Yun-sen Li[1,2]
Zheng-tao Wang[2]
Mian Zhang[2]
Hui Zhou[1]
Ji-jun Chen[3]
Shi-de Luo[3]

New Eremophilane-Type Sesquiterpenes from *Ligularia lapathifolia*

Abstract

Seven new eremophilanolides were isolated from the roots and rhizomes of *Ligularia lapathifolia*. Their structures were established as 3β-angeloyloxy-8β*H*-eremophil-7(11)-ene-12,8α (14β,6α)-diolide, 3β-angeloyloxy-8β-hydroxyeremophil-7(11)-ene-12,8α(14β,6α)-diolide,3β-angeloyloxy-8β-methoxyeremophil-7(11)-ene-12,8α(14β,6α)-diolide,3β-angeloyloxy-8β-ethoxyeremophil-7(11)-ene-12, 8α (14β,6α)-diolide, 3β-angeloyloxy-10β-hydroxyeremophil-8(9),7(11)-diene-12,8(14β,6α)-diolide, 3β-angeloyloxy-8,12-expoy-12α-hydroxy-8β-methoxyeremophil-7(11)-en-14β,6α-olide and 3β-angeloyloxyeremophilan-7,11-dien-14β,6α-olide, by means of spectroscopic analyses. Moreover, application of a photooxygenation reaction on **7** resulted in the generation of **2** with an α,β-unsaturated γ-lactone moiety. This biomimetic transformation supports a biogenetic pathway proposed for **2**.

Key words
Ligularia lapathifolia · Asteraceae · eremophilane-type sesquiterpenes · photooxygenation

Introduction

The genus *Ligularia* (Compositae) contains more than 110 species occurring in China, of which about 40 species have been used as traditional Chinese herbs. *Ligularia lapathifolia* (Franch.) Hand.-Mazz. is mainly distributed in the south-west of China and its roots and rhizomes have been used to treat cough and inflammation by local inhabitants for a long time [1]. In our continuing chemical study on medicinal plants of the genus *Ligularia* [2], [3], seven new eremophilane-type sesquiterpenes were obtained from the EtOH extract of the roots and rhizomes of *Ligularia lapathifolia* (Franch.) Hand.-Mazz. In this paper, the structural elucidations of the compounds **1** – **7** are reported.

Material and Methods

General

Column chromatography (CC): silica gel, 200 – 300 mesh. TLC: precoated silica GF_{254} plates: detection at 254 nm, and by ceric sulfate reagent. Optical rotations: JASCO DEP-370 polarimeter. All melting points were obtained on a Koffler apparatus and are uncorrected. IR spectra were obtained on a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker AM-400 or DRX-500 instrument with TMS as internal standard and $CDCl_3$ or C_5D_5N as solvents. ^{1}H-NMR, ^{1}H-^{1}H COSY, and NOESY spectra were measured at 400.13 or 500.13 MHz; ^{13}C-NMR and DEPT spectra were recorded at 100.6 MHz; the HMBC spectrum was obtained at 500.13 MHz/125.8 MHz. ^{13}C-

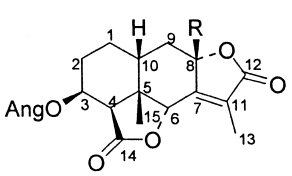
Affiliation
[1] Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, P. R. China
[2] Department of Pharmacognosy, China Pharmaceutical University, Nanjing, P. R. China
[3] Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, P. R. China

Correspondence
Dr. Yun-sen Li · State Key Laboratory of Drug Research · Shanghai Institute of Materia Medica · Chinese Academy of Sciences · 555 Zu Chong Zhi Road · Zhangjiang Hi-Tech Park · Shanghai 201203 · People's Republic of China · Phone: +86-21-50806600 ext 3301 · E-mail: sinodlsia@yahoo.com

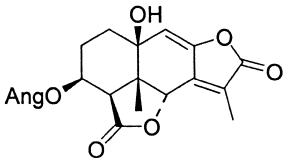
Funding
This research was financially supported by the Natural Science Foundation (NSFC) for outstanding young scientists for Wang Z. T. (39825129)

Received July 28, 2003 · **Accepted** December 12, 2003

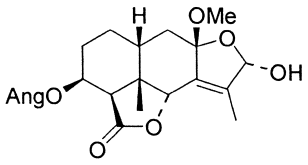
Bibliography
Planta Med 2004; 70: 239–243 · © Georg Thieme Verlag Stuttgart · New York · ISSN 0032-0943 · DOI 10.1055/s-2004-815541



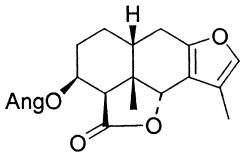
1 R = H
2 R=OH
3 R = OMe
4 R = OEt



5



6



7

NMR assignments were determined by ^{13}C-^{1}H COSY and HMQC spectra. The EIMS and HREIMS were carried out on a VG Auto Spec-3000 spectrometer at 70 eV.

