Eremophilane-Type Sesquiterpenes from the Roots of *Ligularia virgaurea*

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From the roots of *Ligularia virgaurea*, five new eremophilane-type sesquiterpenes were isolated, including three new eremophilenolides, 6β-(angeloyloxy)-1α,8β,10β-trihydroxyeremophil-7(11)-en-12,8α-olide (**1**), 6β-(angeloyloxy)-1β,10β-epoxy-8β-ethoxyeremophil-7(11)-en-12,8α-olide (**2**), and 1β,10β-epoxy-8β-ethoxy-6β-[(2-methylacryloyl)oxy]eremophil-7(11)-en-12,8α-olide (**3**), two new nor-eremophilanes, 3β-[(2-methylacryloyl)oxy]-8-oxo-12-noreremophil-6-en-11-one (**9**), and 9β-hydroxy-8-oxo-12-noreremophil-6-en-11-one (**10**), and six known eremophilanes, namely **4**–**8**, and **11**. Their structures were elucidated by means of spectral methods, such as IR, HR-ESI-MS, and 1D- and 2D-NMR, and by comparison of the spectral data with those reported for structurally related compounds.

Introduction. – *Ligularia* (Compositae) species, widespread throughout China, are important medicinal plants. In *Ligularia* species, sesquiterpenes are found to be the principal secondary metabolites and a number of new ones were isolated recently [1]. *Ligularia virgaurea* (MAXIM.) MATTF. is widely distributed in northwestern China and is used as a traditional folk medicine for the treatment of stomachache and nausea [2]. In continuation of our comparative studies on the influence of different ecological environments on the chemical constituents of *Ligularia* species, we reinvestigated *Ligularia virgaurea* collected from the Gannan Tibetan Autonomous Region (S.A. 2200–3800 m) in the Gansu Province of P. R. China. As a result, eleven compounds (**1**–**11**) were isolated from this species, *i.e.* the five new eremophilanes **1**–**3**, **9**, and **10**, and six known ones, **4**–**8**, and **11**. In this study, we describe their isolation and structure elucidation.

Results and Discussion. – The known compounds **4** [3], **5** [3], **6** [3], **7** [4], **8** [5], and **11** [6] were identified by comparison of their physical and spectral data with those reported in the literature.

Compound **1** was obtained as a colorless gum. The molecular formula of **1** was deduced as $C_{20}H_{28}O_7$ from the HR-ESI-MS (*m*/*z* 398.2173 ($[M+NH_4]^+$)). The IR spectrum showed absorption bands for OH (3390 cm^{-1}), γ-lactone (1764 cm^{-1}), and α,β-unsaturated ester groups (1716 cm^{-1}), and for a C=C bond (1647 cm^{-1}). Careful comparison of the data of the ^{1}H- and ^{13}C-NMR (DEPT) spectra of **1** (*Tables 1* and *2*) with those of related compounds [7][8] led to the conclusion that **1** possesses an eremophilenolide skeleton. The actual structure was inferred and confirmed by the HMBC data (*Fig. 1*). The relative configuration was determined on the basis of NOE experiments and empirical rules. From all these data, the structure of **1** was deduced as

