Regular Article

Eremophilanes from *Ligularia hookeri* Collected in China and Structural Revision of 3*β*-Acyloxyfuranoeremophilan-15,6-olide

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Chemical constituents of *Ligularia hookeri* (Asteraceae) collected in Yunnan and Sichuan Provinces in China were examined for the first time. Seven furanoeremophilanes, five of which were new, as well as known bisabolane- and eudesmane-type sesquiterpenoids, were isolated. Spectroscopic evidence indicates that the previously reported 3*β*-(2′-methylpropenoyloxy)furanoeremophilan-15,6*β*-olide should be revised to 3*β*-(2′-methylpropenoyloxy)furanoeremophilan-15,6*α*-olide.

Key words *Ligularia hookeri*; Asteraceae; eremophilane; sesquiterpene; structure elucidation; internal transcribed spacer

We have been analyzing both the chemical composition and the DNA sequence of evolutionarily neutral regions of *Liguralia* (Asteraceae) plants, mainly from Sichuan and Yunnan Provinces in China, in order to study the mechanism of diversification of secondary metabolites.[1,2] Intra-specific diversity has been revealed in most of the *Ligularia* species hitherto studied and found to be high in some species. The results of DNA analysis have suggested that the diversity in the chemicals has genetic origins in most cases. Furanoeremophilanes and/or eremophilan-8-ones have been isolated from most of the major *Ligularia* species; in particular, furanoeremophilanes are found more often than eremophilan-8-ones. In addition, most of the species that produce furanoeremophilanes appear to be more abundant than those that do not. These observations have led us to propose a hypothesis that furanoeremophilane-producing species or intra-specific populations are ecologically advantageous over eremophilan-8-one-producing ones.[1] For further testing of this hypothesis, analysis of hitherto uninvestigated species is necessary.

L. hookeri (C. B. Clarke) Hand.-Mazz. grows on glassy slopes, scrubland, forest understories, stream banks, and alpine meadows at altitude of approximately 3000 to 4500 m.[3,4] Although the plant occurs widely in the Hengduan Mountains area, to the best of our knowledge, no reports on its root chemicals have been published. Here, we describe the isolation of five new furanoeremophilanes and four known compounds from this species. In the course of the analyses, we realized that 3*β*-(2′-methylpropenoyloxy)-furanoeremophilan-15,6*β*-olide, previously reported by Bohlmann and Knoll,[5] should be revised to 3*β*-(2′-methylpropenoyloxy)furanoeremophilan-15,6*α*-olide.

Table 1. Collection Locality of *L. hookeri* Samples

Sample No.	Specimen No.	Location[a)]	Altitude (m)
1	2002-80	Cangshan (Dali, Yunnan)	3500
2	2003-03	Laojunshan (Jianchuan, Yunnan)	3900
3	2003-70	Bowashan (Daocheng, Sichuan)	4500
4	2003-81	Yading (Daocheng, Sichuan)	4000
5	2004-46	Geza (Shangrila, Yunnan)	4200
6	2004-49	Qianhushan (Shangrila, Yunnan)	3800
7	2008-43	Shikashan (Shangrila, Yunnan)	4300
8	2012-30	Yulin (Kanding, Sichuan)	3900

a) County and province in parenthesis.

Results and Discussion

Eight samples of *L. hookeri* were collected in Yunnan and Sichuan Provinces (Table 1, Fig. 1). Fresh roots of samples 1–4, collected in northwestern Yunnan (Dali/Jianchuan area) and southwestern Sichuan (Daocheng area) in 2002 and 2003,

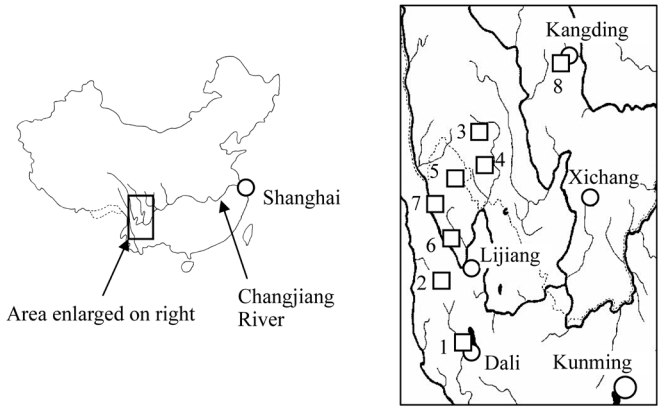

Fig. 1. Locations Where Samples of *L. hookeri* Were Collected (Squares)

Circles indicate major cities. Solid and dotted lines indicate rivers and boundaries of provinces, respectively.

