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Bieremoligularolide and eremoligularin, two novel sesquiterpenoids from *Ligularia muliensis*

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Abstract—From the roots of *Ligularia muliensis*, a novel bieremophilanolide and a new eremophilanolide have been isolated and their structures were elucidated by spectroscopic techniques, including HRMS, IR, UV, 1D-NMR, 2D-NMR, and CD spectra.

In our ongoing investigation of bioactive compounds from the Compositae plants we have studied the dried rhizomes of *Ligularia muliensis* found in mountainous areas in southwestern China. No medicinal use of *L. muliensis* has been reported but most of *Ligularia* plants have been used as folk remedies for their antibiotic, antiphlogistic, and antitussive activities[1] and many bioactive eremophilane-type sesquiterpene lactones have been found.[2,3] We herein report the isolation and structural determination of a novel bieremophilanolide, bieremoligularolide (**1**) and a new eremophilanolide, eremoligularin (**2**) from the roots of *L. muliensis*, as well as their antitumor activities.

The dried rhizomes of *L. muliensis* were extracted successively with a mixed solvent of petroleum ether–Et_2O–acetone (1:1:1) and the extract (67.5 g from 1975 g dried rhizomes) was chromatographed on silica gel columns using a stepwise solvent gradient method and prep.TLC to give the two new sesquiterpenoids bieremoligularolide (**1**, 0.029% yield) and eremoligularin (**2**, 0.006% yield).

Compound **1** was a colorless crystalline material, mp 231–233 °C, $[\alpha]_D^{28}$ +92 (*c* 0.5, CH_3OH). A quasimolecular ion peak at *m/z* 712.4071 $(M+NH_4)^+$ (calcd 712.4055) in HRESIMS showed that the molecular formula was $C_{40}H_{54}O_{10}$. But the peak of the highest mass in its EIMS was at *m/z* 347 (base peak), which was one half of the molecular weight and its ^{13}C NMR spectrum contained only 20 signals which were five CH_3, three CH_2, five CH, and seven C from DEPT spectrum. Thus compound **1** should be a molecule formed from two identical units[4] and seven degrees of unsaturation per unit. The structure of this 'unit' was elucidated as follows: its IR spectrum exhibited strong α,β-unsaturated γ-lactone absorptions at 1771 and 1676 cm^{-1}. The ^{13}C NMR and DEPT spectra at δ 172.1 (C), 157.7 (C), 125.3 (C), together with the UV absorption at λ_{max} (CH_3OH) 218 nm, confirmed the presence of this lactone group. In ^1H NMR[5] and ^{13}C NMR (Table 1) there were signals of a tertiary methyl group [δ_H 1.22 (s), δ_C 18.1 (CH_3)], a secondary methyl group [δ_H 1.01 (d, J = 6.9 Hz), δ_C 13.0 (CH_3)] and an olefinic methyl group [δ_H 1.80 (s), δ_C 8.6 (CH_3)], which were characteristic of an eremophilanolide skeleton. In addition, this unit obviously had an angeloyl moiety from the ^1H NMR data,[5] and IR spectrum confirmed the presence of the α,β-unsaturated ester group (1724 and 1648 cm^{-1}) and also showed an absorption of a hydroxyl group (3463 cm^{-1}). HMBC (Fig. 1) of **1** exhibited that the angeloyl and hydroxyl groups were at the C-6 and C-3, respectively. Other signals were unambiguously assigned by ^1H–^1H COSY, HMQC, and HMBC spectra. So the structure of the unit was determined.

The signal at δ_C 89.1 (sp^3 quarternary carbon) implied that the two identical units joined at C-8 and C-8′

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Table 1. ^{13}C NMR and DEPT data of compound **1** (acetone-d_6) and **2** ($CDCl_3$)[a]

No.	1		No.	2	
	δC	DEPT		δC	DEPT
1,1′	22.0	CH_2	1	28.9	CH_2
2,2′	28.4	CH_2	2	20.3	CH_2
3,3′	70.4	CH	3	29.7	CH_2
4,4′	41.4	CH	4	31.2	CH
5,5′	45.9	C	5	39.2	C
6,6′	78.1	CH	6	89.0	CH
7,7′	157.7	C	7	81.5	C
8,8′	89.1	C	8	212.7	C
9,9′	33.2	CH_2	9	40.1	CH_2
10,10′	38.3	CH	10	36.1	CH
11,11′	125.3	C	11	46.6	CH
12,12′	172.1	C	12	175.1	C
13,13′	8.6	CH_3	13	9.2	CH_3
14,14′	18.1	CH_3	14	17.0	CH_3
15,15′	12.9	CH_3	15	16.4	CH_3
1″	165.4	C			
2″	127.1	C			
3″	139.9	CH			
4″	15.2	CH_3			
5″	19.8	CH_3			

[a] Spectra were recorded on a Varian Mercury-300 MHz spectrometer, chemical shifts (δ) are in ppm.

