Two Novel Asymmetric Eremophilane Dimers from the Roots of *Ligularia virgaurea*

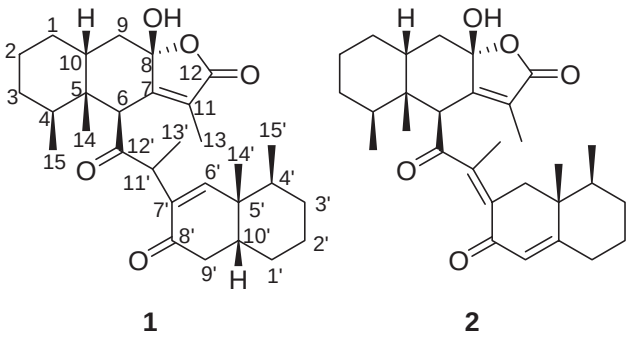
Zhan-Xin Zhang,[1] Chun-Ming Wang,[2] Dong-Qing Fei,[1] and Zhong-Jian Jia*[1]
[1]*College of Chemistry and Chemical Engineering, State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, P. R. China*
[2]*School of Life Sciences, Lanzhou University, Lanzhou 730000, P. R. China*

(Received November 19, 2007; CL-071278; E-mail: jiazj@lzu.edu.cn)

Virgaurol A (**1**) and B (**2**), two novel dimeric eremophilanes in whose structures the two asymmetric sesquiterpene units are connected by a C–C bond directly, have been isolated from the roots of *Ligularia virgaurea*. Their structures were determined by comprehensive spectral analysis. Compound **1** was evaluated for its in vitro cytotoxic activity against human leukemia (HL-60), human hepatoma (SMMC-7721), and human cervical carcinoma (HeLa) cells.

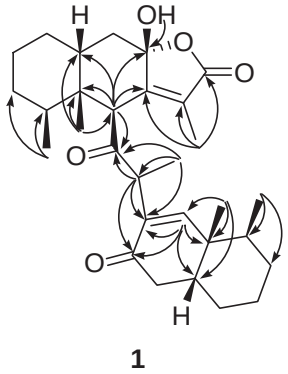
Ligularia (Compositae) species, widespread throughout China, are important medicinal plants, which are receiving phytochemical attention due to the biological and chemical diversities. More than 27 species have long been used as Chinese folk remedies due to their antibiotic, antiphlogistic, and antitumor activities.[1] *Ligularia virgaurea* (Maxim.) Mattf. is widely distributed in northwestern China and has been used as a traditional folk medicine for the treatment of stomachache and nausea.[2] In our long-standing interest in the study of biodiversity and searching for bioactive compounds from *Ligularia* species, those results showed that the components from the same genus, even from the same species, displayed a remarkable differences because of different ecological environments and collection seasons.[3] In the present study, we report the isolation and structure elucidation of two novel eremophilane dimers (**1** and **2**, Figure 1) from *L. virgaurea* collected in Gannan Tibetan Autonomous Region (S.A. 2200–3800 m), Gansu Province, P. R. China, and cytotoxic evaluation of compound **1**.

Virgaurol A (**1**),[4] $[\alpha]_D^{26} + 133$ (c 0.3, $CHCl_3$), possessed a molecular formula of $C_{30}H_{42}O_5$ as evidenced from its HRESIMS (at m/z 505.2929 $[M + Na]^+$, calcd. 505.2924). Furthermore, the molecular ion peak was observed at m/z 482 $[M]^+$ in its EIMS, and significant ion fragments at m/z 249 $[C_{15}H_{21}O_3]^+$, 231 $[C_{15}H_{21}O_3–H_2O]^+$, and 233 $[C_{15}H_{21}O_2]^+$, indicating the occurrence of two C_{15} units in **1**. Its IR absorption bands at 3423, 1760, 1727, and 1661 cm^{-1} indicated the existence of hydroxy, carbonyls, and double bonds. The ^1H NMR spectrum[5] of compound **1** displayed the presence of six methyls at δ_H 1.89 (3H, s, H_3-13), 1.06 (3H, s, H_3-14), 0.85 (3H, d, $J = 6.4$ Hz, H_3-15), 1.28 (3H, d, $J = 7.2$ Hz, H_3-13′), 1.12 (3H, s, H_3-14′), and 0.89 (3H, d, $J = 7.2$ Hz, H_3-15′), an olefinic proton at δ_H 6.66 (1H, s, H-6′), and a hydroxy proton at δ_H 5.58 (1H, s, OH-8), as well as other complicated signals belonging to other methylenes and methines. The ^{13}C NMR (DEPT) data[6] were in good agreement with the above analysis, and exhibited 30 carbon signals consisting of six methyls, eight methylenes, seven methines, and nine quaternary carbons, of which the peaks in the upfield region appeared in duplicate or twice. The ^1H and ^{13}C NMR data, in combination with the molecular composition, highly showed compound **1** to be a dimeric sesquiterpene structure. The final structure of **1** was mainly determined by the extensive study of 2D NMR techniques (especially ^1H–^1H COSY, gHSQC, and gHMBC). The ^1H–^1H COSY spectrum of **1** showed the correlations from H-9 to H-10; H-4 to H_3-15 and H-3; and H-1 to H-2. The gHMBC spectrum (Figure 2) showed long-range correlations between the following protons and carbons: H_3-13 and C-7, C-11, C-12; H_3-14 and C-4, C-5, C-6, C-10; H_3-15 and C-3, C-4, C-5; H-6 and C-4, C-5, C-7, C-8, C-10, C-11; H-9 and C-5, C-7, C-8, C-10; and hydroxy proton and C-8. These observations, in association with characteristic carbon chemical shifts at δ_C 171.1 (ester carbonyl, C-12), 103.9 (acetal, C-8), 154.1 and 126.8 (olefinic, C-7 and C-11), indicated the presence of an 8-hydroxyeremophil-7(11)-en-8,12-olide skeleton (unit I, Figure 3). In addition, the presence of the other sesquiterpene unit II in **1** was revealed by correlations of ^1H–^1H COSY and gHMBC spectra. The ^1H–^1H COSY (from H-9′ to H-10′; H-4′ to H_3-15′ and H-3′; H-1′ to H-2′; and H_3-13′ to H-11′) and gHMBC correlations (H_3-13′ and C-7′, C-11′, C-12′; H_3-14′ and C-4′, C-5′, C-6′, C-10′; H_3-15′ and C-3′, C-4′, C-5′; H-6′ and C-4′, C-5′, C-7′, C-8′, C-10′, C-11′; H-9′ and C-1′, C-5′,



