CL-140745 Received: August 6, 2014 | Accepted: August 16, 2014 | Web Released: August 22, 2014

Isolation and Structure of Three Bislactones, Eremopetasitenin B_4 and Eremofarfugins F and G, from *Ligularia przewalskii* and Revision of the Structure of an Epoxy-lactone Isolated from *Ligularia intermedia*

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Three bislactones belonging to the eremophilane type were isolated from *Ligularia przewalskii* (Asteraceae): an epoxy-lactone (eremopetasitenin B_4) identical to the previously isolated lactone, whose structure was revised in this work; a new enol-lactone (eremofarfugin F); and its diastereoisomer at C-11 (eremofarfugin G). This was identical to the reported lactone, whose stereochemistry was left undetermined. The stereochemistry along with the NMR data of this diastereoisomer was assigned in this study.

Plants belonging to the genus *Ligularia* (Asteraceae) are widely distributed in the Yunnan and Sichuan Province of China.[1] We have been involved in the investigation of the chemical constituents as well as DNA sequences of these plants and have published the results,[2–6] including a review.[7] We collected some samples of *Ligularia przewalskii* (Maxim.) Diels in August 2010. There are some reports on the chemistry of this plant, regarding isolation of eremophilane sesquiterpenoids and other aromatic compounds.[8–11] This paper reports the structure of three eremophilane-type bislactones **1**–**3** found in the extract of *L. przewalskii* (Figure 1).[12] Lactones bearing an epoxide ring at C-7 and C-8 or enol-lactones with a double bond at C-7 and C-8 have been isolated from Asteraceae plants.[2–6,13–18] The configuration at C-11 or the epoxide ring has been a difficult point for structure elucidation, because sometimes detection of NOE signals relating these partial structures is not easy. Therefore, the configurations at C-11 of some compounds have been left undetermined.[17,18] There are papers investigating the photo-oxidation reactions of furanoeremophilanes to isolate such compounds.[19,20] We have previously proposed plausible biosynthetic pathways from furans to epoxy- or enol-lactones, isolated from *Ligularia* plants.[13b] Bakkanes and other related sesquiterpenoids can also be explained by similar discussions.[2b,21]

In this study, we obtained three bislactones **1**–**3** from *L. przewalskii* and determined their structures. Compound **1** was identical to the lactone isolated from *Ligularia intermedia* in 1997, which was assigned the wrong structure.[18] Here, we provide evidence to revise the proposed structure to **1** (Figure 1).[22] Compound **2**[23] is a new enol-lactone and compound **3** was previously reported by Chen et al. in 1997; however, the stereochemistry at C-11 was not assigned.[18] We concluded that this compound has structure **3**.[24]

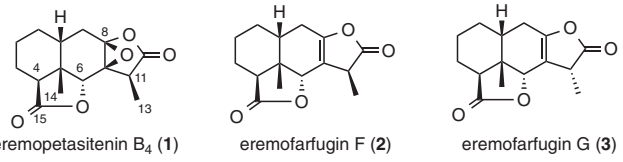


Figure 1. Compounds **1**–**3** isolated in this work.

Compound **1** exhibited a quasi-molecular ion peak at m/z 279 and the molecular formula was determined to be $C_{15}H_{18}O_5$ by HRMS-CI. The IR spectrum showed the presence of an enol- or epoxy-lactone (1817 cm^{-1})[2a,3,5,6] and a γ-lactone (1784 cm^{-1}), which were supported by ^{13}C NMR ($CDCl_3$) signals (δ 174.84 and 174.76). The ^{13}C NMR spectrum indicated the presence of two methyl, four methylene, four methine, and five quaternary carbon atoms (Table 1). The HMBC spectrum indicated that the singlet methyl group correlated to a quaternary carbon (δ 39.8) and three methines, one of which was an oxymethine (δ 77.7). The doublet methyl had correlations to a carbonyl (δ 174.76), an oxygenated quaternary carbon (δ 61.5), and a methine (δ 38.5). COSY (C_6D_6) correlations were also detected as shown in Figure 2 and this compound was deduced to have an eremophilane skeleton. Further long-range correlations between H-6 and C-8 and C-15, and between H_2-9 and C-1, C-7, and C-8 were detected and the planar structure was assigned to 7,8-epoxyeremophilane-(12,8;15,6)-diolide.

