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Unusual Sesquiterpene Lactones from *Ligularia virgaurea* spp. *oligocephala*

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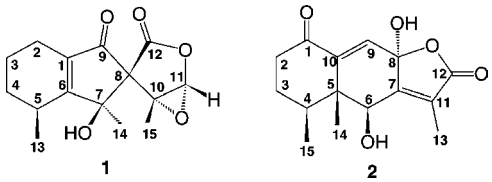
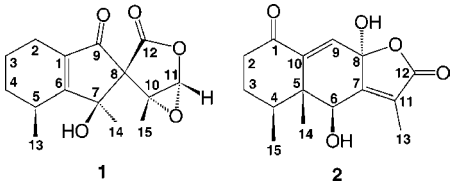
ABSTRACT



Sesquiterpene lactones, ligulolide A (1) and 6β,8α-dihydroxy-1-oxoeremophila-7(11),9(10)-diene-12,8-olide (2), were isolated from *Ligularia virgaurea* spp. *oligocephala*. Their structures were established by analysis of one- and two-dimensional NMR data. The relative stereostructures were determined on the basis of NOESY and single-crystal X-ray experiments. 1 represents a novel sesquiterpene carbon framework, and a possible biosynthetic process is proposed. 2 is a novel eremophilane-type sesquiterpene.

Since 1997, the Compositae plant family has been the subject of several chemical investigations by our group aimed at finding the bioactive sesquiterpenoids. This effort has led to the discovery of some structurally novel sesquiterpenes with important carbon skeleton types of eudesmane, germacrane, eremophilane, and guanane.[1] Therefore, the natural products chemistry of various sesquiterpenoids from higher plants continues to capture our attention. At present, we have isolated some new sesquiterpenoids from the genera belonging to *Artemisia*, *Senecio*, *Carpesium*, *Halenia*, and *Ligularia*.[1] The genus *Ligularia* was found to be an important source of sesquiterpenes of the eremophilane type. In the course of our search for bioactive sesquiterpenoids, we selected *Ligularia virgaurea* spp. *oligocephala*, which has long been used as a traditional folk medicine for the treatment of stomachache and hansea.[2] In this study, we describe the isolation and structural elucidation of the sesquiterpene lactones (**1**, **2**) from the alcoholic extract of the species, as well as their antitumor activities.



Compound **1** is a bicarbocycle sesquiterpene related in structure to bakkenolide A,[3] which has cytotoxic activity,[3d] but possesses a novel carbon framework. New compound **2** has an eremophilane-type carbon skeleton. It has been reported that the eremophilane sesquiterpenes have antihistamine activity.[3d]

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Table 1. NMR Spectral Data for Ligulolide A (**1**) in $CDCl_3$

C/H no.	δ_H[a] mult. (*J* in Hz)	δ_C[b,c]	COSY	HMBC[d]	NOESY
1		139.43 s		2ax, 2eq, 3ax, 3eq, 5ax	
2ax	2.21 m	20.42 t	2eq, 3ax, 3eq	3ax, 3eq, 4ax, 4eq	3ax
2eq	2.16 m		2ax, 3ax, 3eq		3eq
3ax	1.82 m	18.14 t	2ax, 2eq, 3eq, 4ax, 4eq	2ax, 2eq, 4ax, 4eq, 5ax	2ax, 4ax
3eq	1.70 m		2ax, 2eq, 3ax, 4ax, 4eq		2eq, 4eq
4ax	1.85 m	31.07 t	3ax, 3eq, 4eq, 5ax	2ax, 2eq, 3ax, 3eq, 5ax, 13	3ax
4eq	1.58 m		3ax, 3eq, 4ax, 5ax		3eq
5	2.77 br q (7.2)	29.43 d	4ax, 4eq, 13	3ax, 3eq, 4ax, 4eq, 13	4ax, 14
6		178.75 s		2ax, 2eq, 4ax, 4eq, 5ax, 13, 14	
7		82.11 s		14	
8		70.19 s		14, 15	
9		198.13 s		2ax, 2eq	
10		65.33 s		11, 14, 15	
11	5.51 s	82.30 d		15	
12		171.90 s		11	
13	1.34 d (7.2)	19.70 q	5ax	4ax, 4eq, 5ax	4eq, 15
14	1.56 s	15.04 q			5ax
15	1.61 s	24.09 q			11eq, 13

[a] Recorded at 400.13 MHz. [b] Recorded at 100.62 MHz. [c] Multiplicities inferred from by DEPT and HMQC experiments. [d] Protons showing long-range correlation with indicated carbon.