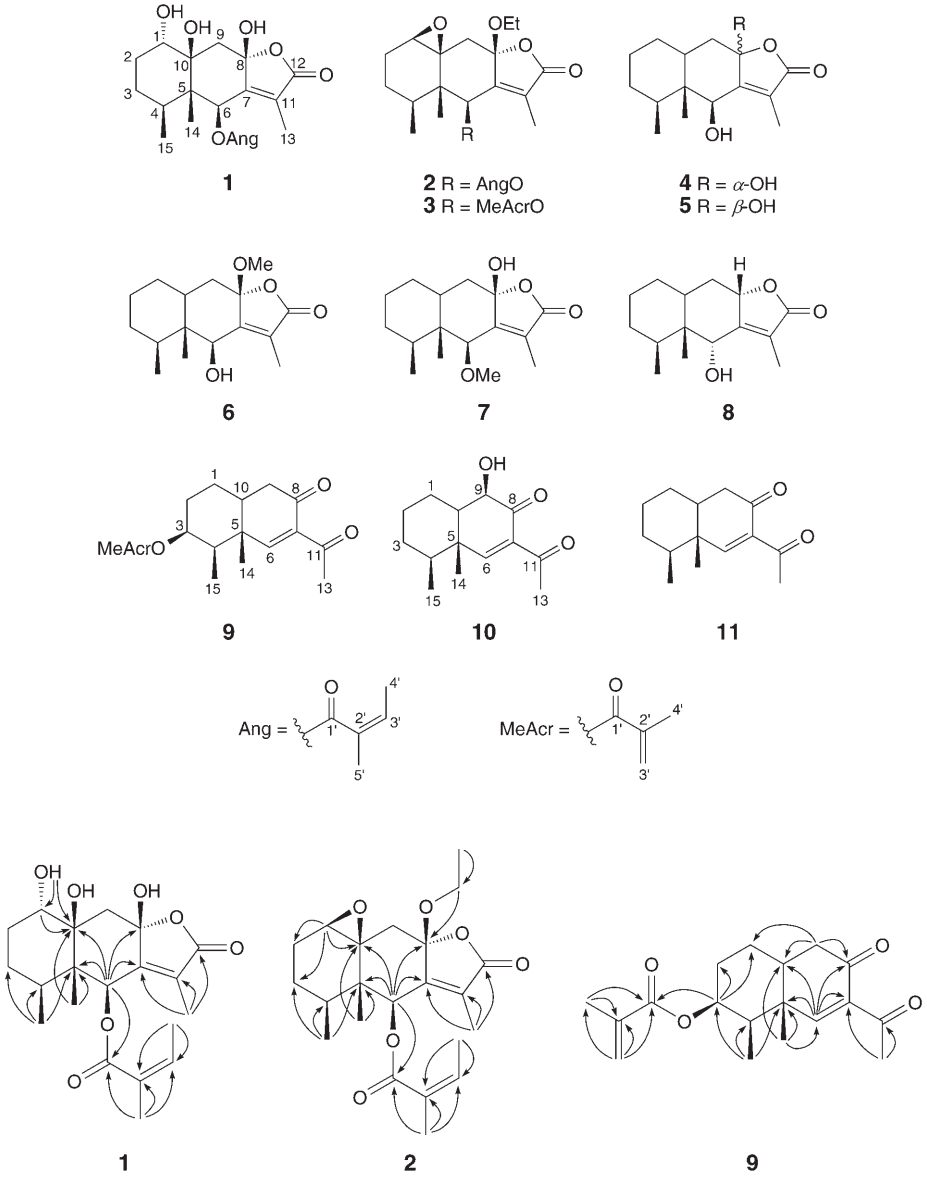

Fig. 1. *Key HMBC correlations* (H → C) *for* **1**, **2**, *and* **9**

6β-(angeloyloxy)-1α,8β,10β-trihydroxyeremophil-7(11)-en-12,8α-olide with as yet unknown absolute configuration.

The ^{1}H- and ^{13}C-NMR (DEPT) spectra of **1** (*Tables 1* and *2*) displayed the signals of OH groups and an angeloyloxy group. The EI-MS showed fragmental peaks at *m*/*z* 362 ([$M - H_2O$]$^+$), 344 ([$M - 2\ H_2O$]$^+$), 280 ([$M - C_5H_8O_2$]$^+$), and 83 ([C_5H_7O]$^+$),

Table 1. *^{1}H-NMR Data of Compounds* **1**–**3**, **9**, *and* **10**. In $CDCl_3$; δ in ppm, J in Hz.

Position	**1**[a])	**2**[b])	**3**[b])	**9**[b])	**10**[a])
1	3.81 (*dd*, *J* = 11.7, 4.8)	3.18 (*d*, *J* = 4.4)	3.19 (*d*, *J* = 3.6)	1.43–1.48 (*m*), 1.59–1.63 (*m*)	2.06–2.09 (*m*), 1.59–1.62 (*m*)
2	1.83–1.88 (*m*), 1.33–1.36 (*m*)	1.94–1.99 (*m*), 1.70–1.73 (*m*)	1.90–1.96 (*m*), 1.65–1.68 (*m*)	1.72–1.76 (*m*), 1.82–1.87 (*m*)	1.58–1.61 (*m*), 1.38–1.42 (*m*)
3	1.60–1.64 (*m*), 1.45–1.49 (*m*)	2.02–2.05 (*m*), 1.36–1.39 (*m*)	2.07–2.10 (*m*), 1.36–1.40 (*m*)	4.84–4.88 (*m*)	1.62–1.64 (*m*), 1.61–1.63 (*m*)
4	1.38–1.41 (*m*)	1.61–1.66 (*m*)	1.60–1.64 (*m*)	2.11–2.17 (*m*)	1.88–1.91 (*m*)
6	5.78 (*s*)	5.77 (*s*)	5.76 (*s*)	7.44 (*s*)	7.69 (*s*)
9	2.68 (*d*, *J* = 14.7), 2.11 (*d*, *J* = 14.7)	1.82 (*d*, *J* = 13.6), 2.28 (*d*, *J* = 13.6)	1.82 (*d*, *J* = 13.6), 2.29 (*d*, *J* = 13.6)	2.51 (br. *d*, *J* = 12.0), 2.61 (br. *d*, *J* = 12.0)	4.49 (*d*, *J* = 12.9)
10				2.11–2.17 (*m*)	1.83–1.85 (*m*)
13	2.01 (*s*)	1.85 (*s*)	1.85 (*s*)	2.47 (*s*)	2.51 (*s*)
14	1.11 (*s*)	1.14 (*s*)	1.15 (*s*)	1.31 (*s*)	1.21 (*s*)
15	0.90 (*d*, *J* = 5.7)	1.05 (*d*, *J* = 7.2)	1.05 (*d*, *J* = 7.6)	1.05 (*d*, *J* = 7.6)	0.97 (*d*, *J* = 6.6)
	AngO	AngO	MeAcrO	MeAcrO	
3′	6.19 (br. *q*, *J* = 7.5)	6.31 (*qq*, *J* = 6.4, 1.6)	6.26 (*d*, *J* = 2.0), 5.78 (*d*, *J* = 2.0)	6.11 (br. *s*), 5.58 (br. *s*)	
4′	2.03 (br. *d*, *J* = 7.5)	2.09 (*dq*, *J* = 6.4, 1.6)	2.03 (br. *s*)	1.96 (br. *s*)	
5′	1.92 (br. *s*)	2.03 (*dq*, *J* = 1.6, 1.6)			
		EtO	EtO		
		3.50 (*q*, *J* = 6.8)	3.48 (*q*, *J* = 6.9)		
		1.25 (*t*, *J* = 6.8)	1.24 (*t*, *J* = 6.9)		
10-OH	3.94 (*s*)				

