Two New Eremophilane-Type Sesquiterpenoids from the Rhizomes of *Ligularia veitchiana* (HEMSL.) GREENM

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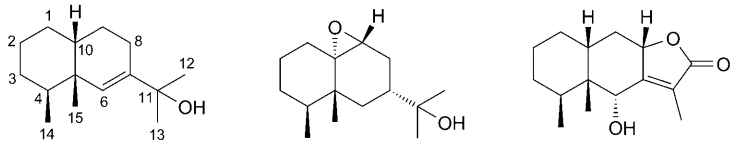
Two new eremophilane-type sesquiterpenoids eremophil-6-en-11-ol (**1**) and (7α,9α,10α)-9,10-epoxy-eremophilan-11-ol (**2**), together with a known eremophilane-type (6α,8α)-6,8-dihydroxyeremophil-7(11)-en-12-oic acid 12,8-lactone (**3**) were isolated from the rhizomes of *Ligularia veitchiana*. The structures of **1** and **2** were established by spectral analysis including ^{1}H- and ^{13}C-NMR, HSQC, HMBC, and HR-ESI-MS data. The compounds **1** and **3** were assessed against lung-cancer (A549) and stomach-cancer (BCG823) cell lines by the MTT method. The results showed that **1** exhibited significant inhibiting activities on the growth of these cancer cells with IC_{50} values between 1 – 100 μg/ml, whereas compound **3** had no effect on the same cell lines.

Introduction. – There are over 100 species of the genus *Ligularia* CASS (Compositae) distributed in China [1]. A number of Ligularia species such as *L. fischeri*, *L. sibirica*, and *L. hodgsoni* are commonly used as herbal remedies to treat bronchitis, cough and asthma, phthisis, and so on in southwest China and other areas [2].

Many phytochemical studies on this plant collected from different regions have revealed the presence of many eremophilane-type sesquiterpenoids showing significant bioactivities and chemically interesting structures [3 – 13]. But there is no report about the chemical components of this plant distributed in Henan Province. To find new active substances, the chemical constituents of *L. veitchiana* collected in the Funiu Mountain area of Henan Province were investigated. In this article, we report the isolation and structure elucidation of two new eremophilane-type sesquiterpenoids eremophil-6-en-11-ol[1]) (**1**) and (7α,9α,10α)-(9,10-epoxyeremophilan-11-ol[1]) (**2**), together with a known eremophilane-type sesquiterpenoid, (6α,8α)-6,8-dihydroxyeremophil-7(11)-en-12-oic acid 12,8-lactone[1]) (**3**) (*Fig. 1*). The structures of **1** and **2** were established by spectral evidences including ^{1}H- and ^{13}C-NMR, HSQC, HMBC, NOESY, and HR-ESI-MS data, and compounds **1** and **3** were assessed against lung-cancer (A549) and stomach-cancer (BCG823) cell lines by the MTT method which established significant inhibiting activities of **1**.

Results and Discussion. – 1. *Structure Elucidation*. Compound **1** was obtained as colorless syrup. Its molecular formula was determined to be $C_{15}H_{26}O$ on the basis of the

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*



1 **2** **3**

Fig. 1. *Compounds* **1**–**3** *isolated from* L. veitchiana *(*HEMSL.*)* GREENM

HR-ESI-MS (*m*/*z* at 245.1875 ($[M+\mathrm{Na}]^+$)) and NMR spectra. The NMR signals of three tertiary Me groups, one secondary Me group, and one olefinic H-atom, and the degree of unsaturation ($U=3$) indicated that **1** was an eremophilane-type sesquiterpenoid [14]. Further NMR data (*Table 1*) and HMBC results (*Fig. 2*) allowed to assign the structure of **1** as eremophil-6-en-11-ol, which is a new compound.

Table. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; $CDCl_3$) *of* **1** *and* **2**. δ in ppm, *J* in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.66, 1.39 (2*m*)	26.6	2.13, 0.87 (2*m*)	28.1
CH_2(2)	1.99, 1.95 (2*m*)	25.1	1.72, 1.51 (2*m*)	22.9
CH_2(3)	1.50, 1.20 (2*m*)	27.1	1.33, 1.12 (2*m*)	23.9
H–C(4)	1.56 (*m*)	33.9	1.34 (*m*)	33.3
C(5)		37.6		35.8
H–C(6) or CH_2(6)	5.32 (*s*)	120.5	1.60 (br. *s*), 1.16 (*m*)	34.5
C(7) or H–C(7)		143.8	1.64 (*m*)	40.8
CH_2(8)	1.71, 1.11 (2*m*)	35.1	2.03, 1.71 (2*m*)	26.1
CH_2(9) or H–C(9)	2.25, 1.96 (2*m*)	27.9	2.95 (*s*)	60.8
H–C(10) or C(10)	1.32 (*m*)	41.8		64.2
C(11)		72.7		72.8
Me(12)[a])	1.13 (*s*)	26.2	1.16 (*s*)	26.1
Me(13)[a])	1.14 (*s*)	27.4	1.18 (*s*)	27.3
Me(14)	0.87 (*d*, *J* = 6.40)	16.0	0.78 (*d*, *J* = 5.89)	15.6
Me(15)	0.91 (*s*)	21.0	0.93 (*s*)	17.1

[a]) Shifts are interchangeable.

