New Eremophilane Sesquiterpenes from *Ligularia japonica*

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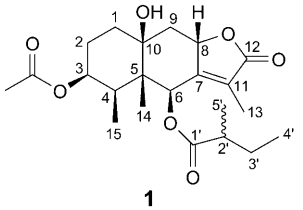
Four new eremophilane-type sesquiterpenes, 3β-(acetyloxy)-6β-(2-methylbutanoyloxy)-10β-hydroxy-eremophil-7(11)-en-12,8α-olide (**1**), 3β-(acetyloxy)-6α,7α-epoxy-11-noreremophil-9(10)-ene-8,11-dione (**2**), 3β-hydroxy-11-noreremophila-6(7),9(10)-diene-8,11-dione (**3**), and 3β-(3-methylbutanoyloxy)-11-noroxoeremophila-6(7),9(10)-diene-8,11-dione (**4**) were isolated from roots of *Ligularia japonica*. The structures of the new compounds were elucidated through spectral studies including HR-EI-MS, IR, and NMR data.

Introduction. – The genus *Ligularia* belongs to the tribe Senecioneae, family Compositae, and comprises more than 110 species native to China. Approximately 40 species have long been used as traditional Chinese herbal medicine for the purposes of invigorating the circulation of blood, clearing heat and toxins, and to ameliorate diuretic and choleretic problems [1]. Eremophilane derivatives, the main constituents of the genus *Ligularia*, were isolated recently. So far, notable activities were reported for eremophilenolides, including antibacterial and cytotoxic properties, as well as inhibition of production of NO and prostaglandin E_2 in macrophages [2–4]. The roots of *Ligularia japonica* have been used for relieving cough and asthma, activating blood and dissolving stasis, abating jaundice in Southeast China. In the present paper, the four new compounds **1**–**4** were isolated and their structures were elucidated based on spectroscopic evidence.

Results and Discussion. – Compound **1**, obtained as a colorless oil, has the molecular formula $C_{22}H_{32}O_7$, as determined by HR-EI-MS (M^+ at m/z 408.2143; calc. 408.2148). The IR spectrum showed absorption bands for OH (3446 cm^{-1}) and C=O (1761, 1738 cm^{-1}) groups. The ^{1}H-NMR spectrum (see *Table 1*) indicated a typical Me *triplet* appearing at δ(H) 0.88 (J = 7.5), three Me *doublet*s at δ(H) 1.01 (J = 6.9), 1.14 (J = 7.2), and 1.89 (J = 2.4), and two Me *singlets* at δ(H) 1.27 and 2.05. The ^{13}C-NMR spectrum displayed 22 signals, of which three are C=O functions (δ(C) 177.7, 176.3, and 172.3). In addition, a tetrasubstituted olefinic bond (δ(C) 158.3 (*s*), 126.2 (*s*)), three O–CH groups (δ(C) 78.4, 74.8, and 71.4), an O–C group (δ(C) 75.6), and six Me groups (δ(C) 21.3, 17.1, 13.3, 13.0, 11.9, and 9.0) could be discerned. The ^{1}H- and ^{13}C-NMR signals of **1** were fully assigned by means of ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments.



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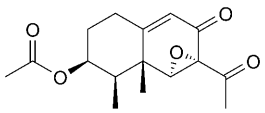

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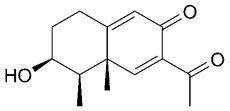

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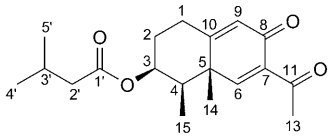

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Table 1. *^{1}H-NMR Data* (300 MHz) *of Compounds* **1**–**4**. δ in ppm; J in Hz.