[a]) Recorded at 300 MHz. [b]) Recorded at 400 MHz.

which confirmed the above conclusions. Apart from the angeloyloxy group, the ^{1}H-NMR spectrum (*Table 1*) of **1** indicated typical signals for three Me groups at δ(H) 2.01 (*s*), 1.11 (*s*), and 0.90 (*d*, *J* = 5.7), two oxygenated methines at δ(H) 3.81 (*dd*, *J* = 11.7, 4.8), 5.78 (*s*), and an OH signal at δ(H) 3.94 (*s*). The ^{13}C-NMR spectrum (*Table 2*) of **1** showed 15 C-atoms, which according to the DEPT spectrum included three Me, three CH_2, and three CH groups (two oxygenated), together with six quaternary C-atoms (one ester CO, one acetal, one oxygenated and two olefinic C-atoms), indicating a sesquiterpene skeleton with an α,β-unsaturated γ-lactone unit and in agreement with an eremophil-7(11)-en-8,12-olide structure [7][8]. In the HMBC spectrum (*Fig. 1*), the long-range correlations between H–C(1) and C(9) and C(10), between H–C(6) and C(4), C(5), C(7), C(8), C(10), C(11), C(14), and C(1′), and between CH_2(9) and C(1), C(5), C(7), C(8), and C(10), together with the characteristic C-atom signals at δ(C) 71.3 (C(1)), 71.4 (C(6)), 77.3 (C(10)) and 103.0 (C(8)), indicated that an OH group was attached to each C(1), C(10) and C(8), in addition to an angeloyloxy group attached to C(6), respectively. Due to biogenetic considerations of eremophilane derivatives isolated from Compositae species, Me(14) and Me(15) were both assigned to have β-orientation [9]. In the ^{1}H-NMR spectrum, the signal of Me(14) (δ(H) 1.11 (*s*)) was shifted downfield compared to Me(15) (δ(H) 0.90 (*d*, *J* = 5.7)), indicating that the

Table 2. *^{13}C-NMR Data of Compounds* **1**–**3**, **9**, *and* **10**. In $CDCl_3$; δ in ppm.

	1[a])	**2**[b])	**3**[b])	**9**[b])	**10**[a])
1	71.3 (*d*)	62.5 (*d*)	62.5 (*d*)	24.4 (*t*)	22.0 (*t*)
2	28.2 (*t*)	20.0 (*t*)	20.2 (*t*)	24.4 (*t*)	20.4 (*t*)
3	28.4 (*t*)	23.8 (*t*)	23.8 (*t*)	73.5 (*d*)	30.5 (*t*)
4	33.3 (*d*)	32.1 (*d*)	32.5 (*d*)	36.7 (*d*)	35.9 (*d*)
5	47.5 (*s*)	43.2 (*s*)	43.2 (*s*)	40.3 (*s*)	40.6 (*s*)
6	71.4 (*d*)	73.1 (*d*)	73.9 (*d*)	163.7 (*d*)	167.6 (*d*)
7	151.4 (*s*)	154.2 (*s*)	153.9 (*s*)	137.3 (*s*)	133.3 (*s*)
8	103.0 (*s*)	104.1 (*s*)	103.9 (*s*)	196.5 (*s*)	199.3 (*s*)
9	37.2 (*t*)	43.5 (*t*)	43.5 (*t*)	40.7 (*t*)	70.2 (*d*)
10	77.3 (*s*)	60.8 (*s*)	60.7 (*s*)	40.3 (*d*)	48.1 (*d*)
11	129.1 (*s*)	126.1 (*s*)	126.1 (*s*)	197.9 (*s*)	196.4 (*s*)
12	170.8 (*s*)	170.9 (*s*)	170.7 (*s*)	–	–
13	8.8 (*q*)	7.9 (*q*)	8.1 (*q*)	30.7 (*q*)	30.4 (*q*)
14	11.0 (*q*)	15.0 (*q*)	14.5 (*q*)	23.8 (*q*)	19.3 (*q*)
15	16.3 (*q*)	15.7 (*q*)	15.9 (*q*)	9.5 (*q*)	16.7 (*q*)
	AngO	AngO	MeAcrO	MeAcrO	
1′	166.5 (*s*)	166.5 (*s*)	166.2 (*s*)	166.7 (*s*)	
2′	126.5 (*s*)	126.3 (*s*)	135.2 (*s*)	136.5 (*s*)	
3′	141.6 (*d*)	141.9 (*d*)	127.5 (*t*)	125.4 (*t*)	
4′	15.9 (*q*)	16.1 (*q*)	18.3 (*q*)	18.3 (*q*)	
5′	20.6 (*q*)	20.6 (*q*)			
		EtO	EtO		
		59.0 (*t*)	58.9 (*t*)		
		15.2 (*q*)	15.1 (*q*)		

