

# Isolation, Characterization and Crystal Structure of Natural Eremophilenolide from *Ligularia sagitta*

Lyi Li<sup>a</sup>, Li-Wen Xu<sup>b</sup>, Yan-Feng Jiang<sup>c</sup>, Chan-Juan Xi<sup>c</sup>, Han-Qing Wang<sup>a</sup>, and You-Rui Suo<sup>d</sup>

<sup>a</sup> Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, 730000, China

<sup>b</sup> State key laboratory of Oxo Synthesis and Selective Oxidation, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, 730000, China

<sup>c</sup> Department of Chemistry, Tsinghua University, Beijing, 100084, China

<sup>d</sup> Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, 810008, China

Reprint requests to Prof. H.-Q. Wang. E-mail: whqwt@hotmail.com. Fax: +86-931-8277088.

Z. Naturforsch. **59b**, 921 – 924 (2004); received January 8, 2004

A new eremophilenolide 1 $\beta$ ,10 $\beta$ -epoxy-6 $\beta$ -acetoxyl-3 $\beta$ -angeloyloxy-8 $\beta$ -hydroxy-eremophilen-7(11)-en-8,12 $\alpha$ -olide (**1**), together with liguhodgsonal (**2**), esculetin (**3**) and  $\beta$ -sitosterol (**4**), was isolated from the aerial parts of *Ligularia sagitta*. The structure of the new constituent (**1**) was elucidated by spectroscopic methods and confirmed by single-crystal X-ray diffraction.

**Key words:** *Ligularia sagitta*, Eremophilenolide, Single-Crystal, X-Ray Diffraction

## Introduction

The genus *Ligularia* (Compositae) is represented by over 100 species in China. Some of them are generally used as substitute of Aster plants to cure phthisis, bronchitis and lumbago, performing a function of diuresis and expectorant in traditional Chinese medicine, especially in Tibetan medicine [1–3]. *Ligularia sagitta* (Maxim.) Matf is widely distributed in northwestern China and has been used as a folk medicine to reduce phlegm, relieve cough, cure pulmonary tuberculosis, urinary track blockages, common cold, and pharyngitis [4]. Previous work on this plant collected in Zhang County of Gansu Province in China showed that it mainly consists of aromatic compounds, diterpenes and eremophilanes. Extensively, the comparative study about the influence of different ecological environments on the chemical constituents of plants, we report here the constituents of *Ligularia sagitta* collected in Mengyuan county of Qinghai Province in China. This paper describes the isolation and structural elucidation of one new eremophilenolide (**1**), along with seven known compounds (**2–4**), the structure of the new eremophilenolide was confirmed by high field NMR techniques and single crystal X-Ray analysis.

## Results and Discussion

The IR band of compound **1** at 1754 cm<sup>-1</sup> verifies the group of unsaturated  $\gamma$ -lactone. The NMR spectral

Table 1. <sup>1</sup>H NMR (400 MHz), <sup>13</sup>C NMR(100 Hz), DEPT data of compound **1** ( $\delta$ , ppm, TMS, CDCl<sub>3</sub>), <sup>1</sup>H and <sup>13</sup>C long-range correlations of compound **1** in HMBC.