	1[a])	**2**[a])	**3**[b])	**4**[b])
CH_2(1)	1.95–2.06 (*m*), 1.33–1.40 (*m*)	2.73–2.84 (*m*), 2.23–2.34 (*m*)	2.82–2.94 (*m*), 2.32–2.40 (*m*)	2.63–2.68 (*m*), 2.28–2.36 (*m*)
CH_2(2)	1.64–1.74 (*m*), 1.65–1.73 (*m*)	2.01–2.09 (*m*), 1.66–1.75 (*m*)	2.10–2.19 (*m*), 1.61–1.69 (*m*)	2.11–2.19 (*m*), 1.64–1.73 (*m*)
H–C(3)	4.88–4.90 (br. *s*)	5.10 (*d*, J = 3.0)	3.93 (*d*, J = 3.0)	5.08 (*d*, J = 3.0)
H–C(4)	1.62–1.72 (*m*)	2.03–2.09 (*m*)	1.52–1.59 (*m*)	1.68–1.76 (*m*)
H–C(6)	5.83 (*s*)	3.72 (*s*)	7.60 (*s*)	7.60 (*s*)
H–C(8)	5.18–5.25 (*m*)			
CH_2(9) or H–C(9)	2.22–2.29 (*m*), 1.93–2.01 (*m*)	5.84 (*s*)	6.13 (*s*)	6.15 (*s*)
Me(13)	1.89 (*d*, J = 2.4)	2.25 (*s*)	2.54 (*s*)	2.55 (*s*)
Me(14)	1.27 (*s*)	1.41 (*s*)	1.40 (*s*)	1.38 (*s*)
Me(15)	1.01 (*d*, J = 6.9)	1.17 (*d*, J = 7.2)	1.30 (*d*, J = 6.9)	1.19 (*d*, J = 6.9)
H–C(2′) or CH_2(2′)	2.43–2.49 (*m*)			2.28 (*d*, J = 6.6)
CH_2(3′) or H–C(3′)	1.61–1.67 (*m*)			2.14–2.20 (*m*)
Me(4′)	0.88 (*t*, J = 7.5)			0.98 (*d*, J = 6.6)
Me(5′)	1.14 (*d*, J = 7.2)			0.98 (*d*, J = 6.6)
AcO	2.05 (*s*)	2.15 (*s*)		

[a]) Measured in CD_3OD. [b]) Measured in $CDCl_3$.

The ^{13}C-NMR data (see *Table 2*) of **1** were nearly superimposable with those of 3β-(acetyloxy)-6β-(2-methylbutanoyloxy)-8β,10β-dihydroxyeremophil-7(11)-en-12,8-olide [5]; the only difference was that in compound **1** a H-atom is linked to C(8) instead of a hemiacetal OH group in the latter, which was deduced from the chemical shift value of H–C(8) (δ(H), 5.18–5.25), the HMBC H–C(6)/C(8), and the COSY correlation H–C(8)/H–C(9) (*Figure*). Thus, **1** was identified as 3β-(acetyloxy)-6β-(2-methylbutanoyloxy)-10β-hydroxyeremophil-7(11)-en-12,8α-olide.

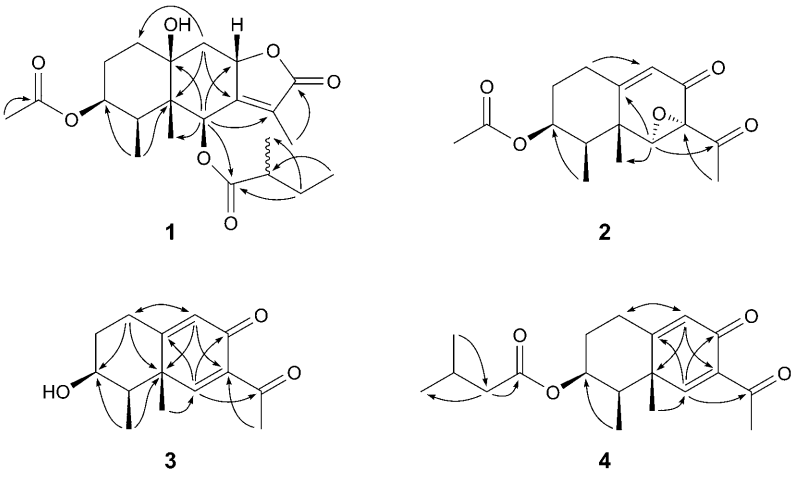

Figure. *^{1}H,^{1}H-COSY* (—) *and key HMBC data* (H→C) of **1**–**4**

Table 2. *^{13}C-NMR Data* (75 MHz) *of Compounds* **1**–**4**. δ in ppm.

	1[a])	**2**[a])	**3**[b])	**4**[b])
C(1)	32.9	29.1	27.6	27.9
C(2)	28.5	32.0	35.9	32.7
C(3)	74.8	75.0	71.3	72.8
C(4)	37.4	41.4	43.4	42.4
C(5)	47.9	42.4	44.3	43.8
C(6)	71.4	67.2	160.1	159.2
C(7)	158.3	65.1	136.2	136.5
C(8)	78.4	192.1	184.1	183.8
C(9)	42.0	121.6	125.1	125.7
C(10)	75.6	168.2	168.7	166.7
C(11)	126.2	203.2	199.7	199.3
C(12)	176.3			
C(13)	9.0	27.9	31.2	31.2
C(14)	13.0	20.7	20.4	20.1
C(15)	13.3	12.5	12.8	12.5
C(1′)	177.7			172.6
C(2′)	42.5			44.1
C(3′)	27.9			26.1
C(4′)	11.9			22.6
C(5′)	17.1			22.6
AcO	172.3, 21.3	172.3, 21.2		

[a]) Measured in CD_3OD. [b]) Measured in $CDCl_3$.