1 **2**

Figure 1. The structures of compounds **1** and **2**.



1

Figure 2. The key gHMBC correlations (from H to C) of **1**.

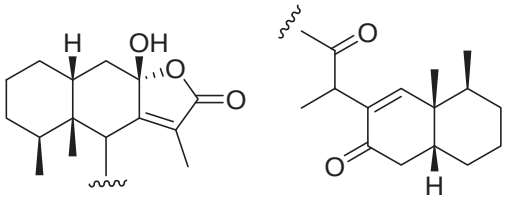


Figure 3. The partial structure of compound **1**.

C-8′, C-10′; and H-11′ and C-6′, C-7′, C-8′, C-12′, C-13′), together with typical carbon chemical shifts at δ_C 200.9 and 208.0 (ketone carbonyl, C-8′ and C-12′), and 160.1 and 135.1 (olefinic, C-6′ and C-7′), indicated that the other eremophilane unit with an 8′-ketone group, a 12′-ketone group and a 6′,7′-double bond was present (unit II, Figure 3). Finally, the connected position of the two units was characterized from the key gHMBC correlation between H-6 and C-12′. Therefore, the units I and II were uniquely connected from C-6 to C-12′ by a single C–C bond directly.

Stereochemically, in the biogenetic consideration of eremophilane derivatives isolated from Compositae species, the methyls at C-4 (or C-4′) and C-5 (or C-5′) were both assigned as the β-orientation.[7] In the NOE different spectra, the enhancement between H_3-14 (or H_3-14′) and H-10 (or H-10′) suggested a cis-fused A/B (A′/B′) ring system. In the ^{1}H NMR spectrum, the chemical shift of H_3-14 at δ_H 1.06 (s) was downfield comparing to H_3-15 at δ_H 0.85 (d, $J = 6.4$ Hz), indicating that OH-8 was β-oriented, which was agreed with the empirical rules reported by Naya et al.[8] In addition, the absence of homoallylic coupling between H-6 and H_3-13 indicated that H-6 was α-oriented.[9] Thus, we concluded that **1** was a novel asymmetric eremophilane sesquiterpene dimer.

The molecular formula of virgaurol B (**2**),[4] $[\alpha]_D^{26} + 13$ (*c* 0.4, $CHCl_3$), was determined to be $C_{30}H_{40}O_5$ by HRESI-MS (at m/z 481.2950 $[M + H]^+$, calcd. 481.2949), indicating 2 mass units less than compound **1**. Most of the signals in ^{1}H[10] and ^{13}C NMR[11] spectra strikingly matched those of **1** and revealed a dimeric sesquiterpene structure. Further analysis its 2D spectra to know: the unit I was identical with **1** and the significant differences were in the unit II. The ^{1}H–^{1}H COSY spectrum indicated correlations from H-4′ to H_3-15′ and H-3′; H-1′ to H-2′. The gHMBC spectrum indicated correlations between H_3-13′ and C-7′, C-11′, C-12′; H_3-14′ and C-4′, C-5′, C-6′, C-10′; H_3-15′ and C-3′, C-4′, C-5′; H-9′ and C-1′, C-5′, C-7′; and H-6′ and C-4′, C-5′, C-7′, C-8′, C-10′, C-11′; These correlations, and representative carbon chemical shifts at δ_C 187.5 and 204.8 (ketone carbonyl, C-8′ and C-12′), 130.7 and 146.6 (olefinic, C-7′ and C-11′), and 124.5 and 173.7 (olefinic, C-9′ and C-10′), established the structure of unit II as eremophil-7′(11′),9′-dien-8′,12′-dione. As **1**, the connected position of the two units was from C-6 to C-12′ by a single C–C bond, which was confirmed by the key gHMBC correlation between H-6 and C-12′. In the NOE different spectrum, the enhancement between H_3-13′ and H-6′ suggested the double bond at C-7′ and C-11′ was Z.