H	<sup>1</sup> H $\delta_H$	C	<sup>13</sup> C $\delta_C$	<sup>1</sup> H- <sup>13</sup> C long-range correlation ( <b>1</b> )
1	3.21 ( <i>d</i> , <i>J</i> = 9.6)	1	60.4 CH	H-2, H-9
2	1.84 ( <i>m</i> ), 2.30 ( <i>m</i> )	2	42.4 CH <sub>2</sub>	H-1, H-4
3	5.11 ( <i>ddd</i> )	3	67.5 CH	H-1, H-2, H-4, H-15
4	2.11 ( <i>m</i> )	4	33.7 CH	H-2, H-3, H-14, H-15
5	–	5	44.4 C	H-4, H-6, H-14, H-15
6	6.04 ( <i>s</i> )	6	72.3 CH	H-14
7	–	7	154.8 C	H-6, H-13
8	–	8	101.2 C	H-9, H-13
9	2.38 ( <i>d</i> , <i>J</i> = 13.6), 2.12 ( <i>d</i> , <i>J</i> = 13.6)	9	24.9 CH <sub>2</sub>	H-1
10	–	10	60.8 C	H-1, H-4, H-14
11	–	11	124.5 C	H-6, H-13
12	–	12	171.1 C	H-13
13	1.81 ( <i>s</i> )	13	7.9 CH <sub>3</sub>	–
14	1.14 ( <i>s</i> )	14	15.2 CH <sub>3</sub>	H-4, H-6, H-15
15	1.02 ( <i>d</i> , <i>J</i> = 6.8)	15	9.4 CH <sub>3</sub>	H-2, H-3, H-14
OAc		OAc		
1'	–	1'	170.9 C	H-2', H-6
2'	2.23 ( <i>s</i> )	2'	20.6 CH <sub>3</sub>	–
OAng		OAng		
1''	–	1''	167.1 C	H-3, H-3'', H-5''
2''	–	2''	127.7 C	H-4'', H-5''
3''	6.08 ( <i>qq</i> )	3''	138.4 CH	H-4'', H-5''
4''	1.97 ( <i>dq</i> )	4''	15.8 CH <sub>3</sub>	H-3'', H-5''
5''	1.95( <i>s</i> )	5''	20.5 CH <sub>3</sub>	H-3'', H-4''

data (Table 1) and EI-MS demonstrate it to be an eremophilenolide with a molecular formula C<sub>22</sub>H<sub>28</sub>O<sub>8</sub>.

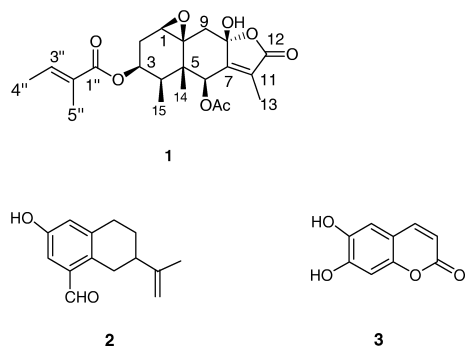


Fig. 1. Structures of the compounds.

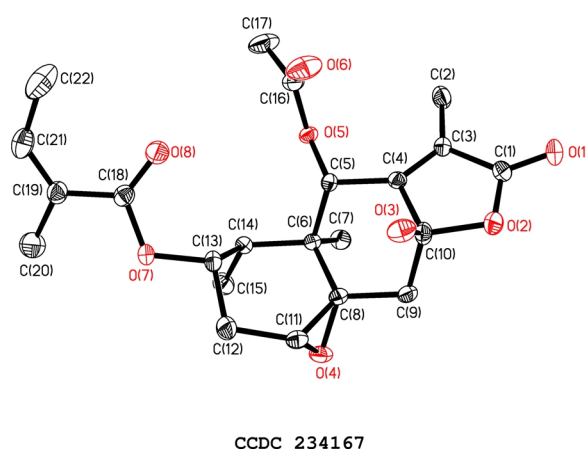


Fig. 2. Molecular structure of title compound at 30% ellipsoid probability.

Twenty-two signals ( $6 \times \text{CH}_3$ ,  $2 \times \text{CH}_2$ ,  $5 \times \text{CH}$ ,  $9 \times \text{C}$ ) are observed in the  $^{13}\text{C}$  NMR spectrum. The typical  $^1\text{H}$  NMR signals at  $\delta$  1.96 (dq, 3H), 1.86 (s, 3H) and 6.07 (qq, 1H) show the presence of an angeloyloxy group, while an acetoxyl group is obviously required by a three-proton singlet at  $\delta$  2.23 (s, 3H). Evidently  $\delta$  5.11 ppm of H-3 and the downfield shift of the H-6 signal at  $\delta$  6.04 ppm including single crystal X-Ray diffraction analysis reveal that these two substituents are attached to the  $3\beta$  and  $6\beta$  positions. The HMBC spectrum of compound **1** reveals the connection of the angeloyloxy group with C-3 and that of the acetoxyl group with C-6 (Table 1). When the chemical shift of Me-15 (d) signal at  $\delta$  1.02 is smaller than that of Me-14 (s) signal at  $\delta$  1.14, the compound is  $\alpha$ -lactone [5]. So C<sub>8</sub> is in  $\beta$  position [6–7].