[a]) Recorded at 75 MHz. [b]) Recorded at 100 MHz.

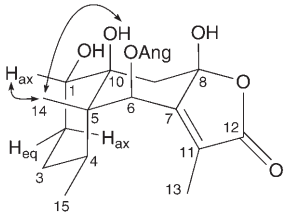

Fig. 2. *Key NOE correlations and relative configuration of compound* **1**

8(12) lactone was α-oriented, which is in accordance with the empirical rules reported by *Naya et al.* [10]. The absence of a homoallylic coupling between H–C(6) and Me(13) showed that H–C(6) is α-oriented [11]. Furthermore, in the NOE difference spectra of **1**, irradiation of Me(14) enhanced the signals of H–C(1) and HO–C(10), indicating that H–C(1) and HO–C(10) are β-oriented. Taking into account that rings *A* and *B* are *cis*-fused, the coupling constants of H–C(1) (*dd*, $J = 11.7, 4.8$) suggest that H–C(1) is axial (*Fig. 2*) [12].

Compound **2** was obtained as a colorless gum. The HR-ESI-MS showed the $[M + Na]^+$ peak at *m*/*z* 413.1942, in accord with the molecular formula $C_{22}H_{30}O_6$. The IR spectrum indicated absorption bands for a γ-lactone (1773 cm^{-1}), an α,β-unsaturated

ester (1722 cm^{-1}) and a C=C bond (1645 cm^{-1}). The ^{1}H- and ^{13}C-NMR (DEPT) spectra (*Tables 1* and *2*) of **2** were very similar to those of **1**, except for the presence of an epoxy group and an EtO group instead of OH groups. The following analysis of the 2D-NMR spectra led to the structure proposal of 6β-(angeloyloxy)-1β,10β-epoxy-8β-ethoxyeremophil-7(11)-en-12,8α-olide for compound **2**.

The ^{1}H- and ^{13}C-NMR spectra of **2** (*Tables 1* and *2*) displayed signals of an EtO and an angeloyloxy group. The EI-MS showed fragmental peaks at *m/z* 344 ($[M-EtOH]^+$, 290 ($[M-C_5H_8O_2]^+$), and 83 ($[C_5H_7O]^+$), which confirmed the above assumptions. In addition, the characteristic signals at δ(H) 3.18 (*d*, *J* = 4.4, 1 H) and δ(C) 62.5 (CH) and 60.8 (C) implied an epoxy group. In the HMBC spectrum (*Fig. 1*), the long-range correlations between H–C(1) and C(2), C(3), C(5), C(9), and C(10), between H–C(6) and C(4), C(5), C(7), C(11), C(14), and C(1′), and between the EtO H-atoms and C(8), indicated that there is an epoxy linkage between C(1) and C(10), an angeloyloxy group attached to C(6), and an EtO group located at C(8), respectively. In the ^{1}H-NMR spectrum, the signal of Me(14) (δ(H) 1.14 (*s*)) was shifted downfield compared to Me(15) (δ(H) 1.05 (*d*, *J* = 7.2)), indicating that the 8(12) lactone is α-oriented [10]. The absence of a homoallylic coupling between H–C(6) and Me(13) showed H–C(6) is also α-oriented [11]. In the NOE difference spectrum of **2**, irradiation of H–C(6) enhanced the signals of H–C(1) (1.6%) and H–C(4) (2.5%), indicating that H–C(1) is α-oriented.

