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One chloro-furoeremophilanoid and two new natural dimers from *Ligularia atroviolacea*

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Abstract

Three new compounds, including one chloro-furoeremophilanoid (**1**), one eremophiladiolide (**2**), a rare dimer of nor-furoeremophilanoid, ligulatrovine A (**3**), and a known furoeremophilanolide (**4**), were isolated from *Ligularia atroviolacea*. The structures of compounds **1–4** were elucidated by spectroscopic methods including 1D and 2D NMR experiments as well as X-ray diffraction study.

Keywords: Natural sesquiterpene; Eremophiladiolide; Dimer; Chloro-furanoeremophilane; X-ray; *Ligularia atroviolacea*

Ligularia species (Asteraceae) are mainly distributed in damp, shaded areas of western China, especially the provinces of Gansu, Sichuan and Yunnan [1]. Recent investigation on the roots of *Ligularia atroviolacea* (Franch.) Hand.-Mazz. has led to the isolation and identification of four eremophilendiolides [2]. Careful examination of the minor constituents of this plant led to the isolation of three new eremophilane derivatives **1–3**, as well as one known furanoeremophilane (**4**) [3]. This article described the isolation, structural elucidation of compounds **1–4**.

Compound **1** was obtained as colorless needles with a melting point of 107–108 °C, $[\alpha]_D^{20} = +0.9$ (*c* 0.2, MeOH). The molecular formula of **1**, $C_{19}H_{25}ClO_5$ was deduced based on its ESIMS (*m/z* 369 for $[M+H]^+$ ion peak), elemental analysis, and ^{13}C NMR data (Table 1). The IR absorption bands of **1** suggested the existence of a hydroxyl (3500 cm^{-1}) group, an ester carbonyl (1736 cm^{-1}) group, and an α-furan keton moiety (1680, 1634, 1560 cm^{-1}). Furthermore, the ^1H NMR spectrum of **1** indicated the presences of three methyl groups (δ 1.05, 1.14 and 1.94) and

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Table 1
NMR spectral data of **1**, **2** and **3** (^{1}H, 400 MHz and ^{13}C, 100 MHz, TMS, δ ppm, J Hz)

Position	**1**[a] ($CDCl_3$)		Position	**2** (Me_2CO-d_6)		**3** ($CDCl_3$)	
	δ_C	δ_H		δ_C	δ_H	δ_C	δ_H
1	62.0	3.94 br s	1, 1′[b]	21.2	2.17 m 2.34 m	31.0	2.76 (t, 7.2)
2	24.3	1.76 (dd, 14, 2.0) 2.59 (t, 14.0)	2, 2′	21.9	1.69 m 2.05 m	23.8	2.24 m
3	23.3	1.44 (d, 13.6) 2.47 (t, 14.0)	3, 3′	136.7	6.83 (t, 3.2)	138.9	7.10 (dt, 12.8, 6.4)
4	31.9	1.05 m	4, 4′	129.9	–	134.4	–
5	50.1	–	5, 5′	44.3	–	127.1	–
6	68.2	7.06 s	6, 6′	82.9	5.12 br s	117.6	–
7	139.6	–	7, 7′	157.4	–	142.3	7.48 (d, 1.6)
8	145.9	–	8, 8′	87.3	–	155.4	–
9	186.3	–	9, 9′	35.2	1.31 m 2.39 m	108.6	7.19 (d, 1.6)
10	80.5	–	10, 10′	32.1	2.58 m	136.9	–
11	121.8	–	11, 11′	128.5	–	169.6	–
12	147.4	7.50 s	12, 12′	170.9	–	18.3	2.38 (d, 1.2)
13	8.53	1.94 s	13, 13′	10.1	1.97 (d, 2.4)	129.7	–
14	16.0	1.05 s	14, 14′	26.2	1.48 s	11.1	2.51 s
15	15.9	1.14 (d, 7.6)	15, 15′	168.4	–	–	–

[a] 6-Isobutyroxy: 1.28 (d, 7.2, H-3′), 1.30 [d, 7.2 Hz, H-4′], 2.73 (qq, 7.2, 7.2 Hz, H-2′); ^{13}C NMR: 176.5 (C-1′), 34.1 (C-2′), 18.6 (C-3′), 19.5 (C-4′).
[b] Positions indicated with prime symbols are applicable to dimers **2** and **3**.

