Sesquiterpenes, Nortriterpenes and Other Constituents from *Ligularia tongolensis*

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Two new eremophilane-type sesquiterpenes, 3β-(2′-methylbutanoyloxy)-8β*H*-eremophil-7(11)-ene-12,8α-(14,6α)-diolide (1) and 8β*H*-eremophil-3,7(11)-diene-12,8α(14,6α)-diolide (2), and two new norursane-type triterpenes, 2α,3β,19α-trihydroxy-28-norurs-12-ene (7) and 2α,3α,19α-trihydroxy-28-norurs-12-ene (8), were isolated from the roots of *Ligularia tongolensis*, together with nine known compounds. The structures of the new compounds were elucidated by spectroscopic methods.

Key words *Ligularia tongolensis*; Compositae; nortriterpene; sesquiterpene; anti-tumor activity

In our ongoing investigation into bioactive compounds from the genus *Ligularia* (Compositae) plants[1—7] we have studied the roots of *Ligularia tongolensis* (FRANCH) HAND-MAZZ collected in mountainous areas (altitude: 3600 m) in southwestern China. *Ligularia tongolensis* is used as a traditional folk medicine in China and its roots can reduce phlegm, relieve coughing and cure pulmonary tuberculosis, urinary track blockages, common cold, and pharyngitis.[8] However, the chemical constituents of this plant have not been reported up until now. In this paper, we report the isolation and structural elucidation of four new compounds (**1**, **2**, **7**, **8**) and nine known compounds **3**,[9] **4**,[10] **5**,[11] **6**,[6] **9**,[12] **10**,[13] **11**,[14] **12**[15] and **13**[14] from its roots. In addition, the cytotoxic activity *in vitro* of compounds **1**, **2**, **3**, **7** and **8** were tested against human hepatoma (SMMC-7721), human embryo liver (L-02) and human leukemia (HL-60) cell lines with 10-hydroxycamptothecine as a standard.

Results and Discussion

Compound **1**, $[\alpha]_D^{20}$ +125.5° ($CHCl_3$), was obtained as a colorless plate, mp 190—191 °C. The IR spectrum of **1** indicated the presence of a typical α,β-unsaturated γ-lactone (1714, 1670 cm^{-1}) and its molecular formula, $C_{20}H_{26}O_6$, was determined by the high resolution second ionization mass spectrometry (HR-SI-MS). Analysis of NMR of **1** indicated the presence of a 2-methylbutanoyloxy group [δ_H 2.38 (m, 1H), 1.66 (m, 1H), 1.48 (m, 1H), 1.14 (d, 3H, J=7.2 Hz), 0.92 (dd, 3H, J=7.5, 7.2 Hz); δ_C 175.5 (C), 41.3 (CH), 26.7 (CH_2), 11.4 (CH_3), 16.4 (CH_3)], and the other 15 carbon signals suggested that **1** was an eremophilenolide-type sesquiterpene and its NMR data were similar to those of the known compound, 3β-angeloyloxy-8β*H*-eremophil-7(11)-ene-12,8α (14,6α)-diolide,[3] except for the presence of a 2-methylbutanoyloxy group in **1** instead of an angeloyloxy group in the known compound. The localization of the 2-methylbutanoyloxy group moiety at C-3 was deduced from the heteronuclear multiple bond correlation (HMBC) spectrum, in which H-3 (δ_H 5.47) gave a long-range coupling with C-1′ (δ_C 175.5). Stereochemically, Me-14 and Me-15 are biogenetically β-orientated.[16] The positive nuclear Overhauser effect spectrometry (NOEs) between Me-15 and H-10 (5.30%) showed a *cis*-fused eremophilane. The coupling pattern observed for H-3 (δ_H 5.47 q, 3.0 Hz) implied that H-3 was an equatorial proton and should be α-orientated.[17,18] H-6 and H-8 were identified as β-orientated from the evidence of positive NOEs between H-6 and H-15 (7.73%), between H-6 and H-8 (4.35%). Therefore, compound **1** was assigned as 3β-(2′-methylbutanoyloxy)-8β*H*-eremophil-7(11)-ene-12,8α (14,6α)-diolide.

The molecular formula of compound **2**, $C_{15}H_{16}O_4$, was determined by the HR-SI-MS, ^{13}C-NMR and DEPT (distortionless enhancement by polarization transfer) data. The NMR and IR data were similar to those of compound **1** except for the presence of a double bond at C-3 in **2** instead of the 2-methylbutanoyloxy group in **1**. The signals of H-3 and C-3 as well as the adjacent C-4 were shifted downfield [H-3 at δ_H 6.85, C-3 at δ_C 136.9 and C-4 at δ_C 129.6], which indicated that a double bond was between C-3 and C-4. In combination with the other NMR data (Tables 1, 2) and HMBC spectrum, compound **2** was confirmed as 8β*H*-eremophil-3,7(11)-dien-12,8α (14,6α)-diolide.