Plant material

The roots and rhizomes of *L. lapathifolia* were collected from Dong Mountain (altitude: 2500 m), Lijiang Prefecture of Yunnan Province, P.R. China, in July 2000, and authenticated by Dr. Main Zhang. A voucher specimen (No. 990005-Li) is deposited in the Herbarium of China Pharmaceutical University.

Extraction and isolation

Dried and powdered roots and rhizomes of *L. lapathifolia* (11.0 kg) were extracted with EtOH (20 L×3) at room temperature. After removal of the solvent under vacuum, an extract of 900.0 g was obtained. The extract was suspended in H_2O and partitioned with $CHCl_3$ and *n*-BuOH successively. 200.0 g of the evaporated $CHCl_3$ part (total 400.0 g) were subjected to repeated chromatography on a silica gel column (200 – 300 mesh, 2.0 kg), eluted with $CHCl_3$ and a gradient of $(Me)_2CO$ in $CHCl_3$ (50 : 1 → 5 : 1). The fraction 1 [$CHCl_3$/$(Me)_2CO$, 30 : 1, 10g] was chromatographed on a silica gel column (200 – 300 mesh, 200 g), eluting with petroleum ether/EtOAc (10 : 1, each: 100 mL) to afford **7** (200 mg, R_f: 0.3). The fraction 5 [$CHCl_3$/$(Me)_2CO$, 20 : 1, 20 g] was chromatographed on a silica gel column (200 – 300 mesh, 400 g), eluting with petroleum ether/EtOAc (5 : 1, each: 100 mL) to furnish fr. 5.3 (1.0 g) and fr. 5.5 (300 mg). Fraction 5.3 was further purified by crystallization from petroleum ether-EtOAc, 5 : 1, to afford **6** (15 mg). After purification (2×) by CC over silica gel 20 (400 mesh) with petroleum-EtOAc (5 : 1), fr. 5.5 afforded **5** (20 mg, R_f: 0.40) and **2** (25 mg, R_f: 0.30). Fraction 3 [$CHCl_3$/$(Me)_2CO$, 10 : 1, 20 g] was chromatographed over a silica gel column (200 – 300 mesh, 600 g) with $CHCl_3$/$(Me)_2CO$ (20 : 1 to 5 : 1, each: 150 mL) to furnish fr. 3.3 (3.0 g) fr. 3.5 (0.5 g) and fr. 3.6 (3.0 g). Fraction 3.4 was further purified by crystallization from petroleum ether-EtOAc, 5 : 1, to afford **1** (50 mg). After purification (2×) by CC over 50 g Sephadex LH-20 with Me_2 CO, fr. 3.5 afforded **3** (10 mg). Fraction 3.6 was further purified on a Sephadex LH-20 column (50 g), eluted with $(Me)_2CO$ (each: 50 mL) to obtain **4** (50 mg).

Photosensitized autoxidation of compound 7

Methylene blue (20 mg) was added to a solution of **7** (10 mg) in methanol (40 mL), and the resulting solution was irradiated with a 200W incandescent lamp under an oxygen atmosphere at 25 °C for 4 h. The residue obtained after removal of the methanol under reduced pressure, was chromatographed on a column of silica gel (10 g). Elution with petroleum ether-ether (5 : 1) gave a product (5 mg) which was found to be identical with **2** (TLC, IR, MS and NMR).