Compound **2**, obtained as a colorless oil, was assigned the molecular formula of $C_{16}H_{20}O_5$ by HR-EI-MS analysis (M^+ at m/z 292.1304; calc. 292.1311). The IR spectrum showed strong signals due to three C=O groups (1732, 1670, 1626 cm^{-1}). The ^{1}H-NMR spectrum (*Table 1*) exhibited resonances of an olefinic H-atom at δ(H) 5.84, an O–CH

group at δ(H) 3.72, a Me *doublet* at δ(H) 1.17 ($J = 7.2$), and three Me *singlet*s at δ(H) 2.25, 2.15, and 1.41. The ^{13}C-NMR (DEPT) spectrum showed four Me and two CH_2 groups, an olefinic bond with signals at δ(C) 168.2 and 121.6, three sp^3 CH groups, including two O–CH groups at δ(C) 75.0 and 67.2, an O–C group at δ(C) 65.1, a C=O group at δ(C) 203.2, an α,β-unsaturated C=O group at δ(C) 192.1, and a C=O group at δ(C) 172.3, presumably due to a carboxylic acid or a carboxylic acid ester.

The ^{13}C-NMR spectrum of **2** (*Table 2*) was nearly identical with that of 1β-(acetyloxy)-6α,7α-epoxy-11-noreremophil-9(10)-ene-8,11-dione [6], indicating that both share an eremophilane skeleton, except that the AcO group of **2** is located at C(3), which was evident from the chemical shift values of H–C(3) (δ(H) 5.10) and C(3) (δ(C) 75.0), and the HMBC Me(15)/C(3) (*Figure*). Thus, the structure of compound **2** was elucidated as 3β-(acetyloxy)-6α,7α-epoxy-11-noreremophil-9(10)-ene-8,11-dione.

Compound **3**, isolated as a colorless gum, has the composition as $C_{14}H_{18}O_3$ according to the HR-EI-MS analysis (M^+ at m/z 234.1259; calc. 234.1256). The IR spectrum (KBr) showed absorption bands for an OH group (3493 cm^{-1}) and two C=O groups (1728, 1659 cm^{-1}). The ^{1}H-NMR spectrum (*Table 1*) displayed two olefinic H-atoms at δ(H) 7.60 and 6.13, an O–CH at δ(H) 3.93, and three Me groups at δ(H) 2.54, 1.40, and 1.30. The ^{13}C-NMR (DEPT) spectrum revealed a C=O group at δ(C) 199.7, an α,β-unsaturated C=O group at δ(C) 184.1, four olefinic C-atoms at δ(C) 160.1, 136.2, 125.1, and 168.7, an O–CH group at δ(C) 71.3, and three Me groups at δ(C) 31.2, 20.4, and 12.8.

Except for the absence of an AcO group, the ^{13}C-NMR signals of **3** were nearly superimposable with those of the skeleton C-atoms of 3β-(acetyloxy)-11-noreremophila-6(7),9(10)-diene-8,11-dione [5], indicating that they share the same sesquiterpene skeleton. The chemical shift value and the coupling constant of H–C(3) (δ(H) 3.93, *d*, $J = 3.0$) revealed that a OH group was located at C(3) with β-configuration. From the above data, the structure of **3** was, therefore, elucidated as 3β-hydroxy-11-noreremophila-6(7),9(10)-diene-8,11-dione.

Compound **4**, obtained as a colorless gum, has the molecular formula $C_{19}H_{26}O_4$, as determined by HR-EI-MS (M^+ at m/z 318.1835; calc. 318.1831). The IR spectrum showed absorption bands for C=O groups (1731, 1695, 1660 cm^{-1}). Comparison of the ^{1}H- and ^{13}C-NMR spectra of **4** and **3** indicated that the only difference was that **4** had an additional 3-methylbutanoyl group, attached to C(3) with β-configuration, based on the chemical shift value and coupling constant of H–C(3) (δ(H) 5.08, *d*, $J = 3.0$), and the HMBC Me(15)/C(3) (*Figure*). As a result, the structure of compound **4** was established as 3β-(3-methylbutanoyloxy)-11-noreremophila-6(7),9(10)-diene-8,11-dione.

Experimental Part

General. All solvents used were of analytical grade (*Shanghai Chemical Plant*). Column chromatography (CC): silica gel *H* (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Ltd.*), *Sephadex LH-20* (25–100 mm; *Pharmacia Fine Chemicals*), *MCI* gel *CHP 20P* (75–150 μm; *Mitsubishi Chemical Ind.*), *D-101* porous resin (*Chemical Factory of Tianjin University*), and *RP-18* (20–45 μm; *Fuji Silysia Chemical Ltd.*). Thin-layer chromatography (TLC): silica gel GF_{254} (*Yantai Huiyou Inc.*). Optical rotations: $CHCl_3$ or MeOH solns.; *Perkin-Elmer PE-241* polarimeter. IR Spectra: *Perkin-Elmer 16-PC-FT-IR* spectrometer; in cm^{-1}. ^{1}H- (300 MHz) and ^{13}C-NMR (75 MHz) Spectra: *Varian Mercury-300*

spectrometer; δ in ppm, J in Hz, with Me_4Si as an internal standard. EI- and HR-EI-MS: *Finnigan MAT-90/95* sector-field mass spectrometer; in m/z.