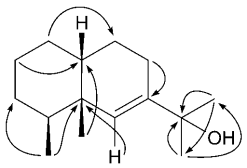


Fig. 2. *Key HMBC data of* **1**

The ^{1}H-NMR spectrum of **1** showed signals due to three tertiary Me groups (δ(H) 1.02 (*s*), 1.13 (*s*), and 1.14 (*s*)), a secondary Me group (δ(H) 0.87 (*d*, *J* = 6.4)), and an olefinic H-atom (δ(H) 5.32 (*s*)). The ^{13}C-NMR and DEPT spectra showed signals assignable to two olefinic C-atoms (δ(C) 143.8 and 120.5), an oxygenated quaternary C-atom (δ(C) 72.7), four Me groups (δ(C) 16.0, 21.0, 26.2, and 27.4), five high-field CH_2 groups (δ(C) 25.1, 26.6, 27.1, 27.9, and 35.1), two high-field CH groups (δ(C) 33.9 and 41.8), and

a high-field quaternary C-atom (δ(C) 37.6). All these data confirmed the eremophilane-type sesquiterpenoid structure of **1**. The C=C bond should be between C(6) and C(7) since the HC(6) signal showed no splitting. The oxygenated quaternary C-atom should be C(11), as supported by HMBC data. According to biogenetic considerations, Me(14) and Me(15) are supposed to be β-oriented [15]. The chemical shift of Me(14) was at higher field than the one of Me(15), so H–C(10) has β-configuration [16]. Further NMR data (*Table 1*) and HMBC results (*Fig. 2*) allowed to assign the structure of **1**.

Compound **2** was obtained as a colorless syrup. Its molecular formula was determined to be $C_{15}H_{26}O_2$ on the basis of the HR-ESI-MS (*m/z* at 261.1820 ($[M+Na]^+$)) and NMR. The NMR data of **2** were very similar to that of **1** except for the presence of two oxygenated quaternary C-atoms and an OCH group and the absence of two olefinic C-atoms and an olefinic H-atom. Additional NMR data (*Table 1*) and HMBC and NOESY results (*Fig. 3*) allowed to assign the structure of **2** as (7α,9α,10α)-9,10-epoxyeremophilan-11-ol.

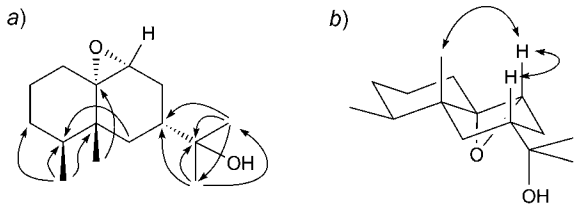


Fig. 3. *a*) *Key HMBC data of* **2**. *b*) *Key NOESY correlations of* **2**

The ^{1}H-NMR data of **2** showed signals due to three tertiary Me groups (δ(H) 0.93 (*s*), 1.16 (*s*), and 1.18 (*s*)), a secondary Me group (δ(H) 0.78 (*d*, $J = 5.89$)), and an oxirane H-atom (δ(H) 2.95 (*s*)). The ^{13}C-NMR data showed signals assignable to two oxygenated quaternary C-atoms (δ(C) 64.2 and 72.8), an oxygenated tertiary C-atom (δ(C) 60.8), four Me groups (δ(C) 15.6, 17.1, 26.1, and 27.3), five high-field CH_2 groups (δ(C) 22.9, 23.9, 26.1, 28.1, and 34.5), two high-field CH groups (δ(C) 33.3 and 40.8), and a high-field quaternary C-atom δ(C) 35.8. The above data were very similar to those of **1**, suggesting that **2** was also an eremophilane-type sesquiterpenoid. The HMBC data (*Fig. 3*) allowed to assign the signal at δ(C) 72.8 to C(11), at δ(C) 33.3 to C(4) due to a correlation with Me(14) at δ(H) 0.78 (*d*)), and at δ(C) 40.8 to C(7). According to the biogenesis, Me(14) and Me(15) should be β-oriented [15]. The epoxy moiety should be at C(9) and C(10). Moreover, the NOESY correlations of H–C(9) with Me(15) and H–C(7) were observed (*Fig. 3*).

2. Biological Studies. Compounds **1** and **3** were evaluated for their cytotoxic properties against lung-cancer (A549) and stomach-cancer (BCG823) cell lines (*Table 2*) by the MTT method. Compound **1** exhibited significant activities, whereas **3** had no effect on these cancer cell lines.

