Four Novel Eremophilanolides from *Ligularia sagitta*

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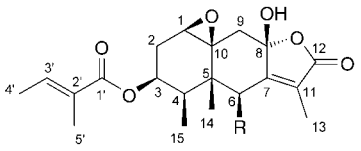
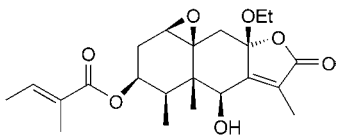
Four new eremophilanolides, isolated from *Ligularia sagitta*, were identified as (1*β*,3*β*,6*β*,8*β*,10*β*)-6-acetoxy-3-(angeloyloxy)-1,10-epoxy-8-hydroxyeremophil-7(11)-en-8,12*α*-olide (**1**), (1*β*,3*β*,6*β*,8*β*,10*β*)-3-(angeloyloxy)-1,10-epoxy-6,8-dihydroxyeremophil-7(11)-en-8,12*α*-olide (**2**), (1*β*,3*β*,6*β*,8*β*,10*β*)-3-(angeloyloxy)-1,10-epoxy-8-ethoxy-6-hydroxyeremophil-7(11)-en-8,12*α*-olide (**3**), and (1*β*,3*β*,8*β*,10*β*)-3-(angeloyloxy)-1,10-epoxy-8-hydroxyeremophil-7(11)-en-8,12*α*-olide (**4**). Their structures were elucidated by spectroscopic methods, including 2D-NMR techniques and chemical transformations.

Introduction. – More than 20 *Ligularia* species are being used in Chinese folk medicines. Their roots, stems, leaves, and flowers are effective anti-inflammation agents, reduce phlegm, relieve cough and pain, and help blood circulation. As mirrored in Chinese pharmacopoeia, these plants have been used for a long time to cure pulmonary tuberculosis, haemoptysis, urinary-tract blockages, rheumatism, common cold, pharyngitis and laryngitis, hepatitia, bronchitis, and asthma [1]. *Ligularia sagitta* (MAXIM.) MATTF. is widely distributed in Northwestern China and has been used as a folk medicine to reduce phlegm, relieve cough, cure pulmonary tuberculosis, urinary-tract blockages, common cold, and pharyngitis [2]. Eremophilane sesquiterpenes and pyrrolizidine alkaloids are the most widespread secondary metabolites of this genus and other plants [3–7]. We have investigated the constituents of *L. sagitta* and found the new eremophilanolides **1**–**4**, whose isolation and structure elucidation will be reportet in this paper.



1 R = AcO
2 R = OH
4 R = H



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Results and Discussion. – The IR band of compound **1** at 1754 cm^{-1} indicated an unsaturated γ-lactone moiety. The NMR spectral data (*Table 1*) and the EI mass spectrum were consistent with an eremophilenolide of the molecular formula $C_{22}H_{28}O_8$. A total of 22 resonances, corresponding to six Me, two CH_2, and five CH groups, as well as nine quaternary C-atoms, were observed in the ^{13}C-NMR spectrum of **1**. In the ^{1}H-NMR spectrum, the three eremophilenolide Me signals were observed at δ_H 1.02 (*d*,

$J = 6.8$ Hz), 1.14 (*s*), and 1.81 (*s*). Moreover, a pair of doublets ($J = 13.6$ Hz) at δ_H 2.38 and 1.83, unambiguously assigned to $CH_2(9)$, indicated that C(8) and C(10) were quarternary, and a doublet at δ_H 3.21 disclosed the presence of a 1,10-epoxy group, as in the cases of structurally similar compounds reported previously [8][9]. The characteristic signals at δ_H 1.96 (*dq*, 3 H), 1.86 (*s*, 3 H), and 6.07 (*qq*, 1 H) showed the presence of an angeloyloxy (=(2-methylbut-2-enoyl)oxy) group, and a singlet (3 H) at δ_H 2.23 was assigned to an AcO group. The signal at δ_H 5.11 (*ddd*, $J = 3.6, 3.6, 7.2$ Hz), arising from H_α–C(3), and the downfield shift of the H_α–C(6) signal at δ_H 6.04 (*s*) revealed that the the angeloyloxy and AcO groups were in 3β and 6β position respectively, in accord with the corresponding HMBC spectrum (*Table 1*). Taking all these data into account, compound **1** was identified as (1β,3β,6β,8β,10β)-6-acetoxy-3-(angeloyloxy)-1,10-epoxy-8-hydroxyeremophil-7(11)-en-8,12α-olide[1]).