3β-Angeloyloxy-8βH-eremophil-7(11)-ene-12,8α(14β,6α)-diolide (**1**)*:* Amorphous powder, m.p. 193 – 194 °C; $[\alpha]_D^{25}$: + 128.8° (*c* 0.41, $CHCl_3$); IR (KBr): ν_{max} = 2954, 1800, 1767, 1714, 1455, 1389, 1355, 1293, 1225, 1137, 1041, 975 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR spectral data, see Tables **1** and **2**; HR-EIMS: found: 360.3915 [M^+] (calcd. for $C_{20}H_{24}O_6$: 360.3920,); EIMS: *m/z* (%) = 360 $[M]^+$, 260 [M – angelic acid]$^+$ (100), 242 (11), 231 (24), 215 (33), 185 (23), 175 (25), 161 (22), 149 (22), 131 (28), 121 (53), 105 (65), 100 [angelic acid]$^+$ (63), 91 (75), 83 (35), 77 (67), 67 (78).

3β-Angeloyloxy-8β-hydroxyeremophil-7(11)-ene-12,8α(14β,6α)-diolide (**2**)*:* Amorphous powder, m.p. 201 – 202 °C; $[\alpha]_D^{25}$: + 136.6° (*c* 0.80, $CHCl_3$); IR (KBr): ν_{max} = 3321, 2949, 1783, 1727, 1645, 1387, 1352, 1308, 1234, 1153, 1086, 972, 929 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR spectral data, see Tables **1** and **2**; HR-EIMS: found: 376.4023 [M^+] (calcd. for $C_{20}H_{24}O_7$: 376.4026); EIMS: *m/z* (%) = 376 $[M]^+$ (23), 276 [M – angelic acid]$^+$ (83), 258 (27), 248 (20), 231 (35), 220 (23), 203 (21), 187 (16), 175 (23), 162 (39), 149 (24), 134 (36), 121 (26), 105 (58), 100 (33), 91 (72), 83 (100), 77 (56), 67 (32).

3β-Angeloyloxy-8β-methoxyeremophil-7(11)-ene-12,8α(14β,6α)-diolide (**3**)*:* Amorphous powder, m.p. 180 – 181 °C; $[\alpha]_D^{25}$: + 71.6° (*c* 0.54, $CHCl_3$); IR (KBr): ν_{max} = 2948, 1767, 1718, 1645, 1452, 1384, 1304, 1229, 1159, 1094, 970, 939 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR spectral data, see Tables **1** and **2**; HR-EIMS: found: 390.4297 [M^+] (calcd. for $C_{21}H_{26}O_7$: 390.4292); EIMS: *m/z* (%) = 390 $[M]^+$, 304 (22), 290 [M – angelic acid]$^+$ (100), 276 (10), 262 (61), 247 (47), 231 (99), 215 (19), 203 (62), 187 (31), 175 (43), 157 (42), 149 (48), 131 (27), 121 (55), 105 (53), 100 (24), 91 (89), 83 (24), 77 (75), 67 (79).

3β-Angeloyloxy-8β-ethoxyeremophil-7(11)-ene-12,8α(14β,6α)-diolide (**4**)*:* Amorphous powder, m.p. 178 – 179 °C; $[\alpha]_D^{25}$: + 79.6° (*c* 0.52, $CHCl_3$); ^{1}H-NMR and ^{13}C-NMR spectral data, see Tables **1** and **2**; HR-EIMS: found: 404.4565 [M^+] (calcd. for $C_{22}H_{28}O_7$: 404.4559); EIMS: *m/z* (%) = 404 $[M]^+$ (4), 304 [M – angelic acid]$^+$ (86), 276 (69), 759 (66), 247 (29), 231 (100), 215 (16), 203 (58), 185 (24), 175 (40), 157 (34), 149 (38), 131 (25), 121 (42), 100 (96), 91 (52), 83 (42), 67 (45).

3β-Angeloyloxy-10β-hydroxyeremophil-8(9),7(11)-diene-12,8(14β,6α)-diolide (**5**)*:* Amorphous powder, m.p. 198 – 199 °C; $[\alpha]_D^{25}$: + 211.2° (*c* 1.00, $CHCl_3$); IR (KBr): ν_{max} = 3387, 2947, 1724, 1707, 1663, 1459, 1383, 1347, 1316, 1229, 1148, 927 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR spectral data, see Tables **1** and **2**; HR-EIMS: found: 374.3902 [M^+] (calcd. for $C_{20}H_{22}O_7$: 374.3907); EIMS: *m/z* (%) = 374 $[M]^+$ (22), 356 [M – H_2O]$^+$ (22), 274 [M – angelic acid]$^+$ (67), 259 (26), 245 (25), 231 (13), 218 (14), 201 (19), 187 (16), 177

(32), 159 (10), 149 (12), 135 (17), 128 (11), 115 (17), 100 (52), 91 (27), 83 (88), 67 (32).