one isobutyroxy group (δ 1.28, 1.30 and 2.73) (Table 1). Except for the isobutyroxy moiety, the ^{13}C NMR and DEPT spectra of **1** demonstrated 15 carbon signals, including six quaternary carbons, four methines, two methylenes and three methyls (Table 1). From above information, **1** was suggested to be a furanoeremophilane similar to a known compound, 6β-angeloyloxy-9-oxo-1α,10β-dihydroxy-furanoeremophilane [4]. However, the differences between **1** and above known furanoeremophilane could be found on the substituents at C-1 and C-6 (Table 1) [4]. The HMBC spectrum of **1** exhibited the correlation between H-6 [δ 7.06 (s, 1H)] and C-1′ (δ 176.5) and thus suggested the location of the isobutyroxy group at C-6. Therefore, the chlorine atom was suggested to present at C-1 position. Furthermore, Me-14 and Me-15 could be assigned to be β orientation based on general biogenetical consideration [5] and supported by the NOESY experiment. In NOSEY spectrum, the correlations between H-1 and H-14, H-4 and H-6 indicated their *cis* relationships. Compound **1** was therefore deduced as 1α-chloro-6β-isobutyroxy-9-oxo-10β-hydroxy-furanoeremophilane. The 3D structure of **1** was constructed by X-ray diffraction experiment (Fig. 1).

The HRESIMS of **2** exhibited a quasimolecular ion peak appeared at m/z 536.2276 $[M+NH_4]^+$ (calc. for 536.2284), which indicated its molecular formula to be $C_{30}H_{30}O_8$. Furthermore, the IR absorption bands appeared at 1765, 1746, and 1675 cm^{-1} suggested the presences of γ-lactones in compound **2**. However, the base peak (m/z 259) revealed that

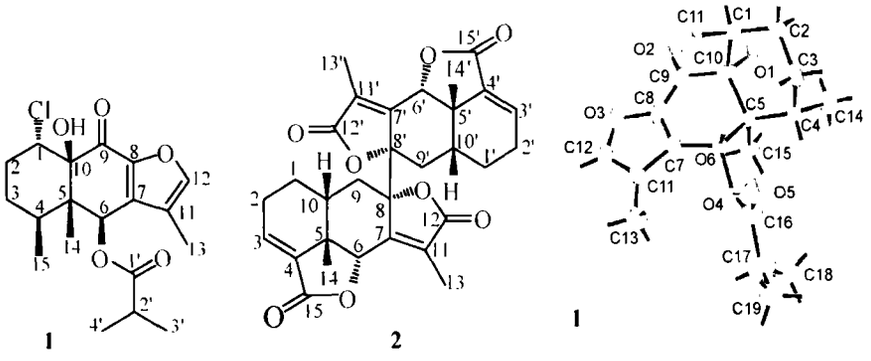


Fig. 1. Structures of compounds **1**, **2** and X-ray diagram of compound **1**.

compound **2** was composed of two halves possessing identical molecular weights. Furthermore, the ^{13}C NMR and DEPT spectra of **2** exhibited only 15 resonances for two methyls, three methylenes, three methines and seven quaternary carbons (Table 1). This implied that compound **2** was formed with two symmetric eremophilanolide units which were directly linked to each other with a C–C bond [6]. Structural elucidation of the half of compound **2** was accomplished by the analyses of its ^{1}H and ^{13}C NMR spectral data (Table 1). The presences of a tertiary methyl group [δ 1.48 (s), δ 26.2 (CH_3)] and an olefinic methyl group [δ 1.97 (d), δ 10.1 (CH_3)] were characteristic of an eremophilanolide. Therefore, the half of **2** was elucidated as an eremophilanolide with a structure similar to 8βH-eremophil-3,7(11)-dien-12,8α;15,6α-diolide [7], except for the absence of a methine signal [δ 4.68 (dd), δ 77.4 (CH)]. Thus, the structure of **2** was determined as a dimer of this known diolide. Furthermore, the diagnostic sp^3 signal appeared at δ 87.3 in the case of **2** implied that the two halves joined at C-8/C-8′ positions. Comprehensive analyses of the NMR data of **2** indicated a H-8β stereochemistry, which was confirmed by the homoallylic coupling between Me-13 and H-6 [7] (Table 1). Therefore, the structure of **2** was elucidated as 8β-[eremophil-3′,7′(11′)-dien-12′,8′α;15′,6′α-diolide]-eremophil-3,7(11)-dien-12,8α;15,6α-diolide.