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

	δ_H	δ_C [a])	HMBC [b])
H–C(1)	3.21 (*d*, $J = 9.6$)	60.4 (*d*)	$CH_2(2)$, $CH_2(9)$
$CH_2(2)$	2.10, 2.27 (2*m*)	42.4 (*t*)	H–C(1), H–C(4)
H–C(3)	5.11 (*ddd*)	67.5 (*d*)	H–C(1), $CH_2(2)$, H–C(4), Me(15)
H–C(4)	2.11 (*m*)	33.7 (*d*)	$CH_2(2)$, H–C(3), H–C(14), Me(15)
C(5)	–	44.4 (*s*)	H–C(4), H–C(6), Me(14), Me(15)
H–C(6)	6.04 (*s*)	72.3 (*d*)	Me(14)
C(7)	—	154.8 (*s*)	H–C(6), Me(13)
C(8)	–	101.2 (*s*)	$CH_2(9)$, Me(13)
$CH_2(9)$	2.38, 1.83 (2*d*, $J = 13.6$ each)	24.9 (*t*)	H–C(1)
C(10)	–	60.8 (*s*)	H–C(1), H–C(4), Me(14)
C(11)	–	124.5 (*s*)	H–C(6), Me(13)
C(12)	–	171.1 (*s*)	Me(13)
Me(13)	1.81 (*s*)	7.9 (*q*)	–
Me(14)	1.14 (*s*)	15.2 (*q*)	H–C(4), H–C(6), Me(15)
Me(15)	1.02 (*d*, $J = 6.8$)	9.4 (*q*)	$CH_2(2)$, H–C(3), Me(14)
Me*C*=O	–	170.9 (*s*)	*Me*C=O, H–C(6)
*Me*C=O	2.23 (*s*)	20.6 (*q*)	–
C(1′)	–	167.1 (*s*)	H–C(3), H–C(3′), H–C(5′)
C(2′)	–	127.7 (*s*)	H–C(4′), H–C(5′)
H–C(3′)	6.08 (*qq*, $J = 6.8, 1.6$)	138.4 (*d*)	H–C(4′), H–C(5′)
Me(4′)	1.86 (*dq*, $J = 6.8, 1.6$)	20.5 (*q*)	H–C(3′), H–C(4′)
Me(5′)	1.97 (*dq*, $J = 1.6, 1.6$)	15.8 (*q*)	H–C(3′), H–C(5′)

[a]) Multiplicities determined by a DEPT experiment. [b]) Observed long-range HMBC (^{1}H,^{13}C) interactions.

Compound **2** gave rise to a molecular-ion peak at m/z 378 in the EI mass spectrum, consistent with a molecular formula of $C_{20}H_{26}O_7$, as confirmed by ^{1}H-, ^{13}C-, and DEPT-NMR. An IR resonance at 1754 cm^{-1} verified an unsaturated γ-lactone moiety. The ^{1}H- and ^{13}C-NMR spectra (*Table 2*) of **2** were similar to those of **1**. A total of 20 signals – five Me, two CH_2, five CH, eight quaternary C-atoms – were observed in the ^{13}C-NMR spectrum. In the ^{1}H-NMR spectrum, the characteristic signals at δ_H 1.95 (*dq*, 3 H), 1.84 (*s*, 3 H), and 6.12 (*qq*, 1 H) showed the presence of an angeloyloxy group in 3β-

[1]) For systematic names, see the *Exper. Part*.

Table 2. *^{1}H- and ^{13}C-NMR Spectral Data of Compounds* **2**–**4**. Spectra recorded in $CDCl_3$ at 400 (^{1}H) and 100 MHz (^{13}C); δ in ppm, J in Hz.