The air-dried whole plant of *Ligularia virgaurea* spp. *oligocephala* (4.0 kg), collected in Huzhu county of Qinghai province of China in August of 2002, was powdered and extracted with 95% ethanol at room temperature. The ethyl acetate-soluble part of the ethanol extract was isolated by column chromatography on silica gel repeatedly to yield pure ligulolide A (**1**)[4] and 6β,8α-dihydroxy-1-oxoeremophila-7(11),9(10)-diene-12,8-olide (**2**).[5]

Compound **1** was a colorless crystalline material, and the molecular formula of $C_{15}H_{18}O_5$ was determined by HR-ESIMS *m/z* at 296.1496 $[M + NH_4]^+$. Inspection of the richly detailed proton and carbon NMR spectra (Table 1), along with IR and UV/vis data,[4] sets the stage for the sesquiterpene lactone structure determination. The gHMQC and the broadband-decoupled ^{13}C NMR spectra established that **1** possessed 7 quaternary, 2 methine, 3 methylene, and 3 methyl carbons, that is, a total of 17 hydrogen attached to 15 carbons. One more proton was inferred from IR spectra showing that a 3441 cm^{-1} band was attributed to a hydroxyl group. This brought the total proton count to 18. Further analysis of the ^{13}C NMR data revealed a ketone carbonyl carbon δ_C 198.13, an ester carbonyl carbon δ_C 171.90, and three oxygenated carbons δ_C 82.11(C), 65.33(C), 82.30(CH). Therefore, the preliminary data suggested that five oxygens were present in compound **1**, amounting to 278 mass units in the molecule.

Extensive analyses of gCOSY and gHMBC data in $CDCl_3$ (Table 1) led to three substructures (Figure 1). Substructure A (C1−C3−C6, C9 and C13) was assembled on the basis of gCOSY correlations (C2−C3−C4−C5−C13 spin system) and gHMBC correlations (H5/C6 and H2/C1, C6, C9). Substructure B (C6−C8−C10, and C14) was deduced on the basis of gHMBC correlations (H14/C6, C7, C8, C10). Substructure C was established by gHMBC correlations (H11/C10, C12 and H15/C8, C10, C11). The presence of

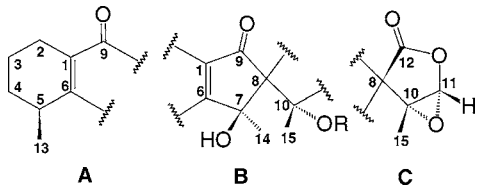

Figure 1. Partial structures from two-dimensional NMR for **1**.

the epoxide ring in substructure C was suggested by typical ^{13}C MNR chemical shifts of C10 (δ_C 65.33) and C11 (δ_C 82.30).[6] Taking into account the seven degrees of unsaturation, compound **1** is a tetracyclic structure with two carbonyl carbons and one double bond. At this point, the task of assembling the three partial structures (**A**−**C**) into a unique structure was complicated because of the incompatibility of **1** with the known bakkenolide A[3] and the long-range heteronuclear of correlations (gHMBC): H2/C9, H14/C6, and H15/C8. However, a plausible structure could be envisioned on the basis of the connections among the substructures. The relative stereochemistry of the ring system

(4) Data for **1**: mp 120 °C; $[\alpha]^{20}_D$ −8° (*c* 0.2, $CHCl_3$); UV (CH_3OH) λ_{max} 240 nm (log ϵ = 5.23); IR (film) ν 3441, 2920, 2851, 1706, 1644, 1460, 1377, 1256, 1071, 670 cm^{-1}; NMR can be found in Table 1; HR-ESIMS *m/z* 296.1496 ($M + NH_4^+$, calcd for $C_{15}H_{22}O_5N$ 296.1492).

(5) Data for **2**: mp 176 °C; $[\alpha]^{20}_D$ −148° (*c* 0.18, $CHCl_3$); UV (CH_3OH) λ_{max} 228 nm (log ϵ = 5.23); IR (film) ν 3443, 2963, 2940, 2879, 1726, 1691, 1449, 1380, 1257, 1088, 1029, 963 cm^{-1}; NMR can be found in Table 2; HR-EIMS *m/z* 278.1117 (M^+), 260.1018 ($M - H_2O$); 246, 189, 159.

