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A novel bieremophilanolide from *Ligularia lapathifolia*

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A new bieremophilanolide was isolated from the roots and rhizomes of *Ligularia lapathifolia*. Its structure was established as 8,8′-bi-3β-angeloyloxy-eremophil-7(11)-*en*-12,8α(14β,6α)-diolide (**1**) by IR, MS, 1D, and 2D NMR experiments.

Keywords: *Ligularia*; Angeloyloxy group; Bieremophilanolide

1. Introduction

The genus *Ligularia* (compositae) contains more than 110 species occurring in China, of which about 40 species have been used as traditional Chinese herbs. *Ligularia lapathifolia* (Franch.) Hand.-Mazz. is mainly distributed in Southwest China, and its roots and rhizomes have been used to treat cough and inflammation by local inhabitants from a long time [1]. In our studies on finding bioactive compounds from the *Ligularia* genus [2–7], a novel bieremophilanolide were obtained from the EtOH extract of the roots and rhizomes of *L. lapathifolia* (Franch.) Hand.-Mazz. In the present study, the isolation and structural assignment of compound **1** have been reported.

2. Results and discussion

The molecular formula of **1** was established as $C_{40}H_{46}O_{12}$ on the basis of HR-EIMS at m/z 718.3121 $[M]^+$ (Calcd 718.3015 for $C_{40}H_{46}O_{12}$). But the peak of the highest mass in

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Table 1. ^{1}H NMR, ^{13}C NMR data of compound **1** in $CDCl_3$ (TMS as an internal standard).

Proton(s)		Carbon	
1	2.00 m	1	21.2
	1.97 m		
2	1.97 m	2	24.8
	1.22 dq (10.2, 3.0)		
3α	5.45 q (3.0)	3	64.4
4α	2.34 d (3.0)	4	42.6
		5	44.5
6β	4.91 q (1.8)	6	83.8
		7	155.7
		8	87.9
9	1.72 m 2.52 m	9	34.9
10β	2.60 m	10	33.8
		11	127.0
		12	170.6
13	2.13 br s	13	10.0
		14	170.8
15	1.55 s	15	22.8
OAng		OAng	
3′	6.06 dq	1′	164.3
4′	1.83 br s	2′	139.8
5′	1.95 dq	3′	129.5
		4′	20.7
		5′	15.7

its EIMS was *m/z* 359 (base peak), which was one-half the molecular weight and its ^{13}C NMR spectrum contained only 20 signals. Thus compound **1** should be a molecule formed from two identical units. The IR spectrum showed absorptions at 1800, 1764, and 1714 cm^{-1} for three ester carbonyl groups. In its ^{1}H NMR and ^{13}C NMR spectra, there are signals for an angeloyloxy group (table 1), which was also supported by the ion fragment peak at *m/z* 618 $[M-\text{anglic acid}]^+$, 518 $[M-2\text{anglic acid}]^+$in the EIMS spectrum. In addition to the five carbons of angeloyloxy moiety, there remain 15 carbon signals in the ^{13}C NMR and DEPT spectra (table 1), which contain two lactonic carbonyl carbons (δ 170.6 and 170.8), two double-bond carbons (δ 155.7 and 127.0), three oxygenated methine carbons (δ 64.4, 77.9 and 87.9) and two methyl carbons (δ 10.0 and 22.8). These data and biogenetic considerations suggested that **1** was an eremophilane-type sesquiterpene. Comparing the NMR spectra of compound **1** with those of 3β-angloyloxy-8β-hydroeremophil-7(11)-*en*-12,8α(14,6α)-diolide (**2**) that was isolated from the same plant [4], the C-8 oxygenated methine signals [δ_C 83.1, δ_H 5.05 (1H, m)] of **2** was replaced by one sp^3 quarternary carbon signal at δ_C 87.9 in NMR spectra of **1**. Except the difference caused by the sp^3 quarternary carbon signal at δ_C 87.9 in **1**, other NMR data of **1** were nearly superimposed on those of **2** [4], indicating that **1** was a derivative of **2**. Along with the NMR signals for C-3 at δ_C 64.4 and δ_H 5.45 (1H, d, $J = 3.0$ Hz) and HMBC correlations [H-3/δ_C 164.3 (C-1′)], the angeloyloxy group was defined as being attached to C-3. Therefore, the structure of the unit of **1** was determined as **2**. The sp^3 quarternary carbon signal at δ_C 87.9 implied that the two identical units joined at C-8 and C-8′ positions and formed dimeric sesquiterpene lactones. Biogenetically, 15-CH_3 and 4-CH_3, COOH of eremophilane-type sesquiterpene from genus *Ligularia* were in β-orientation. The appearance of