Plant Material. The roots of *Ligularia japonica* were collected in Dali City, Yunnan Province, China, and identified by *L.-H. H.* A voucher specimen was deposited with the Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Shanghai.

Extraction and Isolation. The air-dried and powdered roots of *L. japonica* (2.4 kg) were extracted with 95% EtOH (3×20 l) at r.t. After removal of the solvent in vacuum, an extract of 312.5 g was obtained, which was suspended in H_2O and then extracted with $CHCl_3$ ($2\,l \times 3$). After evaporation, the residue of the org. layer (51.0 g) was subjected to CC (*MCI* gel; EtOH/H_2O 20:80, 27:73, 35:65, 43:57, 50:50, 60:40, 80:20, and 100:0) to afford eight fractions (*Fr. A–H*). *Fr. D* (2.1 g) was isolated by CC (SiO_2; $CHCl_3$/MeOH 99:1, 98:2, 95:5, 9:1, 8:2) to give eight fractions (*Fr. D1–D8*). *Fr. D2* (80.1 mg) was separated by CC (*RP-18*; 55% aq. MeOH) to afford **1** (11 mg) and **2** (25 mg). *Fr. F* (5.6 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 99:1, 98:2, 95:5, 9:1, 8:2, 6:4) to afford seven fractions (*Fr. F1–F7*). *Fr. F3* (170.4 mg) was chromatographed on CC (1. *Sephadex LH-20*; 50% aq. MeOH, 2. *RP-18*; 63% aq. MeOH) to provide **3** (18.4 mg) and **4** (20.1 mg).

3β-(Acetyloxy)-6β-(2-methylbutanoyloxy)-10β-hydroxyeremophil-7(11)-en-12,8α-olide (=*(4*R,*4a*S,*5*R,*6*S,*8a*S,*9a*S*)-6-(Acetyloxy)-2,4,4a,5,6,7,8,8a,9,9a-decahydro-8a-hydroxy-3,4a,5-trimethyl-2-oxonaphtho[2,3-*b*]furan-4-yl 2-Methylbutanoate*; **1**). Colorless oil. $[\alpha]_D^{25} = +15.2$ ($c = 0.15$, MeOH). IR (KBr): 3446, 2968, 2939, 1761, 1738, 1462, 1379, 1252, 1140, 1036, 974. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-EI-MS: 408.2143 (M^+, $C_{22}H_{32}O_7^+$; calc. 408.2148).

3β-(Acetyloxy)-6α,7α-epoxy-11-noreremophil-9(10)-ene-8,11-dione (=*(1a*S,*6*S,*7*R,*7a*R,*7b*S*)-1a-Acetyl-1a,2,4,5,6,7,7a,7b-octahydro-7,7a-dimethyl-2-oxonaphtho[1,2-*b*]oxiren-6-yl Acetate*; **2**). Colorless oil. $[\alpha]_D^{25} = +210.8$ ($c = 0.4$, MeOH). IR (KBr): 2942, 1732, 1670, 1626, 1375, 1244, 1216, 1022, 979, 892. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-EI-MS: 292.1304 (M^+, $C_{16}H_{20}O_5^+$; calc. 292.1311).

3β-Hydroxy-11-noreremophila-6(7),9(10)-diene-8,11-dione (=*(4a*R,*5*R,*6*S*)-3-Acetyl-5,6,7,8-tetrahydro-6-hydroxy-4a,5-dimethylnaphthalen-2(4a*H*)-one*; **3**). Colorless gum. $[\alpha]_D^{25} = +39.9$ ($c = 0.3$, MeOH). IR (KBr): 3493, 2933, 1728, 1659, 1448, 1376, 1261, 1122, 977, 958, 892. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-EI-MS: 234.1259 (M^+, $C_{14}H_{18}O_3^+$; calc. 234.1256).

3β-(3-Methylbutanoyloxy)-11-noreremophila-6(7),9(10)-diene-8,11-dione (=*(1*R,*2*S,*8a*R*)-7-Acetyl-1,2,3,4,6,8a-hexahydro-1,8a-dimethyl-6-oxonaphthalen-2-yl 3-Methylbutanoate*; **4**). Colorless gum. $[\alpha]_D^{25} = +9.3$ ($c = 0.3$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-EI-MS: 318.1835 (M^+, $C_{19}H_{26}O_4^+$; calc. 318.1831).

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