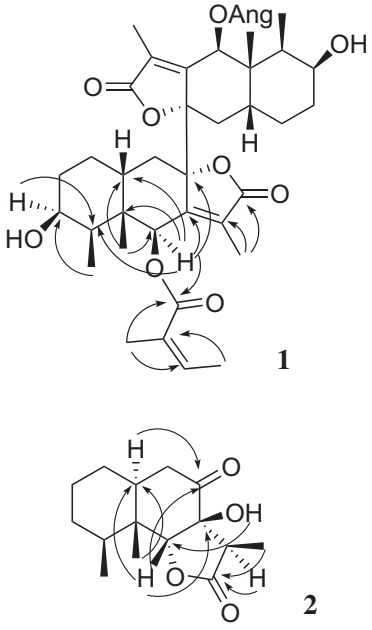


Figure 1. HMBC correlations found for **1** and **2**.

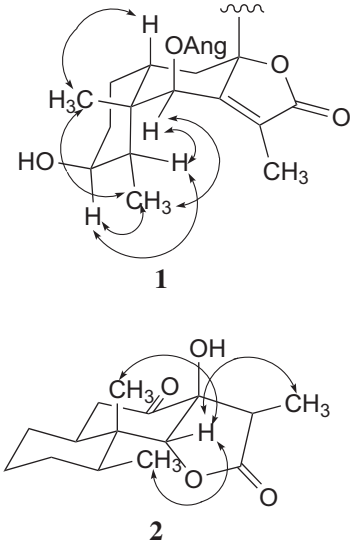
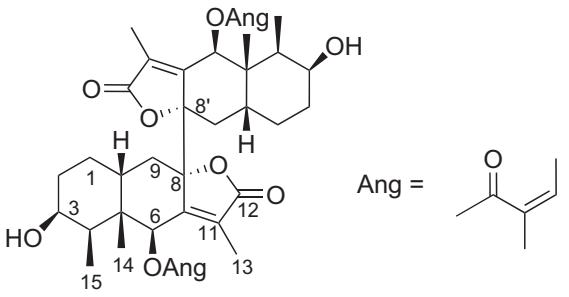


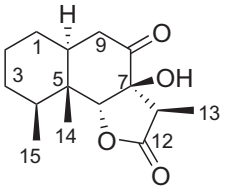
Figure 2. Key NOESY and NOE correlations detected for **1** and **2**.

positions and formed dimeric sesquiterpene lactones. Stereochemically, CH_3-14 and CH_3-15 were in β-orientations.[6] The appearance of correlations between CH_3-14 and CH_3-15; CH_3-10 and H-14; and CH_3-10 and H-15 on NOE difference spectra indicated their *cis* relationship; namely, A/B was a *cis*-fused arrangement. The configurations of the angeloyl at C-6 and of the hydroxyl at C-3 were deduced to be β-orientated from NOESY, in which significant cross peaks between H-6 and H-4α, and CH_3-15; H-3 and H-2α, H-2β, H-4α, and CH_3-15 could be observed. The NOESY spectrum showed that the conformation of molecule is steroidal (Fig. 2), which was supported by the coupling pattern of H-3α (quartet with $J_{3\alpha,2\alpha} = J_{3\alpha,2\beta} = J_{3\alpha,4\alpha} = 3$ Hz).[7] Consequently the other half of the molecule at C-8 position must be β, which was the same direction as the angeloyl at C-6.[7] This was further confirmed by ^1H NMR due to the absence of homoallylic coupling between H-6 and CH_3-13.[6] Therefore the structure of bieremoligularolide was established as depicted in the formula **1**.

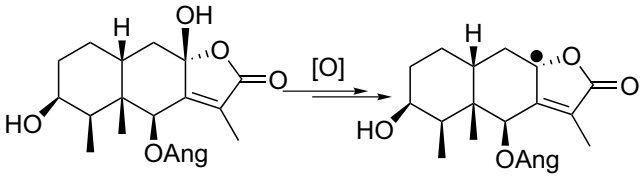
A possible biosynthetic pathway for the bieremoligularolide is shown in Scheme 1. A naturally occurring



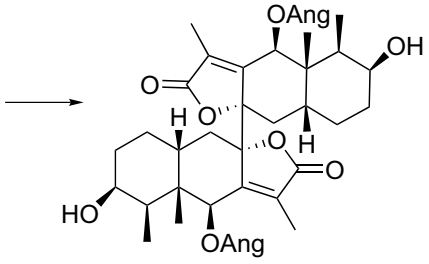
1



2



3



Scheme 1. Plausible biosynthetic pathway for (**1**).