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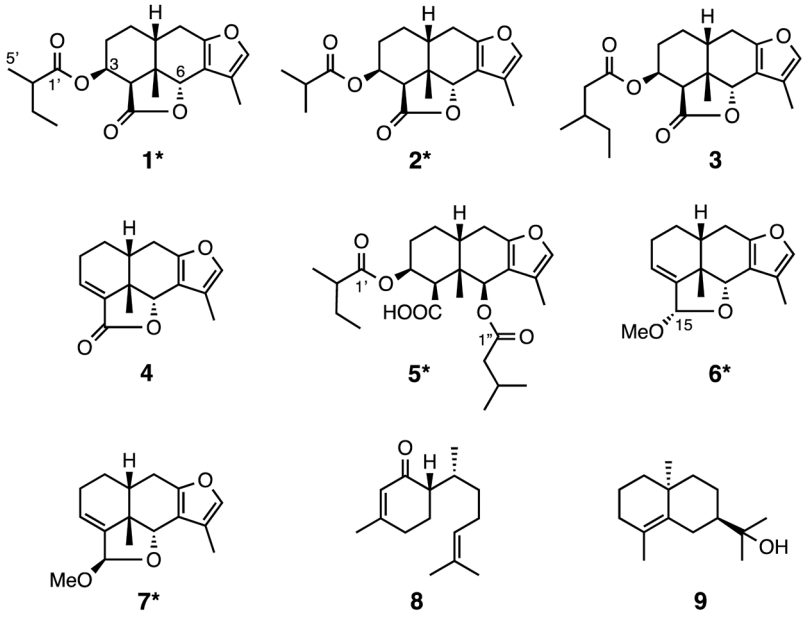

Fig. 2. Compounds Isolated from *L. hookeri* (* Denotes New Compound)

were extracted with EtOH, and the extracts were subjected to TLC and visualization using Ehrlich's reaction.[1,2] They showed several pink spots,[6] indicating the presence of furanoeremophilanes,[1,2] and the spot pattern was almost the same for samples 1–4. *L. hookeri* is a small plant and the quantity of root samples was limited. Therefore, the extracts of samples 1–4 were combined and the compounds were separated by silica gel column chromatography and HPLC. Two new (**1**, **2**) and two known (**3**, **4**) compounds were isolated (Fig. 2). The EtOH extracts of dried roots of samples 5–7, collected in the Shangrila area, Yunnan Province, in 2004 and 2008, showed almost the same TLC pattern as those of samples 1–4. The EtOH extract of sample 8, collected in central Sichuan in 2012, was also analyzed, and four new (**1**, **5**–**7**) and two known (**8**, **9**) compounds were obtained (Fig. 2). The structures of the five new compounds were determined as follows.