Its absolute structure was clarified by single crystal X-Ray diffraction analysis. An ORTER drawing of the title compound showing the molecular conformation and atom-labeling scheme is depicted in Fig. 2.

Table 2. Atomic coordinates ( $10^4$ ) and equivalent displacement parameters ( $\text{\AA}^2 \times 10^3$ ).

Atom	x	y	z	Ueq
O(1)	3747(3)	−1490(2)	2242(1)	48(1)
O(2)	4052(3)	536(2)	2357(1)	41(1)
O(3)	4778(3)	2285(2)	1833(1)	42(1)
O(4)	805(3)	4248(2)	2475(1)	41(1)
O(5)	823(3)	1618(2)	743(1)	28(1)
O(6)	2991(3)	1393(3)	203(1)	63(1)
O(7)	856(3)	5811(2)	685(1)	38(1)
O(8)	1644(4)	4494(3)	−4(1)	53(1)
C(1)	3455(4)	−456(4)	2080(2)	35(1)
C(2)	1578(5)	−999(3)	1250(2)	43(1)
C(3)	2452(5)	−81(3)	1594(2)	30(1)
C(4)	2528(4)	1139(3)	1565(2)	27(1)
C(5)	1751(4)	2105(3)	1205(2)	25(1)
C(6)	634(4)	2910(3)	1588(2)	27(1)
C(7)	−731(4)	2103(3)	1810(2)	36(1)
C(8)	1615(4)	3374(3)	2107(2)	30(1)
C(9)	2483(4)	2401(3)	2454(2)	32(1)
C(10)	3504(5)	1642(3)	2052(2)	34(1)
C(11)	2147(5)	4663(3)	2136(2)	37(1)
C(12)	1823(5)	5575(4)	1655(2)	43(1)
C(13)	1339(5)	4903(3)	1114(2)	34(1)
C(14)	9(4)	3984(3)	1214(2)	29(1)
C(15)	−1486(4)	4609(4)	1434(2)	45(1)
C(16)	1606(5)	1382(4)	241(2)	39(1)
C(17)	488(5)	1096(5)	−232(2)	62(1)
C(18)	1038(4)	5456(4)	125(2)	35(1)
C(19)	420(4)	6391(4)	−279(2)	39(1)
C(20)	−680(6)	7340(5)	−38(2)	72(2)
C(21)	819(5)	6363(4)	−825(2)	55(1)
C(22)	1880(6)	5468(6)	−1136(2)	82(2)

The structure of compound **1** was determined and confirmed by its NMR and X-Ray diffraction analysis which are reported for the first time here. This is also the first isolation of the substance from *Ligularia sagitta*. Many eremophilenolides have been reported to exhibit antibacterial activity [10], further studies into the biological activity of this compound are now underway in our laboratory.

## Experimental Section

### General methods

Melting points were determined on a X-4 micro-melting point apparatus without correction. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Bruker IFS-120HR spectrophotometer.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra, and 2D NMR spectra (H-HCOSY, HMQC, HMBC) were recorded on a Varian INOVA-400 using  $\text{CDCl}_3$ , DMSO as solvent and TMS as the internal standard. EIMS was measured on a VG-ZAB-HS mass spectrometer.