The stereochemistry was determined by NOESY. The A/B *cis* relationship was shown by the NOE between H_3-14 and

Table 1. NMR data of compound **1**

No.	**1** (in C_6D_6)		**1** (in $CDCl_3$)		**1′** (lit.[a]; in $CDCl_3$)	
	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H
1	23.8	α 0.53–0.57 (m)	24.1	—[b]	24.1	α 1.83
	—	β 1.05–1.09 (m)	—	—[b]	—	β 1.4
2	19.6	α 0.67–0.73 (m)	19.9	—[b]	19.8	α 1.76
	—	β 1.00–1.05 (m)	—	—[b]	—	β 1.45
3	19.1	α 1.50–1.55 (m)	18.9	—[b]	18.9	α 1.46
	—	β 0.97–1.03 (m)	—	—[b]	—	β 1.9
4	39.0	1.19–1.22 (m)	39.7	2.21	39.5	2.20
5	39.2	—	39.8	—	39.8	—
6	77.2	3.70 (s)	77.7	4.34	77.7	4.33
7	61.6	—	61.5	—	61.5	—
8	87.0	—	87.1	—	87.1	—
9	21.9	α 1.46 (dd, J = 15.1, 11.0 Hz)	22.2	2.30	22.1	α 2.40
	—	β 1.65 (dd, J = 15.1, 6.4 Hz)	—	2.46	—	β 2.29
10	32.6	1.22–1.25 (m)	32.7	—[b]	32.7	1.96
11	38.8	2.61 (q, J = 7.2 Hz)	38.5	3.01	38.5	3.02
12	174.6	—	174.84	—	174.9	—
13	10.3	1.23 (d, J = 7.2 Hz)	10.2	1.42	10.2	1.40
14	19.9	0.50 (s)	20.4	1.19	20.3	1.19
15	173.8	—	174.76	—	174.9	—

[a]ref 18. [b]Signals not assigned.

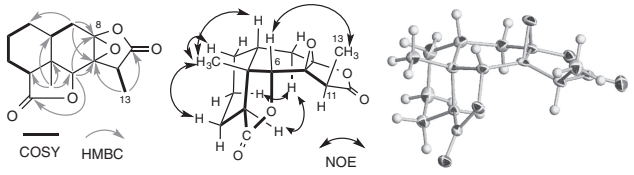


Figure 2. Selected 2D correlations and X-ray structure of **1**.

H-10. The NOE correlations of H_3-14 with H-1β and H-3β indicated that this compound had a steroidal conformation, as shown in Figure 2. Therefore, H-6 should be pseudo β-axial, and the NOE between H_3-13 and H-6 clearly indicated that the methyl group at C-11 was located in the β-orientation. According to our proposal regarding the biosynthesis of epoxy-lactones (vide infra), the epoxide oxygen and the methyl group at C-11 should be oriented on the same side. Thus, the structure of compound **1** was proposed, as depicted in Figure 1 and it was named eremopetasitenin B_4.

In 1997, Chen et al. reported the structure of a bislactone isolated from *L. intermedia* as the 7α,8α-epoxy diastereoisomer of compound **1** (=**1′**).[18] However, the ^{1}H and ^{13}C NMR data in $CDCl_3$ were identical to ours (Table 1). Fortunately, compound **1** crystallized and X-ray analysis was carried out to demonstrate the stereochemistry unambiguously.[25] The results are shown in Figure 2. Apparently, the epoxide oxygen and the methyl group at C-11 are on the upper side of the molecule. Therefore, our proposal for the biosynthesis of these compounds (vide infra) is more likely applied to this compound. The structure of this lactone reported by Chen et al. should therefore be revised.[18]

Compound **2** showed a quasi-molecular ion peak at m/z 263 and the molecular formula was determined to be $C_{15}H_{18}O_4$ by HRMS. The IR spectrum indicated the presence of either an enol-lactone or an epoxy-lactone (1798 cm^{-1})[2a,3,5,6] and a γ-lactone (1778 cm^{-1}) group. ^{13}C NMR indicated the presence of two methyl, four methylene, four methine, and five quaternary carbon atoms (including two carbonyl and two alkene carbons) (Table 2). COSY and HMBC correlations suggested the planar structure to be eremophilanediolide, as shown in Figure 3. As in the case of compound **1**, NOESY indicated the stereochemistry to have the A/B *cis* ring system, because the NOE between H_3-14 and H-10 was observed. The γ-lactone has 4α-H and 6β-H, because the NOE between H-4α and H-9α was observed. The stereochemistry at C-11 was established to be 11α-H, because the NOE between H_3-13 and H-6 was observed. This compound was established as 11αH-eremophil-7-ene-(12,8;15,6α)-diolide and was named eremofarfugin F.

The molecular formula of compound **3** was the same as that of compound **2**. The IR and NMR spectra were similar to those of compound **2**, and 2D NMR indicated that it had the same planar structure as compound **2** (Figure 4). Because the NOE between H-11 and H-6 was detected, the configuration at C-11 was determined to have 11β-H, establishing that compound **3** was the diastereoisomer of compound **2** at C-11. As the NMR data (in $(CD_3)_2CO$) seem to be the same as those described in the literature,[18] compound **3** was concluded to be identical to Chen's lactone reported in 1997 (Tables 2 and 3).[18] However, the stereochemistry at C-11 was not assigned in their report. Thus, this compound was established as 11βH-eremophil-7-ene-(12,8;15,6α)-diolide and was named eremofarfugin G.