Compound **1** showed a quasi-molecular ion peak at *m/z* 347 in its MS and its molecular formula was determined to be $C_{20}H_{26}O_5$ from its high resolution (HR)-MS and ^{13}C-NMR data (Table 2). ^{1}H-NMR data indicated the presence of two singlet methyl (δ 1.20, 1.93), a doublet methyl (δ 1.06), and a triplet methyl (δ 0.85) groups. Signals characteristic of two oxymethine protons (δ 4.58, 5.34) and a trisubstituted furan proton (δ 6.85) were also observed. The ^{13}C-NMR spectrum indicated the presence of four methyl (δ 8.3, 11.6, 16.7, 23.1), four methylene (δ 22.1, 22.7, 24.9, 27.1), six methine (δ 36.8, 41.6, 43.1, 65.4, 81.6, 138.7), and six quaternary (δ 40.6, 115.1, 120.5, 150.8, 171.8, 175.0) carbons, of which two (δ 171.8, 175.0) were carbonyl and four (δ 115.1, 120.5, 138.7, 150.8) were aromatic (Table 2). The degree of unsaturation was eight; hence, this compound was deduced to be tetracyclic. Heteronuclear multiple bond connectivity (HMBC) correlations were observed between H_3-14 and C-4, 5, 6, and 10, between H_3-13 and C-7, 11, and 12, between H_3-5′ and C-1′, between H-6 and C-7, 8, and 15, and between H-12 and C-8. These observations and the correlation spectroscopy (COSY) correlations shown in Fig. 3 suggested that compound **1** was a furanoeremophilane bearing a (15,6)-lactone moiety and a 2′-methylbutanoyloxy group attached to C-3. A nuclear Overhauser effect (NOE) signal between H_3-14 and H-10 clearly showed that rings A and B were *cis*-fused. The orientation of H-6 was determined to be β, because NOE signals were observed between H-6 and H_3-14 and H-10. Both H-3 and H-4 were indicated to be α-oriented, because NOE signals were observed between H-4 and H-2α and H-3. The coupling pattern of H-3 (t-like, 2.2 Hz) supported it to be α-equatorial. Thus, compound **1** was established to be 3β-(2′-methylbutanoyloxy)furan oeremophilan-15,6α-olide.

The spectroscopic data of compound **2** were very similar to those of compound **1** except that compound **2** was smaller than compound **1** by one carbon ($C_{19}H_{24}O_5$). The signals of a 2-methylpropanoyloxy group, instead of those of the 2-methylbutanoyloxy group, were detected in its ^{1}H- and ^{13}C-NMR spectra (Table 2). The two dimensional (2D) correlations were almost the same as those observed for compound **1** (Fig. 3). Compound **2** was established to be 3β-(2′-methylpropanoyloxy)furanoeremophilan-15,6α-olide.

Compound **5** showed a broad absorption around 3200 and 2900 cm^{-1} in its IR spectrum and the molecular formula was determined to be $C_{25}H_{36}O_7$ by HR-MS and ^{13}C-NMR (Table 2). The ^{1}H-NMR spectrum showed a triplet methyl (δ 0.91), three doublet methyl (δ 0.90, 0.92, 1.07), two singlet methyl (δ 1.14, 1.85; one attached to an sp^2 carbon) signals, two oxymethine proton signals (δ 5.64, 6.46), and a singlet peak of a trisubstituted furan (δ 6.91) (Table 2). The 2D correlations shown in Fig. 4 indicated that compound **5** was an eremophilan-15-oic acid bearing a 2′-methylbutanoyloxy at C-3 and a 3”-methylbutanoyloxy group at C-6. The stereochemistry of **5** was determined to be A/B *cis* from the NOE signal between H_3-14 and H-10 (Fig. 4). Both acyloxy groups were judged to be β-oriented because NOE signals were observed between H-6 and H-1α, H-3α, and H-4α. This compound seemed to be a precursor to compound **1** in its biosynthetic pathway.[7–9]

Both compounds **6** and **7** had the same molecular formula of $C_{16}H_{20}O_3$ (*m/z* 260 [M$^+$]). Their NMR spectra indicated the presence of three singlet methyl groups, including one me-

Table 2. NMR Data of Compounds **1**, **2**, **5**, and **13** (in C_6D_6)