3β-Angeloyloxy-8,12-expoy-12α-hydroxy-8β-methoxyeremophil-7(11)-en-14β,6α-olide (**6**)*:* Amorphous powder, m.p. 214 – 215 °C; $[\alpha]_D^{25}$: +56.5° (*c* 0.67, $CHCl_3$); IR (KBr): ν_{max} = 3500, 2940, 1772, 1685, 1457, 1256, 1204, 1094, 1012, 936 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR spectral data, see Tables **1** and **2**; HR-EIMS: found: 392.4423 [M$^+$] (calcd. for $C_{21}H_{28}O_7$: 344.4001); EIMS: *m/z* (%) = 392 [M]$^+$ (6), 292 [M – angelic acid]$^+$ (37), 275 (9), 259 (18), 247 (9), 231 (40), 215 (6), 203 (17), 187 (10), 159 (13), 149 (31), 121 (22), 109 (14), 100 [angelic acid]$^+$ (45), 91 (61), 83 (94), 77 (46), 67 (44).

3β-Angeloyloxyeremophila-7,11-dien-14β,6α-olide (**7**)*:* Amorphous powder, m.p. 140 – 141 °C; $[\alpha]_D^{25}$: +47.4° (*c* 0.44, $CHCl_3$); IR (KBr): ν_{max} = 2946, 2867, 1774, 1714, 1644, 1562, 1456, 1382, 1350, 1232, 1141, 1083, 1041, 1009, 935 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR spectral data, see Tables **1** and **2**; HR-EIMS: found: 344.4008 [M$^+$] (calcd. for $C_{20}H_{24}O_5$: 344.4001); EIMS: *m/z* (%): 344 [M]$^+$ (54), 262 (55), 244 [M – angelic acid]$^+$ (62), 229 (9), 217 (35), 199 (26), 187 (37), 173 (23), 159 (53), 145 (35), 129 (25), 121 (56), 100 (34), 105 (26), 95 (58), 83 (85), 65 (37).

Results and Discussion

The molecular formula of **1** was established as C20H24O6 on the basis of HR-EIMS (*m/z* = 360.3915 [M]+). The IR spectrum showed absorptions at 1800, 1764 and 1714 cm^{-1} for three ester carbonyl groups. In its 1H and 13C-NMR spectra, there are signals for an angeloyloxy group (Tables **1** and **2**). This was also supported by the ion fragment peak at *m/z* = 260 [M – AngOH]+ in the EIMS. In addition to five carbons of the angeloyloxy moiety, there were 15 carbon signals in the ^{13}C-NMR and DEPT spectra (Table **2**), which include two lactone carbonyl (δ = 170.6 and 170.8), two double bond (δ = 155.7 and 127.0), three oxygenated methine (δ = 64.4, 77.9 and 83.8) and two methyl carbons (δ = 10.0 and 22.8). These observations and biogenetic considerations suggested that **1** was an eremophil-7(11)-ene-diolide sesquiterpene. Except for the difference caused by an extra angeloyloxy group in **1**, the ^{1}H- and ^{13}C-NMR data of **1** were nearly superimposable on those of 8β-hydroeremophil-7(11)-ene-12,8α(14,6α)-diolide [4], indicating **1** was an angeloyloxy derivative of this compound. Along with the NMR signals for C-3 at δ_C = 64.4 and δ_H = 5.45 (1H, br s) and HMBC correlations [H-3/δ_C = 164.3 (C-1′)], the angeloyloxy group was defined as being attached to C-3. The stereochemistry of H-3 was assigned as equatorial (α-orientation) due to its small coupling values with three vicinal protons (one axial and two equatorial), which was further validated by the evident cross-peaks between Me-15 and Me-4′ in the NOESY spectrum. Therefore, the structure of **1** was unequivocally determined as 3β-angloyloxy-8β-hydroeremophil-7(11)-ene-12,8α(14,6α)-diolide.