Bond	Dist	Bond	Dist	Bond	Dist
O(1)-C(1)	1.212(4)	O(8)-C(18)	1.205(4)	C(8)-C(9)	1.520(5)
O(2)-C(1)	1.354(4)	C(1)-C(3)	1.468(5)	C(9)-C(10)	1.517(5)
O(2)-C(10)	1.471(4)	C(2)-C(3)	1.478(5)	C(11)-C(12)	1.515(5)
O(3)-C(10)	1.386(4)	C(3)-C(4)	1.331(4)	C(12)-C(13)	1.505(5)
O(4)-C(8)	1.450(4)	C(4)-C(5)	1.493(5)	C(13)-C(14)	1.529(5)
O(4)-C(11)	1.456(5)	C(4)-C(10)	1.501(5)	C(14)-C(15)	1.530(5)
O(5)-C(16)	1.360(5)	C(5)-C(6)	1.566(5)	C(16)-C(17)	1.483(6)
O(5)-C(5)	1.431(4)	C(6)-C(7)	1.544(5)	C(18)-C(19)	1.478(5)
O(6)-C(16)	1.183(5)	C(6)-C(8)	1.545(5)	C(19)-C(21)	1.306(6)
O(7)-C(18)	1.359(4)	C(6)-C(14)	1.548(5)	C(19)-C(20)	1.502(6)
O(7)-C(13)	1.457(4)	C(8)-C(11)	1.476(5)	C(21)-C(22)	1.510(6)
Angle	(°)	Angle	(°)	Angle	(°)
C(1)-O(2)-C(10)	107.8(2)	C(7)-C(6)-C(5)	109.1(3)	C(13)-C(12)-C(11)	109.9(3)
C(8)-O(4)-C(11)	61.1(2)	C(8)-C(6)-C(5)	107.0(3)	O(7)-C(13)-C(12)	108.1(3)
C(16)-O(5)-C(5)	115.7(3)	C(14)-C(6)-C(5)	108.5(3)	O(7)-C(13)-C(14)	109.7(3)
C(18)-O(7)-C(13)	114.9(3)	O(4)-C(8)-C(11)	59.7(2)	C(12)-C(13)-C(14)	113.2(3)
O(1)-C(1)-O(2)	121.2(3)	O(4)-C(8)-C(9)	112.4(3)	C(13)-C(14)-C(15)	112.1(3)
O(1)-C(1)-C(3)	127.8(4)	C(11)-C(8)-C(9)	119.3(3)	C(13)-C(14)-C(6)	108.9(3)
O(2)-C(1)-C(3)	111.0(3)	O(4)-C(8)-C(6)	114.2(3)	C(15)-C(14)-C(6)	115.9(3)
C(4)-C(3)-C(1)	106.7(4)	C(11)-C(8)-C(6)	120.8(3)	O(6)-C(16)-O(5)	123.5(4)
C(4)-C(3)-C(2)	132.2(4)	C(9)-C(8)-C(6)	116.3(3)	O(6)-C(16)-C(17)	125.9(4)
C(1)-C(3)-C(2)	121.1(3)	C(10)-C(9)-C(8)	109.6(3)	O(5)-C(16)-C(17)	110.6(3)
C(3)-C(4)-C(5)	135.1(4)	O(3)-C(10)-O(2)	109.8(3)	O(8)-C(18)-O(7)	122.0(4)
C(3)-C(4)-C(10)	110.6(3)	O(3)-C(10)-C(4)	110.2(3)	O(8)-C(18)-C(19)	126.6(4)
C(5)-C(4)-C(10)	113.9(3)	O(2)-C(10)-C(4)	103.7(3)	O(7)-C(18)-C(19)	111.3(3)
O(5)-C(5)-C(4)	113.5(3)	O(3)-C(10)-C(9)	113.4(3)	C(21)-C(19)-C(18)	120.0(4)
O(5)-C(5)-C(6)	107.0(3)	O(2)-C(10)-C(9)	109.5(3)	C(21)-C(19)-C(20)	122.5(4)
C(4)-C(5)-C(6)	110.4(3)	C(4)-C(10)-C(9)	109.8(3)	C(18)-C(19)-C(20)	117.5(4)
C(7)-C(6)-C(8)	109.6(3)	O(4)-C(11)-C(8)	59.2(2)	C(19)-C(21)-C(22)	129.0(4)
C(7)-C(6)-C(14)	110.8(3)	O(4)-C(11)-C(12)	117.0(3)		
C(8)-C(6)-C(14)	111.8(3)	C(8)-C(11)-C(12)	122.2(3)		

Table 3. Selected bond lengths (Å) and bond angles (°).