The molecular formula of **3** was deduced as $C_{21}H_{28}O_6$ from the HR-ESI-MS (*m/z* 399.1771 ($[M+Na]^+$)). The IR spectrum showed absorption bands for a γ-lactone (1773 cm^{-1}), an α,β-unsaturated ester (1724 cm^{-1}) and a C=C bond (1636 cm^{-1}). Comparing the NMR data (*Tables 1* and *2*) of **3** with those of **2**, the main difference was the presence of a (2-methylacryloyl)oxy group at C(6) (δ(H) 6.26 (*d*, *J* = 2.0, H–C(3′a)), 5.78 (*d*, *J* = 2.0, H–C(3′b)), 2.03 (br. *s*, Me(4′)); δ(C) 166.2, 135.2, 127.5, 18.3) in **3** instead of an angeloyloxy group (δ(H) 6.31 (*qq*, *J* = 6.4, 1.6, H–C(3′)), 2.09 (*dq*, *J* = 6.4, 1.6, Me(4′)), 2.03 (*dq*, *J* = 1.6, 1.6, Me(5′)); δ(C) 166.5, 126.3, 141.9, 16.1, 20.6) as in **2**. The remaining signals were very similar to the ones of **2** (see *Tables 1* and *2*). Thus, the structure of **3** was deduced as 1β,10β-epoxy-8β-ethoxy-6β-[(2-methylacryloyl)oxy]eremophil-7(11)-en-12,8α-olide.

Compound **9** was obtained as a colorless gum. Its molecular formula was determined as $C_{18}H_{24}O_4$ by HR-ESI-MS (*m/z* 327.1568 ($[M+Na]^+$)). The IR spectrum displayed absorption bands for an α,β-unsaturated ester (1714 cm^{-1}), C=O groups (1689, 1680 cm^{-1}) and C=C bonds (1634, 1600 cm^{-1}). The signals of a 2-(methylacryloyl)oxy group were observed in the ^{1}H- and ^{13}C-NMR (DEPT) spectra of **9** (*Tables 1* and *2*). The remaining moieties were further identified as a noreremophilane-type sesquiterpene by extensive analysis of 1D- and 2D-NMR spectral data. Therefore, compound **9** was elucidated as 3β-[(2-methylacryloyl)oxy]-8-oxo-12-noreremophil-6-en-11-one.

Apart from the (2-methylacryloyl)oxy group, the ^{1}H- and ^{13}C-NMR (DEPT) spectra (*Tables 1* and *2*) of **9** indicated the presence of 14 C-atoms, *i.e.*, three Me, three CH_2, and four CH groups, as well as four quaternary C-atoms. In the ^{1}H-NMR spectrum, there were typical signals for three Me groups at δ(H) 2.47 (*s*), 1.31 (*s*), and 1.05 (*d*, *J* = 7.6), an oxygenated methine at δ(H) 4.84–4.88 (*m*), as well as a signal for a C=C–H moiety at δ(H) 7.44 (*s*); in the ^{13}C-NMR spectrum, there were typical signals

for two C=O groups at δ(C) 196.5 and 197.9, a C=C bond at δ(C) 163.7 and 137.3, and an oxygenated methine at δ(C) 73.5. In the HMBC spectrum (*Fig. 1*), the correlations between H–C(6) and C(5), C(14), C(7), C(8) and C(11), and between Me(13) and C(7) and C(11) suggested a 8-oxo-12-nor-eremophil-6-en-11-one derivative. Furthermore, the HMBC correlations between H–C(3) and C(1′) indicated that the 2-(methylacryloyl)oxy group was located at C(3). In the NOE difference spectra of **9**, irradiation of Me(14) enhanced the signal of H–C(10) (1.4%), and irradiation of H–C(3) enhanced the signal of H–C(4) (3.7%), indicating the β-orientation of H–C(10) and the 2-(methylacryloyl)oxy group.

The molecular formula of compound **10** was determined as $C_{14}H_{20}O_3$ by HR-ESI-MS at *m*/*z* 259.1307 ($[M+Na]^+$). The IR spectrum displayed absorption bands for an OH group (3463 cm^{-1}), C=O groups (1692 cm^{-1}) and a C=C bond (1592 cm^{-1}). Careful comparison of the NMR data of **10** with those of **9** showed an identical skeleton structure. In the HMBC spectrum, an oxygenated methine H–C(9) at δ(H) 4.49 (*d*, $J = 12.9$) correlated with C(10) and C(5), and an OH signal at δ(H) 3.61 (br. *s*) correlated with C(9) and C(8), indicating that the OH group was located at C(9). In the NOE difference spectra of **10**, irradiation of Me(14) enhanced the signal of H–C(10) (0.5%), indicating the β-orientation of H–C(10). The large coupling constant observed for H–C(9) with H–C(10) ($J(9\alpha,10\beta) = 12.9$) assigned the β-orientation of the OH group at C(9). Thus, the structure of **10** was elucidated as 9β-hydroxy-8-oxo-12-noreremophil-6-en-11-one.