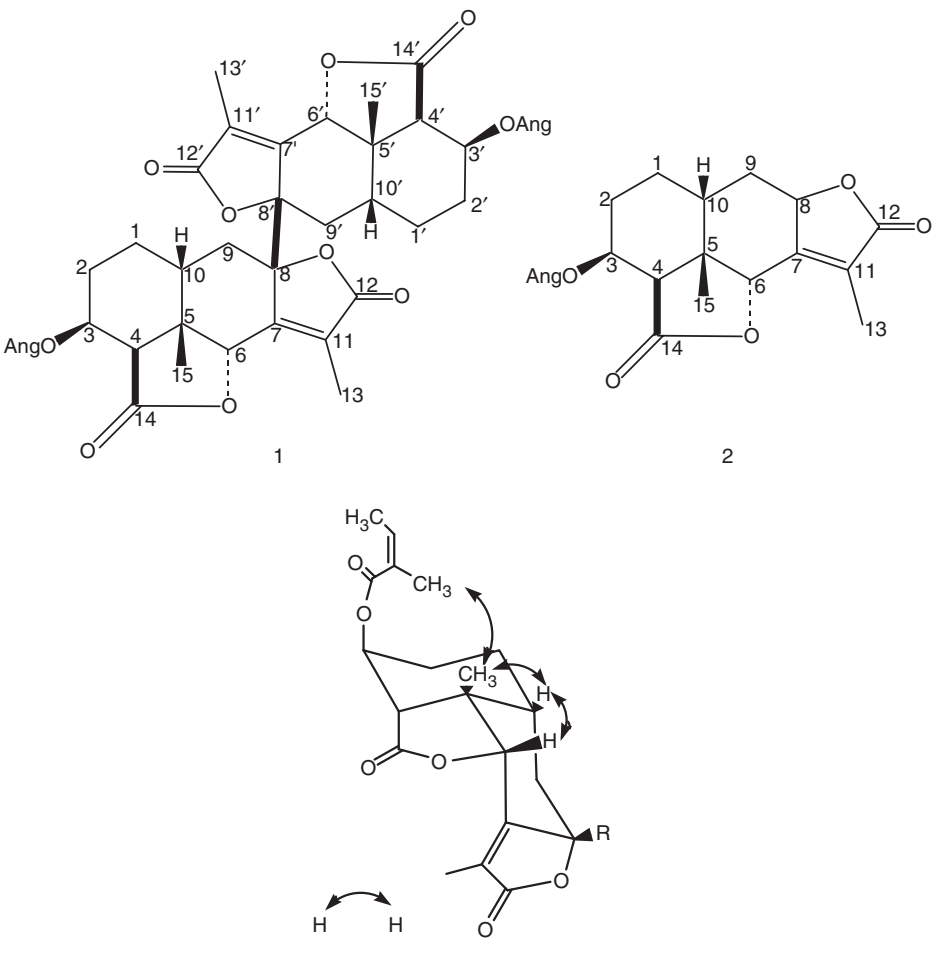


Figure 1. Important NOESY correlations of compound **1**.

correlations between 15-CH_3 and H-10, H-6 and H-10 on a NOESY experiment indicated their *cis* relationship, namely *A*/*B* ring was a *cis*-fused arrangement and H-6 was in β-orientation figures 1 and 2. The stereochemistry of H-6 was further confirmed by ^{1}H NMR due to the presence of homoallylic coupling between H-6 and CH_3-13 (table 1) [9]. The stereochemistry of H-3 was assigned as equatorial bond (α-orientation) due to its small coupling values with three vicinal protons (one axial and two equatorial), which was further validated by the evident cross peaks between Me-15 and Me-4′ in the NOESY spectrum. Moreover, the NOESY spectrum showed that the conformation of molecule is steroidal, 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). Consequently, the other half of the molecule at C-8 position must be in β-orientation. Therefore the structure of bieremophilanolide was established as depicted in the formula **1**.

A possible biosynthetic pathway for 8,8′-bi-3β-angeloyloxy-eremophil-7(11)-*en*-12,8α (14β,6α)-diolide (**1**) is shown in scheme 1. A naturally occurring eremophilanolide **3**, also obtained from the species by us, is perhaps the parent compound of this dimer [8].