#### Plant material

Aerial parts of plant of *L. sagitta* were collected in August, 2002, at Mengyuan County of Qinghai Province, China, and identified by Prof. Yourui Suo of the Northwest Institute of Plateau Biology, CAS.

#### Extraction and isolation of compounds

Air – dried aerial parts of *L. sagitta* (5.8 kg) were powdered and extracted four times at room temperature with 95% EtOH. (each time for 7 days). The resultant extract was concentrated under reduced pressure to give a residue (450 g), then the residue was suspended in H<sub>2</sub>O and extracted, successively, with petroleum ether, CHCl<sub>3</sub>, EtOAc, n-Butanol. CHCl<sub>3</sub> extract was subjected to Column chromatographic (CC) separation over silica gel (1300 g, 200–300 mesh) and eluted with a gradient of petroleum ether – acetone (50:1–1:1) and finally with MeOH. According to the differences in composition indicated by TLC, 20 crude fractions were obtained. From eluant (petroleum, ether-acetone 30:1), pure compound **2** and **4** were afforded. Among F1-20, F8(20:1) was separated on CC over silica gel (100 g, 200–300 mesh) with petroleum ether- acetone (30:1–15:1), and pure compound **1** was obtained. EtOAc extract (10 g) was subjected to separation over silica gel (300 g, 200–300 mesh) and eluted

with a gradient of CHCl<sub>3</sub>-MeOH (100:1–1:1), from eluant (CHCl<sub>3</sub>-MeOH, 30:1), pure compound **3** was obtained.

#### 1β, 10β-epoxy-6β-acetoxy-3β-angeloyloxy-8β-hydroxy-eremophil-7(11)-en-8, 12α-olide (**1**)

Colorless crystal, M. p. 190–191 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup>: –90.0 (*c* = 1.0, CH<sub>2</sub>Cl<sub>2</sub>); – IR (film):  $\tilde{\nu}$  = 3341 (OH), 1754 ( $\gamma$ -lactone), 1727, 1705, 1237, 1219 (ester groups). Its NMR and HMBC data were listed in Table 1. EIMS *m/z* (rel.int) = 420 [M]<sup>+</sup> (8), 378 [M-ketene]<sup>+</sup> (2), 360 [378-H<sub>2</sub>O]<sup>+</sup> (23), 278 (30), 260 (100).

#### Liguhodgsonal (**2**)

Colorless amorphous solid, – IR (film):  $\tilde{\nu}$  = 3272, 2920, 1675, 1609, 1302, 1153, 904. – <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 10.25 (s, 1H, CHO, 14-H), 7.17 (d, *J* = 2.8 Hz, 1H, 3-H), 6.86 (d, *J* = 2.4 Hz, 1H, 1-H), 5.56 (s, OH), 4.78 (d, *J* = 9.2 Hz, 2H, 12-H), 3.43 (dd, *J* = 18.8, 5.2 Hz, 1H, 6 $\alpha$ -H), 1.82 (br s, 3H, 13-Me). – <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  = 192.5 (C-14), 153.5 (C-2), 149.0 (C-11), 139.8 (C-10), 134.7 (C-4), 131.7 (C-5), 121.7 (C-1), 115.7 (C-3), 109.6 (C-12), 41.4 (C-7), 30.8 (C-9), 29.9 (C-6), 27.0 (C-8), 20.7 (C-13).