Compound **1** was tested for in vitro cytotoxic activity against human leukemia (HL-60), human hepatoma (SMMC-7721), and human cervical carcinoma (HeLa) cells according to the sulforhodamine B (SRB) method.[12] It was found that compound **1** showed weak activity against HL-60 (IC_{50} 21.9 μg/mL), SMMC-7721 (47.6 μg/mL), and HeLa (50.8 μg/mL), respectively.

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4 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
5 The ^{1}H NMR data (400 MHz, $CDCl_3$) of **1**: δ 1.89 (3H, s, H_3-13), 1.06 (3H, s, H_3-14), 0.85 (3H, d, $J = 6.4$ Hz, H_3-15), 4.30 (1H, s, H-6), 2.07 (1H, dd, $J = 14.8$, 4.8 Hz, H-9a), 2.03 (1H, dd, $J = 12.8$, 8.0 Hz, H-9b), 5.58 (1H, s, OH-8), 1.28 (3H, d, $J = 7.2$ Hz, H_3-13′), 1.12 (3H, s, H_3-14′), 0.89 (3H, d, $J = 7.2$ Hz, H_3-15′), 6.66 (1H, s, H-6′), 2.64 (1H, dd, $J = 17.6$, 12.0 Hz, H-9′a), 2.27 (1H, dd, $J = 17.6$, 4.8 Hz, H-9′b), 3.80 (1H, q, $J = 7.2$ Hz, H-11′).
6 The ^{13}C NMR (DEPT) data (100 MHz, $CDCl_3$) of **1**: δ 26.7 (t, C-1), 20.3 (t, C-2), 30.4 (t, C-3), 30.2 (d, C-4), 42.4 (s, C-5), 54.1 (d, C-6), 154.1 (s, C-7), 103.9 (s, C-8), 38.8 (t, C-9), 35.4 (d, C-10), 126.8 (s, C-11), 171.1 (s, C-12), 8.9 (q, C-13), 17.3 (q, C-14), 16.3 (q, C-15), 25.6 (t, C-1′), 19.6 (t, C-2′), 30.0 (t, C-3′), 36.2 (d, C-4′), 39.3 (s, C-5′), 160.1 (d, C-6′), 135.1 (s, C-7′), 200.9 (s, C-8′), 39.2 (t, C-9′), 39.3 (d, C-10′), 45.4 (d, C-11′), 208.0 (s, C-12′), 14.7 (q, C-13′), 20.6 (q, C-14′), 15.8 (q, C-15′).
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10 The ^{1}H NMR data (400 MHz, $CDCl_3$) of **2**: δ 1.72 (3H, s, H_3-13), 1.15 (3H, s, H_3-14), 0.76 (3H, d, $J = 6.4$ Hz, H_3-15), 4.17 (1H, s, H-6), 2.16 (1H, dd, $J = 14.0$, 4.4 Hz, H-9a), 2.06 (1H, brd, $J = 13.6$ Hz, H-9b), 6.36 (1H, s, OH-8), 1.99 (3H, s, H_3-13′), 1.07 (3H, s, H_3-14′), 0.99 (3H, d, $J = 6.4$ Hz, H_3-15′), 2.79 (1H, d, $J = 14.8$ Hz, H-6′a), 2.29 (1H, d, $J = 14.8$ Hz, H-6′b), 5.78 (1H, s, H-9′).
11 The ^{13}C NMR (DEPT) data (100 MHz, $CDCl_3$) of **2**: δ 25.7 (t, C-1), 19.7 (t, C-2), 30.5 (t, C-3), 30.0 (d, C-4), 43.1 (s, C-5), 54.7 (d, C-6), 154.2 (s, C-7), 104.3 (s, C-8), 39.3 (t, C-9), 36.1 (d, C-10), 126.6 (s, C-11), 171.2 (s, C-12), 9.0 (q, C-13), 17.6 (q, C-14), 16.3 (q, C-15), 33.1 (t, C-1′), 26.3 (t, C-2′), 30.3 (t, C-3′), 42.6 (d, C-4′), 41.6 (s, C-5′), 39.8 (t, C-6′), 130.7 (s, C-7′), 187.5 (s, C-8′), 124.5 (d, C-9′), 173.7 (s, C-10′), 146.6 (s, C-11′), 204.8 (s, C-12′), 16.8 (q, C-13′), 18.0 (q, C-14′), 15.5 (q, C-15′).
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