The molecular formulas of **2**, **3** and **4** were assigned as $C_{20}H_{24}O_7$, $C_{20}H_{26}O_7$ and $C_{22}H_{28}O_7$, respectively, from their HR-EIMS. These three compounds have the same 3β-angloyloxyeremophil-7(11)-ene-12,8α(14,6α)-diolide sesquiterpene skeleton as **1**, based on the fact that most of their ^{1}H-NMR and ^{13}C-NMR data were nearly identical with those of **1**. An additional hydroxy group connecting with C-8 in 2 instead of the corresponding hydrogen atom in **1** was demonstrated by the presence of a quaternary carbon (IR, 3321 cm^{-1}; δ_C-8 = 103.6) and HMBC correlations (H-10β/C-8; and H-6β/C-8). The relative configuration of the hydroxy functionality at C-8 was assumed to be β since no evident carbon signal difference appears except for C-8 signals shifted downfield by 25.7 ppm compared to those of **1**. Thus, 2 was determined as 3β-angeloyloxy-8β-hydroxyeremophil-7(11)-ene-12,8α(14β,6α)-diolide (**2**).

The signals for an extra methoxy group (δ_C = 50.6 and δ_H = 3.15 3H, s) and a quaternary carbon (δ_C = 105.1) were present in the

Table **1** ^{1}H-NMR data of compounds **1** – **7** (500 MHz, *J* values in Hz in parentheses)

Proton (s)	*1*	*2*	*3*	*4*	*5*	*6*	*7*
1	2.00 m, 1.97 m	1.90 m, 1.76 m	1.92 m, 1.76 m	1.92 m, 1.72 m	1.94 m, 1.70 m	1.95 m, 1.79 m	2.05 m, 1.85 m
2	1.97 m, 1.22 m	1.44 m, 1.39 m	1.34 m, 1.24 m	1.36 m, 1.23 m	1.67 m, 1.53 m	1.39 m, 1.28 m	1.50 m, 1.23 m
3	5.45 br s	5.40 br s	5.45 br s	5.44 br s	5.59 br s	5.42 br s	5.52 br s
4α	2.34 br s	2.54 br s	2.54 br s	2.52 br s	2.18 m	2.59 d (2.5)	2.53 br s
6β	4.91 q (1.8)	5.00 q (1.8)	4.98 q (1.8)	5.01 q (1.8)	5.59 br s	4.70 br s	5.07 s
8	4.07 m						
9	1.72 m, 2.52 m	1.60 m, 2.24 m	1.64 m, 2.27 m	1.62 m, 2.27 m	5.98 br s	1.64 m, 2.18 m	2.68 m
10β	2.60 m	2.30 m	2.31 m	2.29 m		2.18 m	2.36 m
12						5.93 s	7.07 s
13	2.13 br s	1.97br s	1.95 br s	1.98 br s	2.03 br s	2.12 s	2.01 s
15	1.55 s	1.40 s	1.34 s	1.46 s	1.63 s	1.38 s	1.50 s
OMe			3.15 s			3.08 s	
OEt				1.15 t, 3.27 q			
OAng							
3′	6.06 dq	6.03 dq	6.05 dq	6.16 dq	6.09 dq	6.01 dq	6.06 dq
4′	1.83 br s	1.97 dq	1.96 dq	1.98 dq	1.93 dq	1.93 dq	2.00 dq
5′	1.95 dq	1.80 br s	1.82 br s	1.85 br s	1.83 br s	1.83 br s	1.88 br s

All compounds were measured in $CDCl_3$ except for compound **5**. Compound **5** was measured in C_5D_5N-MeOH-d_6.

Table **2** ^{13}C-NMR data of compounds **1** – **7** (125 MHz, measured in $CDCl_3$)

Carbon	*Compound*						
	1	*2*	*3*	*4*	*5*[a]	*6*	*7*
1	21.2	20.5	21.3	21.6	28.6	21.0	22.3
2	24.8	25.0	21.5	24.9	31.9	25.0	22.7
3	64.4	64.7	64.7	64.4	64.7	64.9	65.2
4	42.6	42.4	42.4	42.4	47.6	42.6	43.7
5	44.5	44.2	43.9	44.0	46.8	43.4	40.9
6	83.8	83.4	82.0	83.3	78.9	84.5	82.4
7	155.7	152.5	152.2	152.7	149.3	134.4	120.1
8	87.9	103.6	105.1	105.4	142.4	112.7	150.6
9	34.9	35.4	35.1	34.8	110.7	34.9	25.1
10	33.8	34.8	32.8	32.8	72.3	35.6	37.0
11	127.0	127.0	128.4	128.0	127.7	131.1	114.5
12	170.6	171.6	170.3	170.4	170.2	112.0	138.9
13	10.0	8.8	9.0	8.9	9.6	10.2	8.2
14	170.8	172.2	170.3	172.8	172.6	172.7	172.6
15	22.8	23.0	26.6	26.7	18.1	22.9	23.3
OMe			50.6			49.2	
OEt				59.0			
OAng				15.0			
1′	164.3	166.8	168.3	168.4	166.7	166.8	166.8
2′	139.8	139.5	136.9	140.6	139.3	139.2	138.9
3′	129.5	127.4	129.5	129.4	128.2	127.2	127.5
4′	20.7	20.6	20.7	20.6	21.0	20.6	20.7
5′	15.7	15.7	15.1	15.8	16.0	15.6	15.7

[a] Compound **5** was measured in C_5D_5N-MeOH-d_6.

