Erba elemental analyzer-MOD 1106; M.p.: Kolfler m.p. determining meter (uncorr.); HPLC: Gilson Model 303 Pump and Gilson UV Detector Model 116 with Whatman-partisil 10 ODS C_{18} (9 × 250 mm) column; Silica gel (120–160 mesh/200–300 mesh) for CC and silica GF254 for TLC were supplied by the Qingdao Marine Chemical Factory.

3.2. Plant material

L. songarica was collected in the august of 1997 in the South Suburb of Urumqi, Xinjiang Province, People's Republic of China The plant was indentified by Prof. Guan-Mian Shen from the Xinjiang Institute of Biology and Pedology of Chinese Academy of Science.

3.3. Extraction and isolation

The air-dried roots of the plant (1.5 kg) were pulverized and extracted with petrol (60–90 °C)/Et_2O/MeOH (1 : 1 : 1) four times at room temperature (each time lasting 4 days). A total of extract (60 g) was obtained after concentration in reducted pressure. Then the extract was subjected to CC on silica (120–160 mesh, 500 g), with pretrol/Me_2CO gradient to afford three fractions. Considing different constituents and quantity of these fractions, two fractions were further isolated. The fraction A (7.5 g) eluted with $CHCl_3$/Me_2CO (40 : 1–50 : 1, 50 ml each eluent) was chromatographed on silica gel (200–300 mesh, 80 g) to obtain 3 fractions. Fraction 1 was isolated on a silica gel (200–300 mesh, 80 g) column, eluted with petrol/EtOAc (5 : 1) to afford **1** (40 mg) and **6** (100 mg). From fraction 2, a mixture of **1** and **2** (80 mg) was obtained. The mixture was further separated by HPLC (reverse column) eluting with MeOH : H_2O (7 : 3) to give **1** (30 mg) and **2** (20 mg). Fraction 3 eluted with $CHCl_3$/Me_2CO (20 : 1) and petrol/EtOAc (4 : 1) was chromatographed over a silicl gel (200–300 mesh,15 g) column to get **3** (20 mg). Fraction B (6 g) was chromatographed over a silica gel (200–300 mesh, 15 g) column to get **4** (60 mg). Compound **4** (25 mg) was acetylated to afford **4a**. Compounds **5** and **7** were isolated on a silica gel (200–300 mesh, 20 g) column, eluting with petrol/EtOAc (2 : 1) and Me_2CO from fraction C.

3.4. 5β-Acetoxy-4β,8-diangeloyloxy-2β,3β,10,12-diepoxy-bisabola-7(14)-ene (1)

Colorless needles, m.p. 90–90.5 °C, $[\alpha]_D^{25}$-17.63 ($CHCl_3$, c 0,5), Rf. 0.28 (petrol/EtOAc, 3 : 1), 0.38 (reverse TLC, MeOH/H_2O, 6 : 1). Elemental analysis found: C 66.20, H 7.70, Calcd. for $C_{27}H_{38}O_8$: C 66.10, H 7.81, UV (λ_{max}^{nm}, EtOH) 214, IR (υ_{max}^{KBr}, cm^{-1}): 1744 (OAc), 1707 (C=CCO_2R), 1650 (C=C), 1464, 1381, 1256, 1229, 1169, 1045, 857 (C=CH_2), FAB-MS at m/z (3-NBA as matrix, %): 491 $[M + H]^+$ (9), 391 $[M + H\text{-AngOH}]^+$ (22), 291 $[M + H\text{-}2\times\text{AngOH}]^+$ (7), 231 $[291\text{-AcOH}]^+$ (20), 83 $[C_4H_7CO]^+$ (100), ^1H NMR and ^{13}C NMR data (Table 1).

3.5. 4β-Acetoxy-5β,8-diangeloyloxy-2β,3β,10,12-diepoxy-bisabola-7(14)-ene (2)

Colorless gum, $[\alpha_D^{25}]$ -26.13 ($CHCl_3$, c 0.62), Rf 0.30 (petrol/EtOAc, 3:1), 0.34 (reverse TLC, MeOH/H_2O, 6:1). UV (λ_{max}^{nm}, ETOH) 214, IR (υ_{max}^{KBr}, cm^{-1}) 1746 (OAc), 1717 (C=CCO_2R), 1648 (C=C), 1457, 1379, 1254, 1230, 1157, 1042, 855 (C=CH_2), FAB-MS at m/z (Gly as Matrix) 513 $[M + Na]^+$, 491 $[M + H]^+$, 391 $[M + H\text{-AngOH}]^+$, 291 $[M + H\text{-AngOH}]^+$, 231 $[291\text{-AcOH}]^+$, 83 $[C_4H_7CO]^+$ (100), ^1H NMR and ^{13}C NMR data see Table 1.