	2		**3**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
H–C(1)	3.16 (*d*, *J* = 6.0)	60.4 (*d*)	3.13 (*d*, *J* = 5.6)	60.4 (*d*)	3.21 (*d*, *J* = 6.0)	60.5 (*d*)
CH_2(2)	2.25, 210 (2 *m*)	25.1 (*t*)	2.23, 2.16 (2*m*)	25.0 (*t*)	2.36, 1.77 (2*m*)	42.6 (*t*)
H–C(3)	5.16 (*ddd*, *J* = 3.6, 3.6, 7.2)	68.3 (*d*)	5.11 (*ddd*, *J* = 3.6, 3.6, 7.2)	67.7 (*d*)	5.27 (*ddd*, *J* = 3.6, 3.6, 7.2)	68.4 (*d*)
H–C(4)	2.29 (*d*, *J* = 5.2)	33.6 (*d*)	2.56 (*d*, *J* = 6)	33.5 (*d*)	1.74 (*d*, *J* = 7.2)	40.3 (*d*)
C(5)	–	45.9 (*s*)	–	45.9 (*s*)	–	39.3 (*s*)
H–C(6)[a])	5.07 (br. *s*)	70.9 (*d*)	4.90 (br. *s*)	71.3 (*d*)	2.30, 2.13 (2*d*, *J* = 2.8 each)	25.2 (*t*)
C(7)	–	158.1 (*s*)	–	156.0 (*s*)	–	157.6 (*s*)
C(8)	–	101.4 (*s*)	–	103.7 (*s*)	–	102.7 (*s*)
CH_2(9)	2.14, 1.73 (2*d*, *J* = 13.6 each)	42.3 (*t*)	2.25, 1.75 (2*d*, *J* = 13.6 each)	42.0 (*t*)	3.00, 2.38 (2*d*, *J* = 13.6 each)	34.9 (*t*)
C(10)	–	61.0 (*s*)	–	60.9 (*s*)	–	61.6 (*s*)
C(11)	–	125.3 (*s*)	–	127.2 (*s*)	–	124.7 (*s*)
C(12)	–	172.3 (*s*)	–	171.3 (*s*)	–	172.0 (*s*)
Me(13)	2.02 (br. *s*)	8.6 (*q*)	2.08 (br. *s*)	8.7 (*q*)	1.82 (br. *s*)	8.1 (*q*)
Me(14)	1.06 (*s*)	14.2 (*q*)	1.06 (*s*)	14.3 (*q*)	1.25 (*s*)	20.3 (*q*)
Me(15)	1.01 (*d*, *J* = 6.0)	9.5 (*q*)	1.01 (*d*, *J* = 7.6)	9.4 (*q*)	1.04 (*d*, *J* = 8.8)	9.8 (*q*)
C(1′)	–	168.4 (*s*)	–	167.6 (*s*)	–	167.7 (*s*)
C(2′)	–	127.4 (*s*)	–	127.6 (*s*)	–	127.6 (*s*)
H–C(3)	6.12 (*qq*)	139.9 (*d*)	6.10 (*qq*)	139.1 (*d*)	6.13 (*qq*)	139.0 (*d*)
Me(4′)	1.84 (*dq*)	20.5 (*q*)	1.86 (*dq*)	20.6 (*q*)	1.88 (*dq*)	20.6 (*q*)
Me(5′)	1.95 (*dq*)	16.0 (*q*)	1.96 (*dq*)	15.9 (*q*)	1.98 (*dq*)	15.9 (*q*)
Me*CH_2*O	–	–	3.24–3.48 (*m*)	58.6 (*t*)	–	–
MeCH_2O	–	–	1.17 (*t*, *J* = 7.2)	15.3 (*q*)	–	–

[a]) CH_2(6) for compound **4**.

position, as indicated by the H_α–C(3) resonance at 5.16 ppm (*ddd*, *J* = 3.6, 3.6, 7.2 Hz), an in accord with the corresponding HMBC spectrum (*Table 3*). Taking all these data into account, compound **2** was identified as (1β,3β,6β,8β,10β)-3-(angeloyloxy)-1,10-epoxy-6,8-dihydroxyeremophil-7(11)-en-8,12α-olide[1]).

For compound **3**, a molecular-ion peak at *m*/*z* 406 (EI-MS) and a molecular formula of $C_{22}H_{30}O_7$ was determined by EI-MS, ^{1}H-, ^{13}C-, and DEPT-NMR. Again, an IR band at 1754 cm^{-1} verified an unsaturated γ-lactone group. The ^{1}H- and ^{13}C-NMR spectra (*Table 2*) of **3** were similar to those of **2**. A total of 20 signals – six Me, three CH_2, five CH, and eight quaternary C-atoms – were observed in the ^{13}C-NMR spectrum. The signals at δ_H 1.96 (*dq*, 3 H), 1.86 (*s*, 3 H), and 6.10 (*qq*, 1 H) showed the presence of an angeloyloxy group, and the signal for H–C(3) at δ_H 5.11 (*ddd*, *J* = 3.6, 3.6, 7.2 Hz) revealed that the angeloyloxy substituent was in 3β-position, as corroborated by an HMBC experiment (*Table 3*), which, additionally, helped to position the EtO group at C(8). Taking all these data into account, compound **3** was identified as (1β,3β,6β,8β,10β)-3-(angeloyloxy)-1,10-epoxy-8-ethoxy-6-hydroxyeremophil-7(11)-en-8,12α-olide[1]).

Table 3. *$^1H,^{13}C$ Long-Range HMBC Correlations for Compounds* **2**–**4**. For C- and H-atom positions, see chemical formulae.