No.	**1**		**2**		**5**		**13**	
	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)	δ_C
1	0.77 (m)	22.1	0.74 (m)	22.1	1.02 (m)	26.4	0.72 (m)	22.1
	1.58 (m)	—	1.56 (m)	—	1.02 (m)	—	1.54 (m)	—
2	1.02 (m)	24.9	1.03 (m)	24.8	1.80 (m)	26.7	1.02 (m)	24.7
	1.61 (m)	—	1.61 (m)	—	2.16 (m)	—	1.67 (m)	—
3	5.34 (t-like, 2.2)	65.4	5.31 (br s)	65.6	5.64 (dt, 12.2, 6.1)	69.7	5.38 (br s)	66.3
4	1.59 (m)	43.1	1.56 (m)	43.0	3.30 (br d, 6.1)	49.8	1.62 (m)	43.2
5	—	40.6	—	40.6	—	41.5	—	40.5
6	4.58 (s)	81.6	4.57 (s)	81.5	6.46 (s)	68.2	4.57 (s)	81.6
7	—	115.1	—	115.1	—	114.7	—	115.1
8	—	150.8	—	150.8	—	150.2	—	150.8
9	1.88 (dd, 16.0, 10.0)	22.7	1.87 (dd, 17.6, 9.6)	22.7	2.64 (dd, 17.2, 3.7)	26.3	1.87 (dd, 17.0, 10.6)	22.7
	2.11 (dd, 16.0, 7.0)	—	2.04 (dd, 17.6, 6.8)	—	1.99 (m)	—	2.10 (dd, 17.0, 7.3)	—
10	1.58 (m)	36.8	1.58 (m)	36.7	2.42 (m)	36.9	1.57 (m)	36.8
11	—	120.5	—	120.4	—	119.8	—	120.4
12	6.85 (s)	138.7	6.84 (s)	138.7	6.91 (s)	139.1	6.85 (s)	138.8
13	1.93 (s)	8.3	1.93 (s)	8.2	1.85 (s)	8.9	1.93 (s)	8.3
14	1.20 (s)	23.1	1.19 (s)	23.1	1.14 (s)	19.4	1.18 (s)	23.2
15	—	171.8	—	171.8	—	177.7	—	171.7
1′	—	175.0	—	175.5	—	174.7	—	166.4
2′	2.22 (sext, 7.0)	41.6	2.31 (sept, 7.0)	34.4	2.26 (sext, 7.1)	41.2	—	136.8
3′	1.65 (m)	27.1	1.06 (d, 7.0)	19.1	1.38 (m)	27.0	5.18 (quint, 1.3)	125.4
	1.36 (m)	—	—	—	1.68 (m)	—	6.08 (s)	—
4′	0.85 (t, 6.6)	11.6	1.04 (d, 7.0)	19.0	0.91 (t, 7.1)	11.6	1.93 (d, 1.3)	18.5
5′	1.06 (d, 7.0)	16.7	—	—	1.07 (d, 7.1)	16.6	—	—
1″	—	—	—	—	—	172.7	—	—
2″	—	—	—	—	2.12 (m)	43.4	—	—
3″	—	—	—	—	2.19 (m)	25.7	—	—
4″	—	—	—	—	0.92 (d, 7.1)	22.5	—	—
5″	—	—	—	—	0.90 (d, 7.1)	22.5	—	—

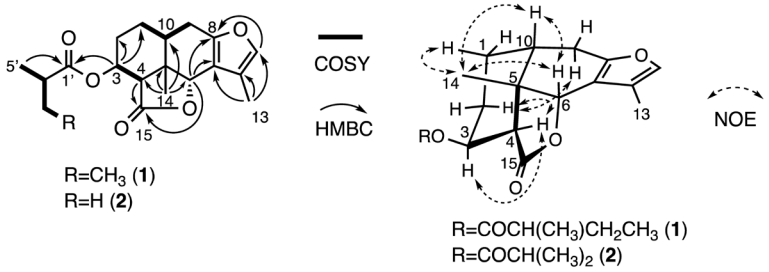

Fig. 3. Selected 2D Correlations Detected for Compounds **1** and **2**

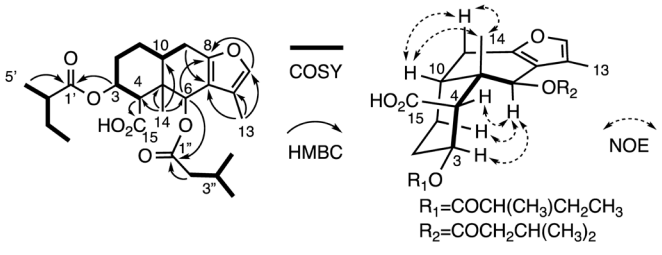

Fig. 4. Selected 2D Correlations Detected for Compound **5**

thoxy group, a trisubstituted furan, and a trisubstituted alkene moiety (Table 3). COSY correlations, including weak ones between H-3 and H-15, H-6 and H_2-9, and H-12 and H_3-13, indicated the same planar structure for **6** and **7** as depicted in Fig. 5. The nuclear Overhauser effect spectroscopy (NOESY) spectrum showed that the only difference between the two was in the configuration at C-15: a NOESY correlation between H-15 and H_3-14 in compound **6** indicated that the methoxy group was α-oriented in **6**, and, hence, it was β-oriented in **7**.