**Esculetin (3)**

$^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$ : 10.18 (s, 1H, OH-7), 9.37 (s, 1H, OH-6), 7.81 (d,  $J = 9.6$  Hz, 1H, 4-H), 6.94 (s, 1H, 5-H), 6.70 (s, 1H, 8-H), 6.11 (d,  $J = 9.6$  Hz, 1H, 3-H).  $^{13}\text{C}\{^1\text{H}\}$  NMR (100 MHz, DMSO)  $\delta$ : 160.8 (C-2), 150.4 (C-7), 148.5 (C-9), 144.5 (C-4), 142.9 (C-6), 112.3 (C-5), 111.5 (C-3), 115.7 (C-3), 110.8 (C-10), 102.6 (C-8).

 **$\beta$ -sitosterol (4)**

Colorless needles, m.p. 134–136 °C. TLC and IR spectrum were identical with that of a standard compound.

**X-ray crystallographic analysis of 1**

CCDC-234167: A crystal of dimensions 0.52 mm  $\times$  0.42 mm  $\times$  0.19 mm was mounted on a glass fiber. The determination of the unit cell and the data collection were performed with a Kagaku R-Axis Rapid IP detector equipped

with a graphite monochromated, operating with Mo-K $\alpha$  radiation ( $\lambda = 0.71073$  Å), at 293(2) K, a total of 2732 reflections ( $R_{\text{int}} = 0.0000$ ) were collected in the range of  $1.76^\circ \leq \theta \leq 27.48^\circ$ , of which 1695 observed reflections with  $I \geq 20(I)$  were used in the succeeding structure determination and refinements. The compound crystallizes in an orthorhombic space group  $P2_12_12_1$  with  $a = 8.5192(3)$ ,  $b = 10.8834(3)$ ,  $c = 23.0838(8)$  Å,  $v = 2140.28(12)$  Å $^3$ ,  $Z = 4$  and with a calculated density of 1.305 Mg/m $^3$ .  $F(000) = 896$ ,  $\mu = 0.099$  mm $^{-1}$ . The crystal structure was solved by direct methods using SHELXS-97 program [8], and refined by  $\delta$  full-matrix least-square refinement on  $F^2$  (SHELXL-97) [9]. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were generated geometrically. The final refinement converged at  $R = 0.0427$  and  $wR = 0.0821$  [ $w = 1/(\sigma^2(F_o^2) + 0.0395P)^2$ , where  $P = (F_o^2 + 2F_c^2)/3$ ],  $S = 1.006$ ,  $(\Delta/\sigma)_{\text{max}} = 0.001$ . The largest peak and deepest hole on the final difference Fourier map are 0.265 and  $-0.233$  e/Å $^3$ , respectively.

- [1] Jiangsu New Medicinal College, A Dictionary of Traditional Chinese Medicine; People's Hygiene Press, Beijing China. p. 7, 154, 549, 2349 (1977).
- [2] Institute of Materia Medica. Chinese Academy of Medical Science, The Handbook of Chinese Materia Medica, Vol. II, p. 203, People's Health Press Beijing (1985).
- [3] H. M. Chen, B. G. Wang, Z. J. Jia, India J. Chem. **35B**, 1304 (1996).
- [4] X. Q. Li, K. Gao, Z. J. Jia, Planta. Med. **69**, 356 (2003).
- [5] K. Sugama, K. Hayashi, H. Mitsuhashi, Phytochemistry **24**, 1531 (1985).
- [6] Y. Zhao, Z. J. Jia, R. X. Tan, L. Yang, Phytochemistry **31(8)**, 2785 (1992).
- [7] F. Bohlmann, J. Jakupovic, U. Warning, M. Grenz, T. V. Chau-Thi, R. M. King, H. Robinson, Bull. Soc. Chim. Bel. **95(9-10)**, 707 (1986).
- [8] G. M. Sheldrick, SHELXS-97. Program for the Solution of Crystal Structures, University of Göttingen, Germany (1997).
- [9] G. M. Sheldrick, SHELXL-97. Program for the Refinement of Crystal Structures, University of Göttingen, Germany (1997).
- [10] M. J. Mao, Z. J. Jia, Plant Med. **68**, 55 (2002).