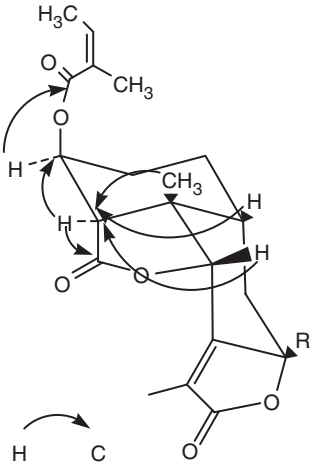
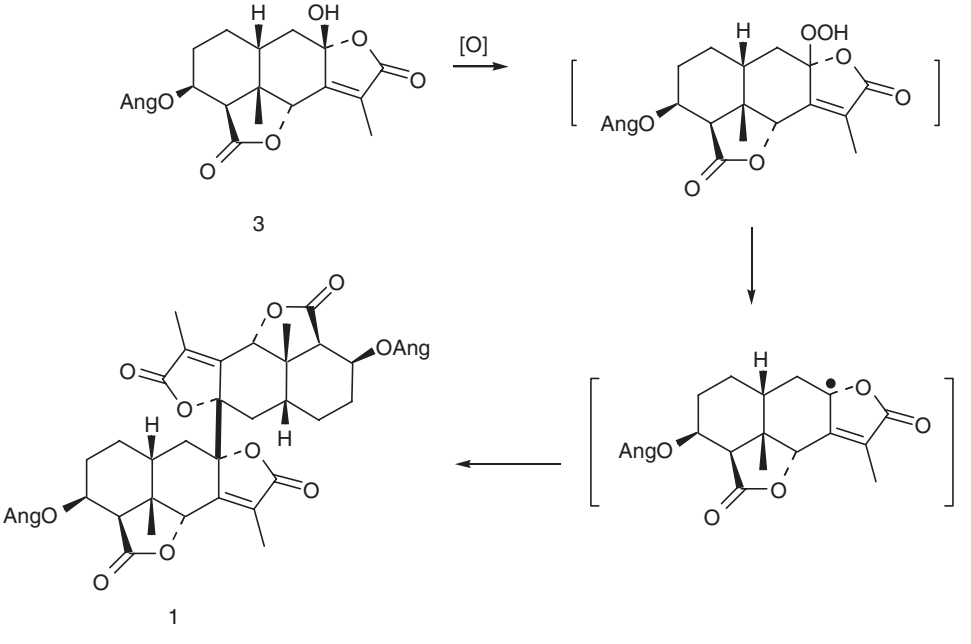


Figure 2. Important HMBC correlations of compound **1**.



Scheme 1. Plausible biosynthetic pathway for compound **1**.

3. Material and methods

Column chromatography (CC): silica gel, 200–300 mesh. TLC: precoated silica GF_{254} plates: detection at 254 nm, and by ceric sulfate reagent. Optical rotations: JASCO DEP-370 polarimeter. IR spectra were obtained on a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were recorded on a DRX-500 instrument with TMS as

an internal standard and $CDCl_3$ as a solvent. ^1H NMR, ^1H–^1H COSY, and NOESY spectra were measured at 400.13 or 500.13 MHz; ^{13}C NMR and DEPT spectra were recorded at 100.6 MHz; HMBC spectrum was obtained at 500.13 MHz/125.8 MHz. ^{13}C NMR assignments were determined by ^{13}C–^1H COSY and HMQC spectra. The EIMS and HREIMS were carried out on a VG Auto Spec-3000 spectrometer at 70 eV. The roots and rhizomes of *L. lapathifolia* were collected from Dong Mountain (altitude: 2500 m), Lijiang Prefecture of Yunnan Province, P.R. China, in July 2000, and authenticated by Dr Main Zhang. A voucher specimen (No. 990005-Li) is deposited in the Herbarium of China Pharmaceutical University.

Dried and powdered roots and rhizomes of *L. lapathifolia* (11.0 kg) were extracted with EtOH (7 days × 3) at room temperature. After removal of solvent *in vacuo*, an extract of 900.0 g was afforded. The extract was suspended in H_2O and partitioned with $CHCl_3$ and *n*-BuOH successively. Of the evaporated $CHCl_3$ part, 200.0 g (total 400.0 g) were subjected to repeated chromatography on a silica gel column (200–300 mesh, 2.0 kg), eluted with $CHCl_3$ and a gradient of $(Me)_2CO$ in $CHCl_3$ (50 : 1–5 : 1). Ten crude fractions were obtained after combining the eluates by TLC monitoring. The third fraction ($CHCl_3$/$(Me)_2CO$ 10 : 1, 20 g) was isolated on CC (silica gel 200–300 mesh, 200 g), eluted with $CHCl_3$/$(Me)_2CO$ (10 : 1, elution volume: 150 mL) and further purified on a Sephadex LH-20 column (50 g), eluted with $(Me)_2CO$ (elution volume: 5 mL) to obtain **1** (250 mg).

Bi-3*β*-angeloyloxy-8*β*-hydroeremophil-7(11)-en-12,8α(14β,6α)-diolide (1): amorphous powder; $[\alpha]_D$ +105.8° (*c* 0.48, $CHCl_3$); IR $(KBr)_{\nu max}$ = 2954, 1800, 1767, 1714, 1455, 1389, 1355, 1293, 1225, 1137, 1041, 975 cm^{-1}; ^1H NMR and ^{13}C NMR spectral data (table 1); HR-EIMS: HR-EIMS at *m/z* 718.3121 $[M]^+$ (Calcd 718.3015 for $C_{40}H_{46}O_{12}$); EIMS *m/z* (%) = 718$[M]^+$, 618[M − anglic acid]$^+$, 518[M − anglic acid]$^+$, 359[1/2M]$^+$ (100), 260[M − angelic acid]$^+$ (98), 242 (11), 231 (24), 215 (33), 185 (23), 175 (25), 161 (22), 149 (22), 131 (28), 121 (53), 105 (65), 100 [angelic acid]$^+$ (63), 91 (75), 83 (35), 77 (67), 67 (78).

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