The molecular formula of ligulatrovine A (**3**), $C_{28}H_{28}O_6$ was deduced by its HR-ESI MS (*m/z* 461.1957 for $[M+H]^+$ ion peak, calc. for 461.1964). The IR spectrum of **3** showed absorption bands for a carboxyl group (3161, 1726 cm^{-1}), double bond (1649 cm^{-1}), and an aromatic ring (1596, 1540, 1456 cm^{-1}). However, the ^{13}C NMR and DEPT spectra of **3** displayed only 14 carbon signals including two methyls, two methelenes, three methines and seven quaternary carbons (Table 1). This suggested that **3** is another symmetric dimer. A methyl group at δ 2.38 [d, H-12/H-12′; δ 18.3 (CH_3)], an olefinic methyl group at δ 2.51 [s, H-14/H-14′; δ 11.1 (CH_3)] were observed in the upfield region of the NMR spectra of **3** (Table 1). Two allylic methylenes were demonstrated by the signals appeared at δ 2.76 [t, H-1/H-1′] and 2.24 [m, H-2/H-2′] in the ^{1}H NMR spectrum of **3**. Furthermore, the downfield part of **3** showed the presence of a 1,2,3,5-tetra substituted aromatic ring at δ 7.19 [d, H-9/H-9′], 7.48 [d, H-7/H-7′] and δ 127.1, 117.6, 142.3, 155.4, 108.6, 136.9. A conjugated olefinic proton at δ 7.10 (dt, H-3/H-3′) and their corresponding carbon signals at δ 138.9, 134.4, as well as a carbon signal due to a carboxyl group at δ 169.6 were also observable in the downfield part of the NMR spectra of **3**. Moreover, it was suggested that the two halves were linked to each other with a C–C double bond, according to a single olefinic carbon signal appeared at δ 129.7 [C-13/C-13′] in its ^{13}C NMR spectrum. Additionally, the ^{1}H–^{1}H COSY spectrum of **3** showed correlations of H-2 to H-1 and H-3, and H-7 to H-9, respectively (Fig. 2). Combined with the observed HMBC data, the structure of **3** could be unambiguously deduced as shown.

Compound **4** had an identical ^{1}H NMR data to that of an artifact furanoeremophil-3-en-15,6α-olide synthesized by Kuroda and co-workers [3]. It is isolated as a natural product for the first time. Biogenetically, furoeremophilanolide **4** is proposed to be the possible precursor of dimer **2**, both existed in the title plant. Though a acid-base catalysed

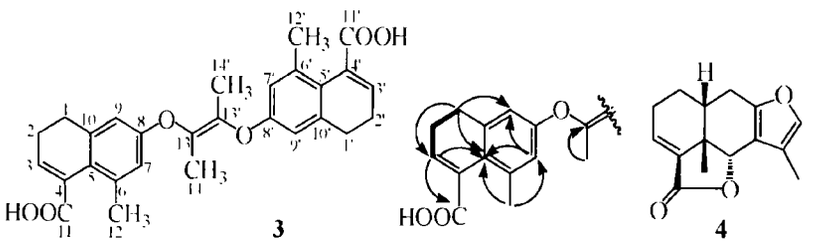
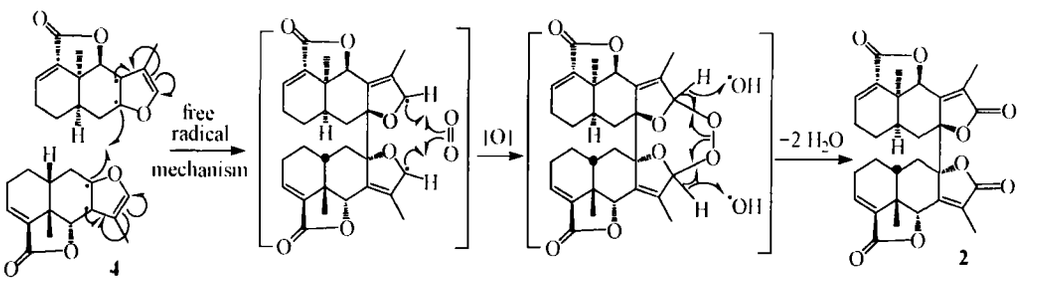


Fig. 2. Structures of **3** and **4**; key ^{1}H–^{1}H COSY (bold) and HMBC (arrow) correlations of **3**.



Scheme 1. Possible biogenetic path of **2** via a free radical mechanism from **4**.

mechanism for C-8 dimer of eremophiladiolides was suggested by Zhao et al. [8], another reasonable free radical mechanism of forming this kind of C-8 dimer is hypothesized as shown in Scheme 1 [9].

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