Table 2. NMR data of compound **2**

No.	**2** (in C_6D_6)		**2** (in $(CD_3)_2CO$)	
	^{13}C	^{1}H	^{13}C	^{1}H
1	24.6	α 0.67 (m)	25.4	α 1.46 (m)
	—	β 1.15 (m)	—	β 1.91 (m)
2	20.3	α 0.79 (m)	21.1	1.62 (m)
	—	β 1.14 (m)	—	1.72 (m)
3	18.9	α 1.60 (m)	19.7	α 1.77 (m)
	—	β 1.12 (m)	—	β 1.51 (qd, J = 12.2, 4.2 Hz)
4	40.9	1.44 (dd, J = 12.4, 3.3 Hz)	41.5	2.52 (dd, J = 12.2, 3.2 Hz)
5	40.3	—	41.6	—
6	78.8	4.07 (s)	80.2	4.80 (br s)
7	112.2	—	113.2	—
8	150.5	—	151.8	—
9	22.2	1.59 (m)	22.8	2.35 (m)
	—	1.59 (m)	—	2.49 (m)
10	35.5	1.27 (m)	36.3	2.31 (m)
11	38.3	2.83 (m)	38.9	3.33 (m)
12	177.9	—	179.2	—
13	13.6	1.01 (d, J = 7.5 Hz)	14.0	1.33 (d, J = 7.6 Hz)
14	19.4	0.63 (s)	19.5	1.21 (s)
15	174.6	—	176.1	—

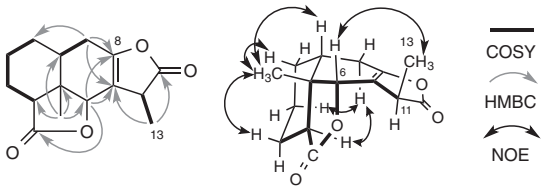


Figure 3. Selected 2D correlations of compound **2**.

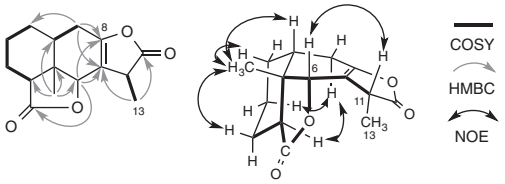


Figure 4. Selected 2D correlations of compound **3**.

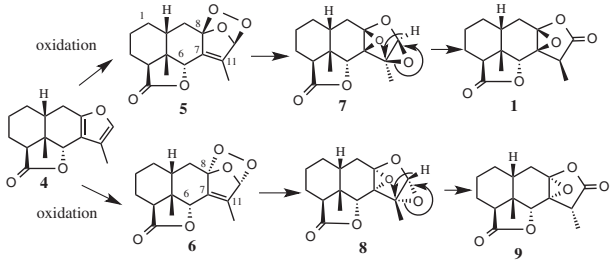
Plausible biosynthetic pathways to compound **1** are shown in Scheme 1. Endoperoxides **5** and **6**, derived from furanoeremophilane **4**, rearrange to **7** and **8**, respectively. One of the two epoxides opens and rearranges to ketone **1** and **9**. Therefore, both the epoxide oxygen and the methyl group at C-11 should always be oriented in the same direction. Compounds **1** and **9** are diastereoisomers concerning the stereochemistry at C-11 and the epoxide. Compound **9** has an α-methyl group at C-11, which has not been isolated yet. Compounds **2** and **3** may be formed by oxidation at C-11 and C-12 of **4**, followed by rearrangement.

In conclusion, we have isolated three bislactones from *Ligularia przewalskii* collected from the Sichuan Province of China. Compound **1** was previously assigned as an α-epoxide;[18] however, we have revised it to a β-epoxide on the basis of NMR and X-ray data and by considering the biosynthetic pathways (Scheme 1). A detailed study on the chemistry and diversity of *L. przewalskii* will be published in due course.