3.6. Ligusongaricone (3)

Colorless grains, m.p. 89.5–91.5 °C; $[\alpha_D^{25}]$ −137.80° ($CHCl_3$, c 0.31); Rf 0.55 (petrol/EtOAc,3:1); IR (ν_{max}^{KBr}, cm^{-1}): 3434 (OH), 1704 (C=O), 1640 (C=C), 1452, 1382, 1029, 888; UV λ^{MeOH} (nm): 214 (log ε 3.6), 225 (log ε 3.1); FAB-MS (Gly as matrix): 235 $[M + H]^+$, 257 $[M + Na]^+$ EI-MS (m/z, %): 234 $[M]^+$, 216 $[M\text{-}H_2O]^+$ (29), 193 $[M\text{-}C_3H_5]^+$ (30), 191 $[193\text{-}2H]^+$ (46), 149 $[191\text{-}C_3H_6]^+$ (39), 134 $[149\text{-}CH_3]^+$ (20), 122 $[149\text{-}C_2H_3]^+$ (30), 107 $[122\text{-}CH_3]^+$ (29), 67 $[C_5H_7]^+$ (59), 55 $[C_4H_7]^+$ (96), 41 $[C_3H_5]^+$ (100); ^1H NMR and ^{13}C NMR data (Table 2).

3.7. Ligusongaricanolide A (4)

Colorless neeles, m.p. 182–183 °C, $[\alpha_D]$ −89.92 ($CHCl_3$, c 0.63), Rf 0.24 (petrol/EtOAc, 6.5 : 1), IR (ν_{max}^{KBr}, cm^{-1}), 3332 (OH), 2966, 2929, 1746, (OCOR), 1719 (C=CCOR), 1692 (sh), 1650 (C=C), 1449, 1270, 1227, 1164, 1134, 1048, 990, 919, 735, EI-MS m/z (int.) 348 $[M]^+$ (16), 330 $[M\text{-}H_2O]^+$ (6), 248 $[M\text{-AngOH}]^+$ (7), 231 $[M\text{-OH}-\text{AngOH}]^+$ (8), 230 $[M\text{-}H_2O-\text{AngOH}]^+$ (6), 174 (3), 140 (11), 109 (35), 83 $[C_4H_7CO]^+$ (100), 55 (40), ^1H NMR and ^{13}C NMR data see Table 3.

3.8. Acetylation of 4 (4a)

Compound **4** (25 mg) was acetylated in the usual manner. PTLC of the reaction mixture afforded 15 mg **4a**, m.p. 100–102 °C, Rf 0.40 (petrol/EtOAc, 6,5 : 1). For ^1H NMR and ^{13}C NMR data see Table 3.

3.9. 2-Hydroxyplatyphyllide (5)

Colorless needles, m.p. 197–198 °C, Rf 0.45 (petrol/EtOAc: 3 : 1). ^1H NMR δ ppm ($CDCl_3$) 7.10 (1 H, d, J = 1.8 Hz, H-3), 6.92 (1 H, d, J = 1.8 Hz H-1), 5.18 (1 H, d, J = 10.8 Hz, H-6), 4.98 (brs, H-12), 4.97 (brs, H-12), 3.10 (dd, J = 17.6, 8 Hz, H-9β), 2.80 (m, H-9α), 2.63 (m, H-8α), 2.21 (m, H-8β), 2.15 (m, 7-H), IR (λ_{max}^{KBr}, cm^{-1}) 3315 (OH), 1736 (CO), 1626, 1620 (sh), 1445, 1349, 1304, 1109, 890, 850 (C=CH_2). EI-MS (m/z, %) 230 (M^+, 23), 202 (M^+-H_2O, 6), 162 (100), 149 (12), 134 (56), 106 (6), 83 (4), 57 (2).

3.10. β-Sitosterol (6)

Colorless needles, m.p. 139–140 °C (petrol/acetone, 20 : 1). Its MS, ^{1}H NMR and ^{13}C NMR data were completely identical to those reported for β-sitosterol.

3.11. Daucosterol (7)

Colorless wax, all respects (m.p., mass, IR, ^{1}H NMR and ^{13}C NMR) were fitted to literature values or an authentic sample.

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Prof. Zhong-Jian Jia
Institute of Organic Chemistry
Lanzhou University
Lanzhou, Gansu, 730000
P.R. China
zhengrl@lzu.edu.cn