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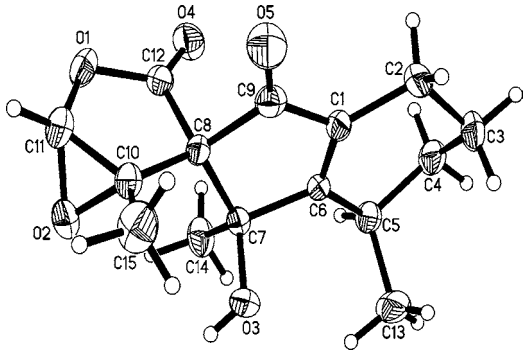

Figure 2. X-ray crystal structure for **1**.

in **1** was established by analysis of key NOESY correlations. The strong correlation of H-5 (δ 2.77) to H-14 (δ 1.56) indicated a cis junction of H-5 and CH_3-14; the correlation of H-15 (δ 1.61) to H-11 (δ 5.51) and H-13 (δ 1.34) indicated that 7-OH, CH_3-13, and CH_3-15 were in β-orientation together with CH_3-14, while the epoxide ring was in α-configuration. However, the B/C ring junction was not shown. The structure of **1** was firmly confirmed by X-ray crystallography[7] (Figure 2). The site of CH_3-14 of **1** turned out to be different from that of bakkenolide A and consists of a unique skeleton. Compound **1** was named ligulolide A after the genus *Ligularia*. A possible biosynthetic pathway for ligulolide A (**1**) is shown in Figure 3. A naturally

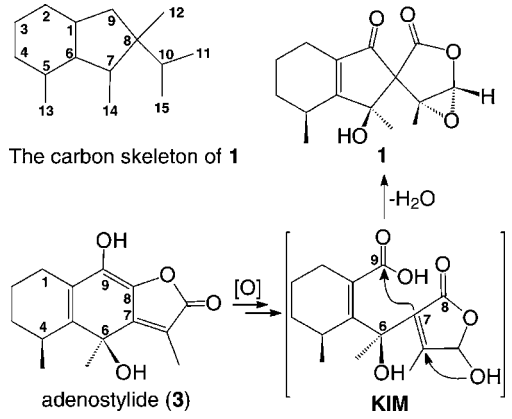

Figure 3. Plausible biosynthetic pathway for **1**.

occurring adenostylide (**3**),[8] also obtained from the species by us, is perhaps the parent compound for novel sesquiterpene **1**. First, adenostylide was oxygenated, and then the olefinic bond $\Delta^{8,9}$ was cleaved to yield a key intermediate

(7) Crystal data for ligulolide A (**1**): $C_{15}H_{18}O_5$, M_r = 278.29, monoclinic, space group $P2(1)2(1)2(1)$, a = 6.286(1), b = 14.650(3), c = 14.890(3) Å, V = 1371.2(4) Å^3, Z = 4, D_{calc} = 1.348 g/cm^3. The final R value was 0.0424 (R_w = 0.0994 for 2005 reflections [$I > 2\sigma(I)$]).

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Table 2. NMR Spectral Data for **2** in CD_3COCD_3

C/H no.	δ_H[a] mult. (J in Hz)	δ_C[b,c]
1		203.0 s
2ax	2.43 m	40.5 t
2eq	2.15 m	
3ax	1.72 m	29.5 t
3eq	1.75 m	
4	2.44 m	41.8 d
5		51.5 s
6	4.92 d (1.6)	74.6 d
7		159.4 s
8		100.6 s
9	6.22 s	126.4 d
10		149.7 s
11		124.0 s
12		172.0 s
13	1.95 d (1.6)	8.6 q
14	0.85 s	12.6 q
15	1.21 d (6.8)	18.1 q

[a] Recorded at 400.13 MHz. [b] Recorded at 100.62 MHz. [c] Multiplicities inferred from by DEPT experiment.

molecule as a hemiacetal (KIM), which changed into compound **1** at the end via several changes, including an important nucleophilic addition reaction in the internal molecule (Figure 3).

The molecular formula of compound **2**,[5] obtained as a white crystalline material, was assigned as $C_{15}H_{18}O_5$ by HR-EIMS m/z at 278.1117 $[M]^+$. The ^{1}H NMR spectrum (Table 2) showed characteristic signals for a furano-elemophila-type sesquiterpene,[9] including a singlet olefinic proton at δ 6.22 (H-9), singlet methyl protons at δ 0.85 (H_3-14), and two doublet methyl protons at δ 1.95 and 1.21 (H_3-13 and H_3-15). An oxygenated methine proton was observed at δ 4.92 (H-6), suggesting hydroxyl functionality. Fifteen carbon signals appeared in the broadband-decoupled ^{13}C NMR spectrum, including a ketone carbonyl carbon signal at δ 203.0 (C-1), an ester carbonyl carbon signal at δ 172.0 (C-12), and olefinic carbon signals at δ 159.4 (C-7) and 124.0 (C-11), indicated an α,β-unsaturated ester in the furan ring and at δ 149.7 (C-10) and 126.4 (C-9) due to an α,β-unsaturated ketone.[8] The signal at δ 74.6 (C-6) indicated a hydroxymethine carbon, and the signal at δ 100.6 (C-8) implied a dioxyquatenary carbon. These groups were linked to the structure of compound **2** by comparing data of the ^{1}H and ^{13}C NMR with those in the literature,[9] and **2** was elucidated as 6β,8α-dihydroxy-1-oxoeremophila-7(11),9(10)-diene-12,8-olide.