NMR spectra of **3**. These data showed that **3** was a methoxy derivative of **2**. The position of the methoxy group was determined as attaching to C-8 as supported by HMBC correlations (MeO/C-8; H-10β/C-8; H-6β/C-8) and NOESY cross-peaks (MeO/H-10β; MeO/H-6β). Therefore, the structure of **3** was defined as 3β-angeloyloxy-8β-methoxyeremophil-7(11)-ene-12,8α(14β,6α)-diolide.

Besides the signals for the 3β-angloyloxyeremophil-7(11)-ene-12,8α(14,6α)-diolide moiety, the ^{1}H- and ^{13}C-NMR spectra of **4** disclosed the presence of an additional ethoxy group [δ_C = 59.0 and 15.0; δ_H = 3.27 q (2H) and 1.15 t (3H)]. Its structure was unequivocally determined as 3β-angeloyloxy-8β-ethoxyeremophil-7(11)-ene-12,8α(14,6α)-diolide by HMQC, HMBC and NOESY spectroscopic analysis.

The molecular formula $C_{20}H_{22}O_7$ for compound **5** was determined by the HR-EIMS. The IR spectrum revealed the absorptions for hydroxy (3387 cm^{-1}) and three carbonyl groups (1724, 1707, 1663 cm^{-1}). Besides with the signals for an angeloyloxy group, its ^{1}H- and ^{13}C-NMR spectra showed the presence of two lactone carbonyls ($\delta_{C\text{-}12}$ = 170.2 and $\delta_{C\text{-}14}$ = 172.6), two double bonds ($\delta_{C\text{-}7}$ = 149.3 and $\delta_{C\text{-}11}$ = 127.7; $\delta_{C\text{-}8}$ = 142.4 and $\delta_{C\text{-}9}$ = 110.7, $\delta_{H\text{-}9}$ = 5.98 s), two oxygenated methine groups ($\delta_{C\text{-}6}$ = 78.9 and $\delta_{C\text{-}3}$ = 64.9; $\delta_{H\text{-}6\beta}$ = 5.59 and $\delta_{H\text{-}3\alpha}$ = 5.59), an oxygenated quaternary carbon ($\delta_{C\text{-}10}$ = 72.3), and two tertiary methyls. From these findings and biogenetic considerations, the structure of **5** was deduced as an eremophil-7(11)-ene-diolide sesquiterpene. Except for the difference caused by an extra angeloyloxy group in **5**, the ^{1}H- and ^{13}C-NMR data of **5** were nearly superimposable on those of 10β-hydroxyeremophil-7(11)-ene-12,8α(14,6α)-diolide [4], indicating that **5** was an angeloyloxy derivative of this compound. Along with the NMR signals for C-3 at δ_C = 64.4 and δ_H = 5.59 (1H, br s) and HMBC correlations [H-3/δ_C = 166.7 (C-1′)], it was deduced that the angeloyloxy group was attached to C-3. Thus, the structure of **5** was unequivocally determined as 3β-angeloyloxyl-10β-hydroxyeremophil-7(11)-ene-12,8α(14, 6α)-diolide.

Compound **6** was assigned the molecular formula as $C_{21}H_{28}O_7$ by HR-EIMS analysis (*m/z* = 392.4423 [M]$^+$). Its IR spectrum showed the presence of hydroxy (3500 cm^{-1}) and two carbonyl groups (1772, 1685 cm^{-1}). Its ^{1}H- and ^{13}C-NMR spectra displayed the signals for a methoxy (δ_C = 49.2 and δ_H = 3.08 3H) and an angeloyloxy group. While its ^{13}C-NMR data compared with those of **5**, the disappearance of the 8(9)-ene and 12-carbonyl group and the presence of two oxygenated carbons ($\delta_{C\text{-}8}$ = 112.7 and $\delta_{C\text{-}12}$ = 112.0) indicated that a hydroxy and a methoxy group were connected with the 12 and 8 positions, respectively. In its NOESY spectrum, strong correlations were observed between H-10β (δ = 2.18, m) and δ = 3.08 (3H, s), H-12 (δ = 5.93, s) and δ = 3.08 (3H, s), suggesting the methoxy group and H-12 have the β-orientation. Thus, **6** was identified as 3β-angeloyloxy-8,12-expoy-12α-hydroxy-8β-methoxyeremophil-7(11)-ene-14,6α-olide.