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Experimental Part

General. TLC: SiO_2 *GF*$_{254}$ (10–40 μ; *Qingdao Marine Chemical Factory*); reversed phase *RP-18 F*$_{254}$*s* (size 20 × 20 cm, Schichtdicke 0.25 mm; *E. Merck Factory*, Germany); detection at 254 nm or by heating after spraying with 5% H_2SO_4 in EtOH (*v*/*v*). Column chromatography (CC): SiO_2 (200–300 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China). $[\alpha]_D$: *Perkin-Elmer-341* polarimeter. IR Spectra: *Nicolet-NEXUS-670 FT-IR* spectrometer; in cm^{-1}. NMR Spectra: *Varian Mercury plus-400 or -300* NMR spectrometer (^1H at 400 or 300 MHz; ^{13}C at 100 or 75 MHz); Me_4Si as internal standard. MS: *HP5988A GC/MS* instrument for EI, and *Bruker-APEX-II* instrument with glycerol as the matrix for HR-ESI; in *m*/*z* (rel. %).

Plant Material. The roots of *Ligularia virgaurea* (MAXIM.) MATTF. were collected in Gannan Tibetan Autonomous Region, Gansu Province, P. R. China, in August 2005. It was identified by Prof. *Guo-Liang Zhang*, School of Life Sciences, Lanzhou University. A voucher specimen (No. 20050801) was deposited in College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation. The air-dried roots of *L. virgaurea* (3.8 kg) were pulverized and extracted with petroleum ether (60–90°) (PE)/Et_2O/MeOH (1:1:1) (6 d × 3 times) at r.t. The solvent was evaporated giving an extract (256 g) which was subjected to CC (SiO_2 (200–300 mesh, 2000 g); PE/acetone (AC) 30:1, 15:1, 8:1, 5:1, 3:1, 1:1, and 0:1): eight fractions. *Fr. 4* (41.0 g, eluted with PE/AC 8:1) was subjected to CC ($CHCl_3$/AcOEt 60:1, 40:1, 20:1, and 10:1): *Fr. 4.1–4.6*. *Fr. 4.1* (8.0 g, eluted with $CHCl_3$/AcOEt 60:1) was subjected to CC (PE/AC 20:1, 10:1, and 5:1): *Fr. 4.1.1–4.1.4*. *Fr. 4.1.2* (348 mg, eluted with PE/AC 20:1) was further purified by repeated CC (PE/AC 25:1) to afford a crude compound which was subjected to prep. TLC (SiO_2 *GF*$_{254}$ (10–40 μ), 25 × 25 cm, PE/AC 18:1) to afford compound **11** (85 mg, R_f 0.28). *Fr. 4.1.3* (18 mg, eluted with PE/AC 10:1) was subjected to reversed-phase prep. TLC (*RP-18*, MeOH/H_2O, 3:1) to afford compound **10** (3 mg, R_f 0.49). *Fr. 4.2* (6.5 g, eluted

with $CHCl_3$/AcOEt 40:1) was subjected to CC (PE/AC 20:1, 10:1, 5:1, and 3:1): *Fr. 4.2.1–4.2.5*. *Fr. 4.2.2* (1.3 g, eluted with 10:1) was further separated by repeated CC (PE/AcOEt 8:1) to afford crude compounds (58 mg) which were purified by TLC (SiO_2 GF_{254} (10–40 μ), 25 × 25 cm, PE/AcOEt 12:1) to afford compounds **2** (30 mg, R_f 0.28) and **3** (10 mg, R_f 0.35). *Fr. 4.3* (6.0 g, eluted with $CHCl_3$/AcOEt 40:1) was subjected to CC (PE/AC 10:1, 5:1, and 3:1): *Fr. 4.3.1–4.3.3*. *Fr. 4.3.2* (1.85 g, eluted with PE/AC 5:1) was subjected to CC (PE/AC 15:1) to afford crude **9** (56 mg) which was separated by prep. TLC (SiO_2 GF_{254} (10–40 μ), 25 × 25 cm, PE/AC 8:1) to afford compound **9** (5 mg). *Fr. 4.4* (18.5 g, eluted with $CHCl_3$/AcOEt 20:1) was subjected to CC (PE/AC 10:1, 7:1, 5:1, and 3:1): *Fr. 4.4.1–4.4.12*. *Fr. 4.4.10* (0.56 g, eluted with PE/AC 5:1) was subjected to repeated CC (PE/AcOEt 5:1) and then worked-up by crystallization to afford **7** (30 mg). A number of colorless crystals of compound **8** (>850 mg) was obtained from *Fr. 5* by crystallization. *Fr. 5* (32.0 g, eluted with PE/AC 8:1 to 5:1) was subjected to CC ($CHCl_3$/AC 50:1, 30:1, 15:1, 8:1, and 5:1): *Fr. 5.1–5.5*. *Fr. 5.1* (6.6 g, eluted with $CHCl_3$/AC 50:1) was further subjected to CC (PE/AC 20:1, 10:1, 5:1, and 3:1): *Fr. 5.1.1–5.1.6*. *Fr. 5.1.1* (2.2 g, eluted with PE/AC 20:1) was purified by repeated CC (PE/AcOEt 20:1) to afford **12** (5 mg). From *Fr. 6*, mixed crystals of **4**, **5**, and **6** (>1 g) were obtained. The partial mixture was purified by TLC (SiO_2 GF_{254} (10–40 μ), 25 × 25 cm, PE/AC 2:1) to afford **4** and **5** (R_f 0.49), and **6** (R_f 0.65). *Fr. 7* (2.6 g, eluted with PE/AC 3:1) was subjected to CC (PE/AC 10:1, 5:1 and 2:1): *Fr. 7.1–Fr. 7.3*. *Fr. 7.3* (50 mg, eluted with PE/AC 2:1) was subjected to CC (PE/AcOEt 3:1 and 1:1): *Fr. 7.3.1–Fr. 7.3.3*. *Fr. 7.3.2* (9 mg, eluted with PE/AcOEt 3:1) was subjected to reversed-phase prep. TLC (*RP-18*, MeOH/H_2O, 3:2) to afford compound **1** (3 mg, R_f 0.16).