Other compounds isolated were 3β-(3′-methylpentanoyloxy)-furanoeremophilan-15,6α-olide (**3**)[10] and furanoeremophil-3-en-15,6α-olide (**4**),[10] bisabola-2,10-dien-1-one (**8**),[11] and eudesm-4-en-11-ol (**9**).[12]

Compounds **1** and **2** are different in the substituent of the 3β-acyloxy group from the previously reported compounds bearing 3β-angeloyloxy and 3β-(2′-methylpropenoyloxy) groups.[5] Compounds **1** and **2** have H-6β. In the course of the structural determination of these compounds, we realized that the structures of a pair of compounds **10** and **11** should be

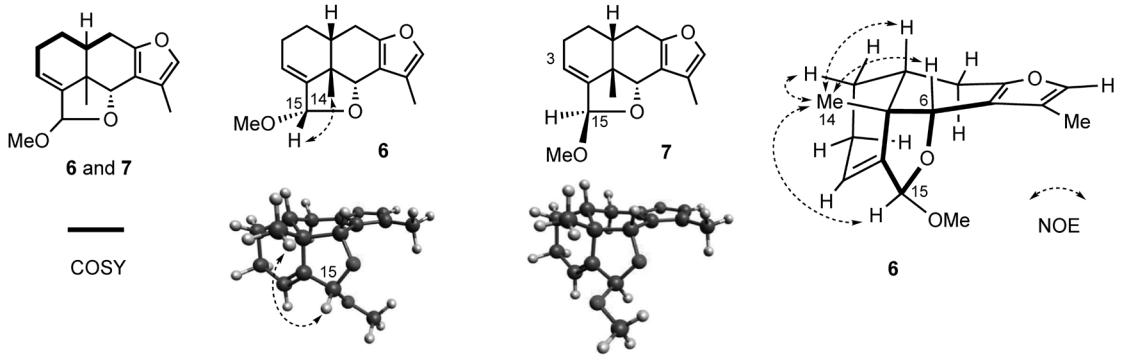

Fig. 5. Major 2D Correlations and Conformations of Compounds **6** and **7**

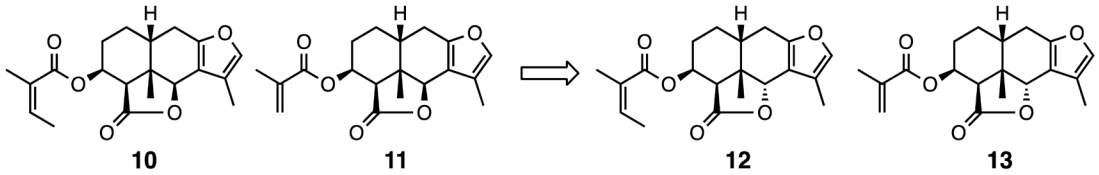

Fig. 6. Bohlmann and Knoll's Compounds, **10** and **11**, and Revised Structures, **12** and **13**

Table 3. NMR Data of Compounds **6** and **7** (in C_6D_6)

No.	6	7
	δ_H mult. (*J* in Hz)	δ_H mult. (*J* in Hz)
1	1.67 (m)	1.74 (m)
	1.12 (m)	1.12 (m)
2	1.83 (m)	1.74 (m)
	1.77 (m)	1.74 (m)
3	5.68 (td, 2.7, 1.7)	5.45 (t, 2.5)
6	4.75 (s)	5.01 (s)
9	2.28 (dd, 16.6, 11.0)	2.23 (dd, 16.4, 10.5)
	2.19 (dd, 16.6, 6.9)	2.17 (dd, 16.4, 6.9)
10	1.85 (m)	1.80 (m)
12	6.94 (s)	6.93 (s)
13	2.12 (s)	2.08 (d, 1.2)
14	1.15 (s)	1.46 (s)
15	5.51 (q, 1.7)	5.11 (s)
OMe	3.16 (s)	3.33 (s)