Table 2. IC_{50} *Values* [μg/ml] *of* **1** *and* **3**

	1	**3**	Cisplatin[a])
A549	10.27	> 100	2.67
BCG823	31.34	> 100	1.09

[a]) Pos. control

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Experimental Part

General. Column Chromatography (CC): silica gel (SiO_2; 200–300 mesh and 300–400 mesh, *GF254*, *Qingdao Marine Chemical Factory*, Qingdao, China), visualized by spraying 5% phosphomolybdic acid soln. followed by heating. NMR Spectra: *Bruker DRX-400* spectrometer; at 400 (^{1}H) and 100 (^{13}C) MHz; $CDCl_3$ soln.; δ in ppm, J in Hz. HR-ESI-MS (Na^+ positive-ion mode): *VG-Auto-spec-3000* spectrometer; in m/z.

Plant Material. The rhizomes of *L. veitchiana* (HEMSL.) GREENM were collected in Funiu Mountain area of Henan Province in China and air-dried. A voucher specimen was deposited with the Herbarium at the School of Pharmacy, Zhengzhou University.

Extraction and Isolation. The dried rhizomes of *L. veitchiana* (HEMSL.) GREENM (670 g) were crushed to powder. The powder was soaked in EtOH at r.t. overnight and then filtered. The filtrate was concentrated, and the extract (23 g) obtained was subjected to CC (SiO_2, gradient $CHCl_3$/AcOEt/MeOH 10:0:0 → 10:1:0, 10:10:0, and 0:10:5 → 0:10:10): *Fractions I–V. Fr. I* was further purified repeatedly by CC (SiO_2, hexane/AcOEt 10:1 → 1:1): **1** (20 mg) and **2** (5 mg). *Fr. II* was further purified by recrystallization: **3** (15 mg).

Eremophil-6-en-11-ol (= *(4a*R,*8*S,*8a*R*)-3,4,4a,5,6,7,8,8a-Octahydro-α,α,8,8a-tetramethylnaphthalene-2-methanol*; **1**). Colorless syrup. ^{1}H- and ^{13}C-NMR: *Table 1*; assigned by HSQC and HMBC. HMBC: *Fig. 2*. HR-ESI-MS (pos.): 245.1875 ($[M+Na]^+$, $C_{15}H_{26}NaO^+$; calc. 245.1881).

(7α,9α,10α)-9,10-Epoxyeremophilan-11-ol (= *(1a*R,*3*R,*4a*R,*5*S,*8a*S)-1a,2,4,4a,5,6,7,8-Octahydro-α,α,4a,5-tetramethyl-3H-*naphth[1,8*a-b*]oxirene-3-methanol*; **2**). Colorless syrup. ^{1}H- and ^{13}C-NMR: *Table 1*; assigned by ^{1}H,^{1}H-COSY, HSQC, and HMBC. HMBC and NOESY: *Fig. 3*. HR-ESI-MS (pos.): 261.1820 ($[M+Na]^+$, $C_{15}H_{26}NaO_2^+$; calc. 261.1830).

(6α,8α)-6,8-Dihydroxyeremophil-7(11)-en-12-oic Acid 12,8-Lactone [17] (= *(4*R,*4a*R,*5*S,*8a*R,*9a*S*)-4a,5,6,7,8,8a,9,9a-Octahydro-4-hydroxy-3,4a,5-trimethylnaphtho[2,3*-b*]furan-2(4*H*)-one*; **3**). Colorless crystals. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.14 (*m*, 1 H); 4.74 (*s*, 1 H); 2.10–2.16 (*m*, 2 H); 1.90 (*t*, J = 1.74, 3.06, 3 H); 1.69–1.79 (*m*, 3 H); 1.35–1.50 (*m*, 6 H); 1.16 (*s*, 3 H); 0.82 (*d*, J = 5.4). ^{13}C-NMR ($CDCl_3$, 100 MHz): 8.7; 16.3; 16.4; 20.0; 25.8; 29.4; 30.6; 33.8; 35.0; 42.9; 70.0; 78.4; 121.7; 161.4; 174.8.

Biological Studies: The cytotoxicities of compounds **1** and **3** were investigated by means of the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay with the lung-cancer (A549) and stomach-cancer (BCG823) cell lines. Cancer cell lines were obtained from the Shanghai Institute of Life Science of the Chinese Academy of Science and cultured according to the supplier's instruction. The cells were seeded in 96-well plates, incubated with 10% bovine serum at 37° and with 5% CO_2 for 24 h, and treated with compounds **1** and **3** at different concentrations for 72 h. Cisplatin was used as a positive control. The absorbance of the extracted MTT was measured at 490 nm. The experiments were carried out in triplicate. The percentage survival rates of cells exposed to the compounds were calculated by assuming the survival rate of untreated cells to be 100%.

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