Table 3. NMR data of compound **3**

No.	**3** (in C_6D_6)		**3** (in $(CD_3)_2CO$)		**3** (lit.[a]; in $(CD_3)_2CO$)	
	^{13}C	^1H	^{13}C	^1H	^{13}C[b]	^1H
1	24.7	α 0.69 (m)	25.4	α 1.47 (m)	25.4	1.20–2.60 (m)
		— β 1.18 (m)		— β 1.92 (m)		— 1.20–2.60 (m)
2	20.2	α 0.79 (m)	21.0	1.66 (m)	21.0	1.20–2.60 (m)
		— β 1.15 (m)		— 1.72 (m)		— 1.20–2.60 (m)
3	18.9	α 1.59 (m)	19.7	α 1.77 (m)	19.8	1.20–2.60 (m)
		— β 1.14 (m)		— β 1.50 (qd, $J = 12.2$, 4.1 Hz)		— —
4	40.9	1.51 (m)	41.7	2.57 (dd, $J = 12.2$, 3.2 Hz)	41.6	1.20–2.60 (m)
5	40.7	—	41.9	—	41.6	—
6	81.1	3.88 (s)	82.6	4.84 (br s)	82.6	4.85 (d, $J = 1.2$ Hz)
7	111.7	—	112.8	—	112.6	—
8	151.2	—	152.6	—	152.4	—
9	22.1	1.59 (m)	22.8	α 2.32 (m)	22.8	1.20–2.60 (m)
		— 1.59 (m)		— β 2.56 (m)		— 1.20–2.60 (m)
10	35.7	1.27 (m)	36.8	2.31 (m)	36.7	1.20–2.60 (m)
11	40.0	2.59 (m)	40.5	3.43 (m)	40.5	3.45 (m)
12	177.7	—	179.1	—	178.9	—
13	15.5	1.25 (d, $J = 7.6$ Hz)	15.7	1.29 (d, $J = 7.6$ Hz)	15.7	1.30 (d, $J = 7.2$ Hz)
14	19.3	0.63 (s)	19.6	1.19 (s)	19.6	1.20 (s)
15	174.4	—	176.0	—	175.9	—

[a]ref 18. [b]Original assignment incorrect and reassigned in this study.



Scheme 1. Plausible biosynthetic pathways to compound **1** and its diastereoisomer **9**.

We thank Mrs. Guowen Hu of the Kunming Institute of Botany for research coordination. This work was partly supported by a Grant-in-Aid for Scientific Research from JSPS (Nos. 21404009 and 25303010).

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12 Plant material: Sample was collected in 2010 in Sichuan Province, China and voucher specimen No. 201076 was deposited in the Herbarium of Kunming Institute of Botany. The sample was identified by X. Gong, one of the authors. The sample (12.4 g) was extracted with EtOAc and concentrated to afford an extract (713.6 mg), which was separated by silica gel column chromatography and HPLC to give **1** (57.8 mg), **2** (1.7 mg), and **3** (4.2 mg).

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22 Compound **1**: Mp: >200 °C (decomp); $[\alpha]_D^{24}$: −13.0 (*c* 0.43, EtOAc); FTIR (KBr): 1817, 1784 cm^{-1}; MS (CI, 70 eV): $m/z = 279$ $[M + H]^+$, 261, 233 (100), 205; HRMS-CI: m/z $[M + H]^+$ calcd for $C_{15}H_{19}O_5$: 279.1233; found: 279.1226.

23 Compound **2**: $[\alpha]_D^{17}$: −43.6 (*c* 0.17, EtOH); FTIR (KBr) 1798, 1778 cm^{-1}; MS (CI, 70 eV): $m/z = 263$ $[M + H]^+$, 235 (100), 218, 190; HRMS-CI: m/z $[M + H]^+$ calcd for $C_{15}H_{19}O_4$: 263.1283; found: 263.1283; CD (EtOH): $[\theta]$ +10600 (214 nm), +1700 (256 nm), −400 (310 nm).

24 Compound **3**: $[\alpha]_D^{18}$: −32.1 (*c* 0.30, EtOH); MS (CI, 70 eV): $m/z =$ 263 $[M + H]^+$ (100), 235, 218, 190; HRMS-CI: m/z $[M + H]^+$ calcd for $C_{15}H_{19}O_4$: 263.1283; found: 263.1283.

25 X-ray crystallographic analysis of compound **1**: All diagrams and calculations were performed using maXus (Bruker Nonius, Delft & MacScience, Japan). $C_{15}H_{19}O_5$ Mo Kα radiation, $\lambda = 0.71073$ Å, 3707 measured reflections, 3242 independent reflections, Program(s) used to refine the structure: SHELXL-97 (Sheldrick, 1997);[26] refinement of F^2, full-matrix least-squares refinement. Crystal data: triclinic, *P*1, $a = 6.935(3)$ Å, $b = 9.838(4)$ Å, $c = 10.245(4)$ Å, $\alpha =$ 74.720(5)°, $\beta = 89.138(5)$°, $\gamma = 84.052(4)$°, $R = 0.0389$. Crystallographic data for compound **1** have been deposited in Cambridge Crystallographic Data Center as supplementary publication number CCDC 982800. Copies of the data can be obtained, free of charge, via http://www.ccdc.cam.ac.uk/data_request/cif, or by mailing the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, U.K. (Fax: +44 1223 336033 or e-mail: data_request@ccdc.cam.ac.uk).

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