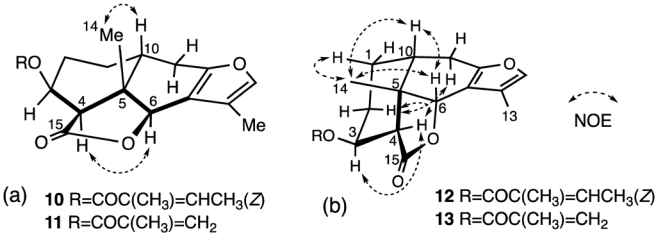

Fig. 7. (a) Supposed Conformation and Expected NOEs for Compounds **10** and **11** Proposed by Bohlmann and Knoll; (b) Observed NOEs for Compounds **12** and **13** (Revised Structures)

revised to **12** and **13**, respectively, as shown in Fig. 6. Compounds **10** and **11** bearing H-6*α* were first reported by Bohlmann and Knoll.[5] Compound **12** was chemically derived from 3*β*,6*β*-bis(acyloxy)-15-oic acid by Kuroda *et al.*[7,8] The H-6*β* structure of **12** was established from the NOE signal between H-6 and H_3-14.[13] The agreement of the chemical shift values of H-6 between Bohlmann and Knoll's **10** and Kuroda *et al.*'s **12** suggested that the former was actually the latter. Besides, the lactone would not be formed easily with H-6*α* because **10** has a highly strained tricyclic ring system (Fig. 7). Later, Jakupovic and Bohlmann also showed an H-6*β* structure **12**, citing Bohlmann and Knoll,[5] but without any comment.[14] In 2004, Li *et al.* reported the isolation of a compound, described its full spectroscopic data, and identified it to be **12**.[15] A year later, Hanai *et al.* also isolated the compound from *L. tongolensis* and identified it to be **12**,[10] citing Bohlmann and Knoll's report[5] erroneously.[16] Thus, the four compounds, Bohlmann and Knoll's **10**,[5] Li *et al.*'s **12**,[15] Kuroda *et al.*'s **12**[7,8] and Hanai *et al.*'s **12**[10] are one and the same. We believe that **11** should also be revised to **13** accordingly. Because no spectroscopic data have been published for **13**, it is worth recording the data, measured on material isolated from *L. tongolensis*,[10] here (Table 2 and Experimental).

The base sequence of the ITS1-5.8S-ITS2 region of the nuclear ribosomal RNA gene cluster was determined in order to assess the genetic diversity in the samples. The results are summarized in Table 4. No distinct difference was seen among the samples. This result was consistent with the chemical similarity among the samples.

Compounds **1**–**7** are furanoeremophilanes with an oxygen functionality at C-15. 15-Oxygenated furanoeremophilanes have been obtained from *L. cymbulifera* (W. W. Smith) Hand.-Mazz.,[10] *L. vellerea* (Franch.) Hand.-Mazz.,[17,18] and *L. tongolensis* (Franch.) Hand.-Mazz.[10] These species, as well as *L. hookeri*, occur in northwestern Yunnan to southwestern Sichuan Province. 15-Oxygenated eremophilanolides have been obtained also from *L. przewalskii* (Franch.) Hand.-Mazz. in Ganxu Province,[19] although eremophilane sesquiterpenes in most of the major *Ligularia* species in northern Sichuan to Ganxu/Qinghai area are not 15-oxygenated. Further chemical and genetic analyses are necessary to determine whether there is any relationship among *L. hookeri* and other species producing 15-oxygenated furanoeremophilanes.