6β-(Angeloyloxy)-1α,8β,10β-trihydroxyeremophil-7(11)-en-12,8α-olide (= rel-*(4*R*,4a*R*,5*R*,8*R*,8a*R*,9a*R*)-2,4,4a,5,6,7,8,8a,9,9a-Decahydro-8,8a,9a-trihydroxy-3,4a,5-trimethyl-2-oxonaphtho[2,3-*b*]furan-4-yl (2*Z*)-2-Methylbut-2-enoate*; **1**): Colorless gum. $[\alpha]_D^{20} = +36$ ($c = 0.6$, $CHCl_3$). IR: 3390, 2924, 1764, 1716, 1647, 1460, 1385, 1227, 1152, 1040, 977, 758. ^{1}H- and ^{13}C-NMR (DEPT): *Tables 1* and *2*. EI-MS: 380 (1, M^+), 362 (8, $[M - H_2O]^+$), 344 (10, $[M - 2\,H_2O]^+$), 280 (15, $[M - C_5H_8O_2]^+$), 262 (40, $[M - C_5H_8O_2 - H_2O]^+$), 83 (100, $[C_5H_7O]^+$). HR-ESI-MS: 398.2173 ($[M + NH_4]^+$, $C_{20}H_{32}NO_7^+$; calc. 398.2173).

6β-(Angeloyloxy)-1β,10β-epoxy-8β-ethoxyeremophil-7(11)-en-12,8α-olide (= rel-*(1a*R*,4*S*,4a*S*,5*S*,8a*S*,9a*S*)-8a-Ethoxy-2,3,4,4a,5,7,8a,9-octahydro-4,4a,6-trimethyl-7-oxo-1a*H*-oxireno[8,8a]naphtho[2,3-*b*]furan-5-yl (2*Z*)-2-Methylbut-2-enoate*; **2**): Colorless gum. $[\alpha]_D^{20} = -58$ ($c = 1.0$, $CHCl_3$). IR: 2977, 2932, 1773, 1722, 1645, 1227, 1148, 1056, 1016, 923. ^{1}H- and ^{13}C-NMR (DEPT): *Tables 1* and *2*. EI-MS: 390 (2, M^+), 344 (1, $[M - EtOH]^+$), 307 (2, $[M - C_5H_7O]^+$), 290 (4, $[M - C_5H_8O_2]^+$), 261 (4, $[M - EtOH - C_5H_7O]^+$), 244 (8, $[M - EtOH - C_5H_8O_2]^+$), 83 (100, $[C_5H_7O]^+$). HR-ESI-MS: 413.1942 ($[M + Na]^+$, $C_{22}H_{30}NaO_6^+$; calc. 413.1935).

1β,10β-Epoxy-8β-ethoxy-6β-[(2-methylacryloyl)oxy]eremophil-7(11)-en-12,8α-olide (= rel-*(1a*R*,4-*S*,4a*S*,5*S*,8a*S*,9a*S*)-8a-Ethoxy-2,3,4,4a,5,7,8a,9-octahydro-4,4a,6-trimethyl-7-oxo-1a*H*-oxireno[8,8a]naphtho[2,3-*b*]furan-5-yl 2-Methylprop-2-enoate*; **3**): Colorless gum. $[\alpha]_D^{20} = -50$ ($c = 0.6$, $CHCl_3$). IR: 2978, 2934, 1773, 1724, 1636, 1152, 1015, 923. ^{1}H- and ^{13}C-NMR (DEPT): *Tables 1* and *2*. EI-MS: 376 (2, M^+), 330 (2, $[M - EtOH]^+$), 307 (3, $[M - C_4H_5O]^+$), 290 (5, $[M - C_4H_6O_2]^+$), 261 (7, $[M - EtOH - C_4H_5O]^+$), 244 (12, $[M - EtOH - C_4H_6O_2]^+$), 69 (100, $[C_4H_5O]^+$). HR-ESI-MS: 399.1771 ($[M + Na]^+$, $C_{21}H_{28}NaO_6^+$; calc. 399.1778).