C-Atom position	H-Atom position		
	2	**3**	**4**
1	2, 9, 14	2, 9, 14	2, 9, 14
2	1, 4	1, 4	1
3	1, 2, 4, 15	1, 2, 4, 15	1, 2, 4, 6, 15
4	2, 6, 14, 15	2, 14, 15	2, 14, 15
5	6, 9, 14, 15	6, 9, 14, 15	4, 6, 9, 14, 15
6	14	14	1
7	6, 9, 13	6, 9, 13	9, 13
8	9	9, 13, O–CH_2	6, 9, 13
9	1	1	14
10	1, 2, 9, 14	1, 2, 9, 14	1, 2, 6, 9, 14
11	6, 13	6, 13	9, 13
12	13	13	13
14	6	6	9
15	2, 3, 4, 14	2, 3, 4, 14	3, 4, 14
1′	3, 4′, 5′	3,	4′,
2′	4′, 5′	4′, 5′	4′, 5′
3′	4′, 5′	4′, 5′	4′, 5′
4′	3′	3′	3′
5′	3′	3′	3′
CH_3CH_2O	–	CH_3CH_2O	–
CH_3*C*H_2O	–	CH_3CH_2O	–

Compound **4** gave rise to a molecular-ion peak at *m*/*z* 362 (EI-MS) and a molecular formula of $C_{20}H_{26}O_6$. Again, an IR band at 1754 cm^{-1} verified an unsaturated γ-lactone. The ^{1}H- and ^{13}C-NMR spectra (*Table 2*) were similar to those of compounds **2** and **3**, with a total of 20 signals (five Me, two CH_2, five CH, and eight quaternary C-atoms) in the ^{13}C-NMR spectrum. In the ^{1}H-NMR spectrum, the signals at δ_H 1.98 (*dq*, 3 H), 1.88 (*s*, 3 H), and 6.13 (*qq*, 1 H) indicated an angeloyloxy group in 3β-position (assigned as above). Taking all these data into account, compound **4** was identified as (1β,3β,8β,10β)-3-(angeloyloxy)-1,10-epoxy-8-hydroxyeremophil-7(11)-en-8,12α-olide[1]).

Experimental Part

General. Melting points (m.p.) were determined on an *X-4* micro-melting-point apparatus; uncorrected. Optical rotations were measured on a *Perkin-Elmer 241* polarimeter. IR Spectra were recorded on a *Bruker IFS-120 HR* spectrophotometer, in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR spectra (^{1}H,^{1}H-COSY, HMQC, HMBC) were recorded on a *Varian INOVA-400* apparatus in $CDCl_3$ with $SiMe_4$ as internal standard; chemical shifts δ in ppm, coupling constants *J* in Hz. EI Mass spectra were recorded on a *VG-ZAB-HS* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. The aerial parts of *Ligularia sagitta* were collected in the Mengyuan County, Qinghai Province, China, in August 2002, and were identified by Prof. *Yourui Suo* of the Northwest Institute of Plateau Biology, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried aerial parts of *L. sagitta* (5.8 kg) were powdered and extracted at r.t. with 95% aq. EtOH (4 × 7 d). The combined extracts were concentrated under reduced pressure to afford a residue (450 g), which was suspended in H_2O and successively extracted with petroleum ether (PE), $CHCl_3$, AcOEt, and BuOH. The $CHCl_3$ extract was subjected to column chromatographic (CC) separation (1.3 kg of

SiO_2, 200–300 mesh; PE/acetone 50:1, 40:1, 30:1, 20:1, 15:1, 10:1, 8:1, 6:1, 4:1, 2:1, and 1:1, then MeOH). Based on TLC analysis, a total of 20 crude fractions (*F*) were obtained. *F8* (eluted with PE/acetone 20:1) was subjected to CC (100 g SiO_2, 200–300 mesh; PE/acetone 30:1, 25:1, 20:1,15:1), which led to the isolation of pure **1** (150 mg).

The original PE extract was subjected to CC (1.3 kg SiO_2, 200–300 mesh; PE/acetone 100:1, 80:1, 60:1, 50:1, 40:1, 30:1, 20:1, 15:1, 10:1, 8:1, 6:1, 4:1, and 2:1, then MeOH). Based on TLC analysis, a total of 15 crude fractions were obtained. *F13* (2 g) was subjected to CC (100 g SiO_2, 200–300 mesh; PE/acetone 30:1, 25:1, 20:1,15:1,10:1, 8:1, 5:1, 2:1, and 1:1), which resulted in the isolation of compound **4** (35 mg). Additionally, *F8* (4 g), which was purified by CC (100g SiO_2, 200–300 mesh; PE/acetone 80:1, 60:1, 50:1,40:1, 30:1, 20:1, 10:1, 5:1, and 1:1), afforded pure **2** (25 mg). Finally, compound **3** (20 mg) was obtained from *F10* (3 g) by CC (100 g SiO_2, 200–300 mesh; PE/acetone 50:1, 40:1, 30:1, 20:1, 10:1, 5:1, 2:1, and 1:1).