Table 4. Differences in the ITS1-5.8S-ITS2 Sequences among *L. hookeri* Samples[a)]

Sample No.	ITS1								5.8S	ITS2							
			1	2	2	2	2	2				1	1	1	2		2
	2	2	3	0	1	3	5	5	2	2	7	0	5	7	0		1
	1	6	2	9	0	2	1	7	2	7	3	7	5	6	6	b)	8
1	Y	Y	Y	c)	c)	G	T	Y	C	Y	Y	C	Y	T	T	—	W
2[d)]	Y	Y	Y	c)	c)	G	T	Y	M	Y	Y	Y	Y	T	T	—	W
3	Y	Y	Y	c)	c)	K	Y	Y	M	Y	Y	C	Y	T	T	A	T
4	C	Y	Y	c)	c)	K	Y	C	C	C	Y	M	C	T	T	A	T
5	Y	Y	Y	c)	c)	G	T	Y	M	Y	Y	C	Y	T	Y	A	T
6	C	T	C	c)	c)	G	T	C	C	C	C	C	C	T	T	—	T
7	Y	Y	Y	c)	c)	G	T	Y	M	Y	Y	C	Y	Y	T	—	T
8	C	T	C	c)	c)	K	Y	C	C	C	C	C	C	T	T	T	T
Ref[e)]	C	T	C	A	T	G	T	T	A	T	C	T	C	T	T	—	G

a) K=G+T; M=A+C; Y=C+T; W=A+T; —, none. *b*) Two sequences with and without an insertion of A or T between the 217 and the 218 positions of ITS2 were present. *c*) Two sequences with and without AT at 209–210 of ITS1 were present. *d*) Determined for a sample sympatrically collected in 2016. *e*) A *L. hookeri* sequence in the database (DQ272327).

Conclusion

Furanoeremophilanes were isolated from *L. hookeri*, from which this is the first chemical report. All the isolated furanoeremophilanes were 15-oxygenated, as in major *Ligularia* species in Shangrila area, Yunnan Province. No distinct variation was found in the eight collected samples. It is interesting to note that all the compounds bearing a 15,6-lactone moiety isolated in this work and previously from other *Ligularia* had H-6β.[10,17–20)] The structures of Bohlmann and Knoll's compounds[5)] **10** and **11** should be revised to **12** and **13**, respectively, with respect to the configuration at H-6.

Experimental

General Specific rotations, a JASCO DIP-370 digital polarimeter; NMR, a Varian Unity 600 (600 MHz for ^{1}H; 150 MHz for ^{13}C) and a Unity 200 (200 MHz for ^{1}H; 50 MHz for ^{13}C) spectrometer; IR spectra, a SHIMADZU FT/IR-8400S (measured with samples absorbed on powdered KBr by the diffusion reflection method); MS, a JEOL JMS-700 MStation; HPLC, Chemcopak Nucleosil 50-5 (4.6×250 mm) (a JASCO pump system) with a solvent system of hexane-ethyl acetate or TSK-GEL G1000H$_{HR}$ (300×7.8 mm) with ethyl acetate; Column chromatography (CC), on silica gel BW-127ZH or BW-300 (Fuji Silysia, Japan); Analytical TLC, Merck Kieselgel 60 F$_{254}$, 0.2 mm thickness with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde and HCl).

Plant Materials Samples were collected in August 2002 (sample 1), 2003 (samples 2–4), 2004 (samples 5, 6), 2008 (sample 7), and 2012 (sample 8) at the locations shown in Table 1 and Fig. 1. Each sample was identified by X. G. (one of the authors). The voucher specimen numbers were 2002-80, 2003-03, 2003-70, 2003-81, 2004-46, 2004-49, 2008-43, and 2012-30 for samples 1–8, respectively (Kunming Institute of Botany).

Extraction and Isolation The fresh roots of samples 1–4 (50, 55, 180, 65 g, respectively) were extracted with EtOH. The extracts were combined (4.0 g) and subjected to silica gel column chromatography (hexane–EtOAc, gradient) and HPLC (Nucleosil 50-5, hexane–EtOAc) to isolate compounds **1** (17.9 mg), **2** (2.2 mg), **3** (12.0 mg), and **4** (7.4 mg).

The dried roots of sample 8 (10.2 g) were extracted with EtOH to give extracts (838 mg), which were separated by silica gel column chromatography (hexane–EtOAc, gradient) and HPLC (Cosmosil 5SL-II, hexane–EtOAc; YMC-Pack Diol-120-NP, hexane–EtOAc; TSK gel G1000H$_{HR}$, EtOAc; Inertsil Diol 5 μm, hexane–EtOAc) to isolate compounds **1** (7.6 mg), **5** (7.7 mg), **6** (0.37 mg), **7** (0.08 mg), **8** (0.6 mg), and **9** (0.3 mg).