Compound **7** was assigned the molecular formula as $C_{20}H_{24}O_5$ by HR-EIMS analysis (*m/z* = 344.4008 [M]$^+$). In the ^{1}H- and ^{13}C-NMR spectra the characteristic signals for a furan ring [δ_C = 120.1 (C-7), 150.6 (C-8), 114.5 (C-11), 138.9 (C-12);

δ_H = 7.07, (H-12, 1H, s)] and an angeloyloxy moiety displayed, suggesting **7** to be a furanosesquiterpene. By comparison of its NMR data with those of a known sesquiterpene, furanoeremophilan-14β,6α-olide [6], compound **7** was identified as 3β-angeloyloxyeremophila-7,11-dien-14β,6α-olide due to the presence of an oxygenated methine carbon signal at $\delta_{C\text{-}3}$ = 65.2 in **7** instead of the methylene carbon signal at $\delta_{C\text{-}3}$ = 23.3 in **10**.

Due to the co-occurrence of compounds **2** and **7** within the plant, it was proposed that the biosynthesis of the α,β-unsaturated five-membered lactone ring of **2** proceeds via the intermediacy of a furan ring. This conversion was demonstrated through a biomimetic photochemical reaction [7].

Oxidation of furans with singlet oxygen has had widespread application in organic synthesis [7], [8], [9]. This oxidation, which take place through the Diels-Alder type of cycloaddition, to form an initial 1,4-endoperoxide leads to the α,β-unsaturated five-membered lactonic ring [7]. Using **7** as a precursor, **2** was stereoselectively synthesized through photochemical transformation in acetone solution. This conversion further supported the proposed biogenetic pathway.

References

[1] Yunnan Medicinal Materials Company Ed. Items of Traditional Chinese Medicinal Material Resources in Yunnan Province. Science Press, Beijing: 1993: p 577

[2] Li YS, Luo SD, Zhang M, Chen JJ, Wang ZT. Constituents of *Ligularia vellerea* (Franch) Hand-Mazz. Zhongguo Zhongyao Zazhi 2001; 26: 835 – 7

[3] Li YS, Wang ZT, Zhang M, Chen JJ. Study on identification of terpenes from *Ligularia kanaitzensis*. Zhongguo Yaoxue Zazhi 2002; 37: 12 – 4

[4] Saito I, Kuo YH, Matsuura T. Tetrahedron letters. 1986; 27: 2757 – 60

[5] Zhao Y, Jia ZJ, Peng H. Eight new eremophilane derivatives from the roots of *Ligularia przewalskii*. J Nat Prod 1995; 58: 1358 – 64

[6] Cheng HM, Cai MS, Jia ZJ. Sesquiterpenes from the roots of *Ligularia intermedia*. Phytochemistry 1997; 45: 1441 – 4

[7] Ishizaki Y, Tanahashi Y, Tsuyuki T, Takeyoshi T, Tori K. Furanoerephilan-14,6α-olide. A new furanosesquiterpene lactone of an eremophilane-type from *Ligularia hodgsoni* Hook, f. Bull Chem Soc 1979; 52: 1182 – 6

[8] Schap Ap, Zaklika KA, Kaskar B. Mechanisms of photooxygenation. 2. Formation of 1, 2- dioxetanes via 9, 10-dicyanoanthrancene-electron-transfer process. Journal of the American Chemistry Society 1980; 102: 389 – 391

[9] Foote CS, Wuesthoff MT, Wexler OS. Photosensitized oxygenation of alkyl-substituted furans. Tetrahedron 1967; 23: 2583

[10] Wasserman HH, Ives JL. Syntheses photochimiques de dioxa-1,6-spiro [4,5]decanes pheromones de *Pharavespula vulgaris* L. Tetrahedron 1981; 37: 1875 – 1882