sesquiterpene lactone **3**, also obtained from the species by us, is perhaps the parent compound of this dimer.[8]

Compound **2** was a colorless crystal, mp 192–195 °C, $[\alpha]_D^{28}$ −8 (*c* 0.89, CH_3OH). Its molecular formula $C_{15}H_{22}O_4$ was determined by HRESIMS, accounting for five degrees of unsaturation. The IR spectrum showed 3461 cm^{-1} for a hydroxyl group, 1774 cm^{-1} for a saturated γ-lactone, and 1709 cm^{-1} for a ketone carbonyl. The ^{13}C NMR and DEPT spectra (Table 1) disclosed an ester carbonyl group at δ 175.1 and an oxygenated methine carbon at δ 89.0, indicating the presence of a lactone ring. Besides, a ketone carbonyl at δ 212.7 and a quarternary carbon bearing a hydroxyl group at δ 81.5 could be observed. In the ^1H NMR spectrum,[9] one tertiary and two secondary methyl signals were present at δ 1.15 (s), 0.84 (d, *J* = 7.0 Hz), 1.04 (d, *J* = 7.6 Hz). These observations and ^1H–^1H COSY, HMBC (Fig. 1) suggested that **2** was an eremophilanolide derivative with 6,12-olide, 7-hydroxyl, and 8-oxo.

The relative stereostructure was determined by the NOESY and NOE difference spectra, in which the correlations between H-6 and CH_3-14; H-6 and CH_3-15; and H-6 and CH_3-13 were detected (Fig. 2). The absolute configuration was determined by its CD spectrum, in which a positive Cotton effect by the C-8 carbonyl group was shown at 292 nm (Δε +4.5) and a negative effect by the saturated γ-lactone at 216 nm (Δε −4.2). Application of the octant rule[10] and lactone sector rule[11] to **2** indicated that the A/B ring of **2** was a *trans* arrangement, the lactone ring was *cis*-fused and in α-orientation and the hydroxyl was in β-orientation.[12,13] Thus, the total structure was established as depicted in the formula **2**.

Cytotoxic activity of compounds **1** and **2** was assayed by SRB method against human leukemia cell (HL-60), human hepatoma cell (SMMC-7721) and human cervical carcinoma cell (HeLa). Compound **1** showed strong cytotoxicity: IC_{50} = 5.5, 16.1, and 8.9 μM against HL-60, SMMC-7721, and HeLa cells, whereas **2** showed no cytotoxicity against the above three cells (IC_{50} > 100 μM).

Acknowledgements

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5. Compound **1**: $C_{40}H_{54}O_{10}$ EIMS *m/z* 347, 264, 247, 229, 175, 124, 100, 83; IR: 3463, 1771, 1724, 1676, 1648 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6): δ 1.01 (3H, d, *J* = 6.9 Hz, H-15), 1.22 (3H, s, H-14), 1.36 (1H, m, H-10), 1.62 (1H, dq, *J* = 10.2, 3 Hz, H-2β), 1.71 (1H, m, H-2α), 1.80 (3H, s, H-13), 1.83 (1H, m, H-1β), 1.86 (1H, qd, *J* = 6.9, 3 Hz, H-4), 1.91 (1H, m, H-9β), 1.92 (3H, dq, *J* = 7.2, 1.5 Hz, H-4″), 1.95 (3H, dq, *J* = 1.5, 1.5 Hz, H-5″), 2.26 (1H, m, H-1), 2.77 (1H, t, *J* = 10.6 Hz, H-9α), 3.81 (1H, q, *J* = 3 Hz, H-3), 5.17 (1H, s, H-6), 6.21 (1H, qq, *J* = 7.2, 1.5 Hz, H-3″).
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9. Compound **2**: $C_{15}H_{22}O_4$ EIMS *m/z* 266 [M^+], IR: 3461, 1774, 1709 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.84 (3H, d, *J* = 7.0 Hz, H-15), 1.04 (3H, d, *J* = 7.6 Hz, H-13), 1.15

(3H, s, H-14), 1.25–1.34 (5H, m, H-2, H-3, and H-4 overlapping), 1.50–1.65 (2H, m, H-1), 2.33 (1H, dd, $J = 16.4$, 3.6 Hz, H-9α), 2.47 (1H, dd, $J = 16.4$, 13.0 Hz, H-9β), 2.55 (1H, m, H-10), 3.01 (1H, q, $J = 7.6$ Hz, H-11), 3.93 (1H, s, OH), 4.62 (1H, s, H-6).

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