(1β,3β,6β,8β,10β)-6-Acetoxy-3-(angeloyloxy)-1,10-epoxy-8-hydroxyeremophil-7(11)-en-8,12α-olide (= *(1a*R,*3*S,*4*R,*4a*S,*5*S,*8a*S,*9a*S*)-5-Acetoxy-2,3,4,4a,5,7,8a,9-octahydro-8a-hydroxy-4,4a,6-trimethyl-7-oxo-1a*H-*oxireno[2′,3′:8,8a]naphtho[2,3-*b*]furan-3-yl (*E*)-2-Methylbut-2-enoate*; **1**). Yield: 150 mg. Colorless crystals. M.p. 190–191°. $[\alpha]_D^{20} = -90.0$ ($c = 1.0$, CH_2Cl_2). IR (KBr): 3341 (OH), 1754 (γ-lactone), 1727, 1705, 1237, 1219. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 420 (8, M^+), 378 (2, $[M - C_2H_2O]^+$), 360 (23, $[M - C_2H_2O - H_2O]^+$), 278 (30), 260 (100).

(1β,3β,6β,8β,10β)-3-(Angeloyloxy)-1,10-epoxy-6,8-dihydroxyeremophil-7(11)-en-8,12α-olide (= *(1a*R,*3*S,*4*R,*4a*S,*5*S,*8a*S,*9a*S*)-2,3,4,4a,5,7,8a,9-Octahydro-5,8a-dihydroxy-4,4a,6-trimethyl-7-oxo-1a*H-*oxireno[2′,3′:8,8a]naphtho[2,3-*b*]furan-3-yl (*E*)-2-Methylbut-2-enoate*; **2**). Yield: 25 mg. Colorless gum. IR (KBr): 3481 (OH), 1756 (γ-lactone), 1714, 1700, 1235. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*. EI-MS: 378 (10, M^+), 360 (9, $[M - H_2O]^+$), 278 (52, $[M - C_5H_8O_2]^+$), 260 (100, $[M - C_5H_8O_2 - H_2O]^+$).

(1β,3β,6β,8β,10β)-3-(Angeloyloxy)-1,10-epoxy-8-ethoxy-6-hydroxyeremophil-7(11)-en-8,12α-olide (= *(1a*R,*3*S,*4*R,*4a*S,*5*S,*8a*S,*9a*S*)-8a-Ethoxy-2,3,4,4a,5,7,8a,9-octahydro-5-hydroxy-4,4a,6-trimethyl-7-oxo-1a*H-*oxireno[2′,3′:8,8a]naphtho[2,3-*b*]furan-3-yl (*E*)-2-Methylbut-2-enoate*; **3**). Yield: 20 mg. Crystalline needles. M.p. 166–168°. IR (KBr): 3454 (OH), 1742 (γ-lactone), 1716, 1703, 1232. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*. EI-MS: 406 (18, M^+), 360 (59, $[M - C_2H_5OH]^+$), 306 (19, $[M - C_5H_8O_2]^+$), 277 (36, $[M - C_2H_5OH - C_5H_7O]^+$), 260 (100, $[M - C_2H_5OH - C_5H_8O_2]^+$).

(1β,3β,8β,10β)-3-(Angeloyloxy)-1,10-epoxy-8-hydroxyeremophil-7(11)-en-8,12α-olide (= *(1a*R,*3*S,*4*R,*4a*S,*5*S,*8a*S,*9a*S*)-2,3,4,4a,5,7,8a,9-Octahydro-8a-hydroxy-4,4a,6-trimethyl-7-oxo-1a*H-*oxireno[2′,3′:8,8a]naphtho[2,3-*b*]furan-3-yl (*E*)-2-Methylbut-2-enoate*; **4**). Yield: 35 mg. Colorless gum. IR (KBr): 3361 (OH), 1771 (γ-lactone), 1714, 1701, 1233. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*. EI-MS: 362 (4, M^+), 279 (55, $[M - C_5H_7O]^+$), 262 (51, $[M - C_5H_8O_2]^+$), 245 (100, $[M - C_5H_8O_2 - OH]^+$).

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