3β-[(2-Methylacryloyl)oxy]-8-oxo-12-noreremophil-6-en-11-one (= rel-*(1*R*,2*S*,8a*R*)-7-Acetyl-1,2,3,4,4a,5,6,8a-octahydro-1,8a-dimethyl-6-oxonaphthalen-2-yl 2-Methylprop-2-enoate*; **9**): Colorless gum. $[\alpha]_D^{20} = +38$ ($c = 0.5$, $CHCl_3$). IR: 2929, 2878, 1714, 1689, 1680, 1634, 1600, 1165. ^{1}H- and ^{13}C-NMR (DEPT): *Tables 1* and *2*. EI-MS: 304 (1, M^+), 289 (1, $[M - Me]^+$), 276 (1, $[M - CO]^+$), 235 (3, $[M - C_4H_5O]^+$), 218 (3, $[M - C_4H_6O_2]^+$), 69 (100, $[C_4H_5O]^+$). HR-ESI-MS: 327.1568 ($[M + Na]^+$, $C_{18}H_{24}NaO_4^+$; calc. 327.1567).

9β-Hydroxy-8-oxo-12-noreremophil-6-en-11-one (= rel-*(1*R*,4a*S*,5*S*)-3-Acetyl-4a,5,6,7,8,8a-hexahydro-1-hydroxy-4a,5-dimethylnaphthalen-2(1*H*)-one*; **10**): Colorless gum. $[\alpha]_D^{20} = -35$ ($c = 0.3$, $CHCl_3$). IR: 3463, 2924, 1692, 1592, 1249, 1097, 1031, 957, 903. ^{1}H- and ^{13}C-NMR (DEPT): *Tables 1* and *2*. EI-MS: 236 (6, M^+), 221 (2, $[M - Me]^+$), 218 (2, $[M - H_2O]^+$), 208 (2, $[M - CO]^+$), 43 (100). HR-ESI-MS: 259.1307 ($[M + Na]^+$, $C_{14}H_{20}NaO_3^+$; calc. 259.1305).

REFERENCES

[1] P.-L. Li, C.-M. Wang, Z.-X. Zhang, Z.-J. Jia, *Tetrahedron* **2007**, *63*, 12665; Z.-X. Zhang, C.-J. Lin, P.-L. Li, Z.-J. Jia, *Planta Med.* **2007**, *73*, 585; X. Gao, C.-J. Lin, W.-D. Xie, T. Shen, Z.-J. Jia, *Helv. Chim. Acta* **2006**, *89*, 1387; B.-G. Wang, Z.-J. Jia, X.-P. Yang, *Planta Med.* **1997**, *63*, 577; H.-R. Peng, Y.-P. Shi, Z.-J. Jia, *Planta Med.* **1997**, *63*, 335; Z.-J. Jia, H.-M. Chen, *Phytochemistry* **1991**, *30*, 3132.
[2] Z.-Y. Wu, 'Flora Xizangica', Science Press, Beijing, 1985, Vol. 4, p. 836.
[3] K. Sugama, K. Hayashi, H. Mitsuhashi, *Phytochemistry* **1985**, *24*, 1531.
[4] W.-S. Wang, K. Gao, L. Yang, Z.-J. Jia, *Planta Med.* **2000**, *66*, 189.
[5] Q.-H. Wu, C.-M. Liu, Y.-J. Chen, K. Gao, *Helv. Chim. Acta* **2006**, *89*, 915.
[6] Y. Goto, Y. Kojima, T. Nakayama, M. Terazawa, *Phytochemistry* **2001**, *57*, 109.
[7] J.-Q. Xu, Y.-S. Li, Y.-M. Li, S.-H. Jiang, C.-H. Tan, D.-Y. Zhu, *Planta Med.* **2006**, *72*, 567.
[8] E.-W. Li, J. Pan, K. Gao, Z.-J. Jia, *Planta Med.* **2005**, *71*, 1140.
[9] Y. Zhao, S. Parsons, B. A. Smart, R. Tan, Z. Jia, H. Sun, D. W. H. Rankin, *Tetrahedron* **1997**, *53*, 6195.
[10] K. Naya, N. Nogi, Y. Makiyama, H. Takashina, T. Imagawa, *Bull. Chem. Soc. Jpn.* **1977**, *50*, 3002.
[11] Y. Moriyama, T. Takahashi, *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3196.
[12] G. Delgado, P. E. Garcia, R. I. Roldan, R. Bye, E. Linares, *Nat. Prod. Lett.* **1996**, *8*, 145.

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