3β-(2′-Methylbutanoyloxy)furanoeremophilan-15,6α-olide (**1**)

Oil. IR (FT) cm^{-1}: 1769, 1732. Chemical ionization (CI)-MS *m/z*: 347.1848 (Calcd for $C_{20}H_{27}O_5$: 347.1858). MS (CI) *m/z*: 347 $(M+H)^+$, 245 (100). $[\alpha]_D^{22}$ +39.0 (*c*=0.78, $CHCl_3$). ^{1}H- and ^{13}C-NMR data are in Table 2.

3β-(2′-Methylpropanoyloxy)furanoeremophilan-15,6α-olide (**2**)

Oil. IR (FT) cm^{-1}: 1772, 1730, 1636. CI-MS *m/z*: 333.1695 $(M+H)^+$ (Calcd for $C_{19}H_{25}O_5$: 333.1702). MS (CI) *m/z*: 333 $(M+H)^+$, 245 (100), 89, 56. $[\alpha]_D^{25}$ +24.6 (*c*=0.22, $CHCl_3$). CD (EtOH) θ (nm): +1800 (222). ^{1}H- and ^{13}C-NMR data are in Table 2.

3β-(2′-Methylbutanoyloxy)-6β-(3′-methylbutanoyloxy)-furanoeremophilan-15-oic Acid (**5**)

Oil. IR (FT) cm^{-1}: 3282, 2964, 1736, 1709, 1185. Electron ionization (EI)-MS *m/z*: 448.2460 $(M)^+$ (Calcd for $C_{25}H_{36}O_7$: 448.2461). MS (EI) *m/z*: 448 $(M)^+$, 346 (100), 244, 85. $[\alpha]_D^{19}$ −13.1 (*c*=0.77, $CHCl_3$). ^{1}H- and ^{13}C-NMR data are in Table 2.

15,6α-Epoxy-15α-methoxyfuranoeremophil-3-ene (**6**)

Oil. IR (FT) cm^{-1}: 2921, 1453, 1081, 963. EI-MS *m/z*: 260.1411 $(M)^+$ (Calcd for $C_{16}H_{20}O_3$: 260.1412). MS (EI) *m/z*: 260 $(M)^+$, 229, 137, 88 (100). $[\alpha]_D^{16}$ +49.4 (*c*=0.037, $CHCl_3$). ^{1}H-NMR data are in Table 3.

15,6α-Epoxy-15β-methoxyfuranoeremophil-3-ene (**7**)

Oil. IR (FT) cm^{-1}: 2923, 1464, 1260, 1107, 806. EI-MS *m/z*: 260.1411 $(M)^+$ (Calcd for $C_{16}H_{20}O_3$: 260.1412). MS (EI) *m/z*: 260 $(M)^+$, 229, 137, 88 (100). $[\alpha]_D^{20}$ +38.0 (*c*=0.007, $CHCl_3$). ^{1}H-NMR data are in Table 3.

3β-(2′-Methylpropenoyloxy)furanoeremophilan-15,6α-olide (**13**)

Oil. IR (FT) cm^{-1}: 1770, 1720, 1650. CI-MS *m/z*: 331.1550 $(M+H)^+$ (Calcd for $C_{19}H_{23}O_5$: 331.1546). MS (CI) *m/z*: 331 $(M+H)^+$, 245 (100). $[\alpha]_D^{21}$ +15.4 (*c*=1.3, $CHCl_3$). ^{1}H- and ^{13}C-NMR data are in Table 2.

DNA Analysis DNA was purified from dried leaves using a DNeasy Plant Mini Kit (QIAGEN). PCR was carried out

with primers L5 and L6[21] and HotStarTaq *plus* Master Mix Kit, and the products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostics, Switzerland) following agarose gel electrophoresis. Sequencing reactions were carried out with primers L1–L4[21] and a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, U.S.A.) and analyzed using a 3130xl or a 3300 Genetic Analyzer (Applied Biosystems).

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Conflict of Interest The authors declare no conflict of interest.

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