(9) (a) Tori, M.; Kawahara, M.; Sono, M. *Phytochemistry* **1998**, *47*, 401−409. (b) Zhang, S. M.; Zhao, G. L.; Li, R.; Lin, G. Q. *Phytochemistry* **1998**, *48*, 519−524. (c) Perez, A. L.; Vidals, P.; Cardenas, J.; Vivar, A. R. D. *Phytochemistry* **1991**, *30*, 905−908. (d) Jia, Z. J.; Zhao, Y.; Tan, R. X. *J. Nat. Prod.* **1993**, *56*, 494−499. (e) Bohlmann, F.; Zdero, C.; Jakupovic, J.; Grenz, M.; Castro, V.; King, M. R.; Robinson, H.; Vincent, L. P. D. *Phytochemistry* **1986**, *25*, 1151−1159. (f) Jia, Z. J.; Chen, H. M. *Phytochemistry* **1991**, *30*, 3132−3134. (g) Mericli, A. H.; Mericli, F.; Jakupovic, J.; Bohlmann, F.; Dominguez, X. A.; Vega, H. S. *Phytochemistry* **1989**, *28*, 1149−1153. (h) Massiot, G.; Nuzillars, J. M.; Men-Olivier, L. L.; Aclinou, P.; Benkouider, A.; Khelifa, A. *Phytochemistry* **1990**, *29*, 2207−2210.

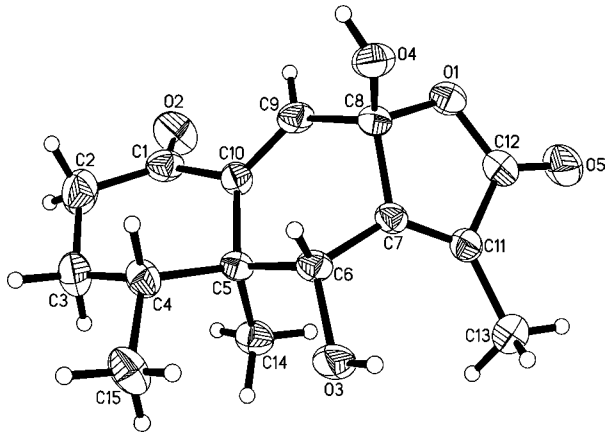

Figure 4. X-ray crystal structure for **2**.

The relative stereostructure of the new eremophilane lactone **2**[10] was firmly established by X-ray crystallography (Figure 4).

Because only very small quantities of ligulolide A (**1**) and 6β,8α-dihydroxy-1-oxoeremophila-7(11),9(10)-diene-12,8-olide **(2)** were obtained from the species, it was not possible to screen their bioactivity against a lot of tumor cell lines. Compounds **1** and **2** were assayed only against both human promyelocytic leukemia (HL-60) and human ovarian (HO-8910) cells. **1** showed strong cytotoxicity (IC_{50} = 19.43 μM against HL-60 and IC_{50} > 100 μM against HO-8910) in vitro, whereas **2** showed no cytotoxicity against both tumor cells (IC_{50} > 100 μM). It seems likely that total synthesis will be required to access sufficient quantities of these novel sesquiterpenes for further pharmaceutical evaluation.

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Supporting Information Available: One-dimensional ^{1}H and ^{13}C NMR (DEPT) and X-ray crystallographic data of **1** and **2**, together with HR-ESIMS, COSY, and HMBC of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Crystal data for 6β,8α-dihydroxy-1-oxoeremophila-7(11),9(10)-diene-12,8-olide (**2**): $C_{15}H_{18}O_5$, M_r = 278.29, monoclinic, space group $P2(1)2(1)2(1)$, a = 8.883(2), b = 10.216(2), c = 15.107(3) Å, V = 1371.0(4) Å^3, Z = 4, D_{calc} = 1.348 g/cm^3. The final R value was 0.0371 (R_w = 0.0748 for 2134 reflections [$I > 2\sigma(I)$].