The molecular formula $C_{29}H_{48}O_3$ for compound **7** was determined by the high resolution electrospray ionization mass spectrometry (HR-ESI-MS) and the data of ^{13}C-NMR and DEPT. Its IR spectrum showed strong hydroxyl bands at

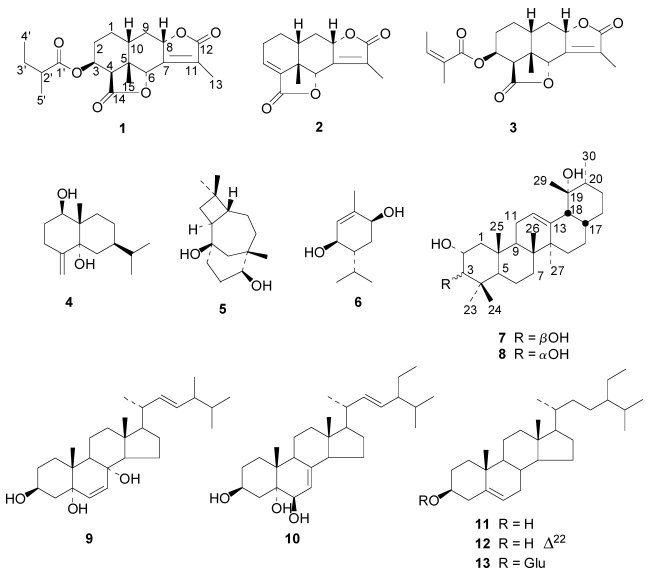

Fig. 1. Structures of Compounds **1**—**13**

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3334 cm^{-1} and a double bond band at 1687 cm^{-1}. The ^{1}H-, ^{13}C-NMR and DEPT spectra of **7** showed the presence of seven methyls, eight methylenes, eight methines and six quaternary, among which, two sp^2 carbon atoms of a carbon–carbon double bond, two methines bearing an oxygen and a quaternary carbon bearing an oxygen indicated that compound **7** is a nor-methyl pentacyclic triterpene structure with a double bond and three hydroxyls. Furthermore, the pair of characteristic double bond signals at δ_C 129.6 (CH) and 140.5 (C) in the ^{13}C-NMR spectrum is suggested to be a urs-12-ene skeleton.[19] However, the most significant difference between **7** and the known urs-12-ene compounds was the absence of the proton and carbon signals of CH_3-28 attached to C-17 and the appearance of one methine C-17 (δ_C 39.3, CH) instead of one quarternary carbon (δ_C 33—47, C) in the known urs-12-ene derivatives.[20,21] A broad singlet at δ_H 2.50 in the ^{1}H-NMR spectrum should be assigned to the H-18 of urs-12-ene with 19α-hydroxyl substitution and the proton of H-17, which was confirmed by the correlation between H-18 and H-17 observed in ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY). So **7** was deduced to have a 28-norurs-12-ene-skeleton. Except for 19-hydroxyl, the other two oxygenated methines in the ^{13}C-NMR spectrum were attributed to C-2 and C-3 by analysis of HMBC data (Fig. 1). The coupling constant (J=9.6 Hz) between H-2 and H-3 confirmed that H-2 and H-3 were both axial with respectively α and β-configuration. The orientations of H-2 and H-3 were also confirmed by the NOEs experiment. Strong NOEs were observed between H-2_{ax} and β Me-24 (4.8%), and between H-2_{ax} and β Me-25 (6.7%), indicating that these three proton systems were on the β side of the A-ring. NOEs between H-3_{ax} and α Me-23 (5.0%) indicated that these two proton systems were on the α side of the A-ring. On the other hand, strong NOEs measured between H-18 and H-12 (10%) and between H-18 and β Me-29 (3.0%) indicated the orthogonal disposition of the E-ring with respect to the D-ring, as observed in musancropic and musangic acids.[22,23] Finally, NOEs were detected between β Me-25 and β Me-26, as expected. Thus, the structure of **7** was elucidated as 2α,3β,19α-trihydroxy-28-norurs-12-ene.

We also obtained compound **8**, and the molecular formula $C_{29}H_{48}O_3$ was shown by accurate mass measurement at HR-SI-MS. The IR spectrum of **8** showed hydroxyl bands at 3388 cm^{-1}, a trisubstituted double bond band at 1678 cm^{-1}, which was very similar to that of **7**. The ^{1}H-, ^{13}C-NMR and DEPT spectral data (Tables 1, 2) were also similar to those of **7**, except that the resonance signals of the hydroxylated carbons C-2 and C-3 of **8** were shifted upfield at δ 67.5 for C-2 and δ 80.4 for C-3, but compound **7** was at δ 69.8 for C-2 and δ 84.8 for C-3. Furthermore, the ^{1}H-NMR spectrum of **8** showed signals at δ 3.92 (m, H-2β), δ 3.30 (br s, H-3β), which suggested the α-configuration for the two hydroxyl groups on ring A. Compounds reported with a 2α,3α-diol system[23] had the same chemical shifts for C-2 and C-3 as those of compound **8**. This also confirmed the configuration of 2α,3α-diol for compound **8**.

The relative stereochemistry was further confirmed by NOE difference measurements. The H-18 proton, which was axial with respect to the E-ring but equatorial on the D-ring, gave a strong NOEs with H-12 (12.9%) and H-17 (10.8%). The α-*cis* stereochemistry of the hydroxyl groups at C-2 and C-3 was also verified, as the β H-2_{ax} showed NOEs with the following spin systems: β Me-25 (9.0%), Me-24 (5.1%) and β H-3_{eq} (8.9%). Finally, H-3_{eq} gave similar NOEs with α Me-23 (5.0%) and β Me-24 (4.3%). Accordingly, compound **8** is 2α,3α,19α-trihydroxy-28-norurs-12-ene.

Using the sulorhodamine B (SRB) method the anti-tumor activities of compounds **1**, **2**, **3**, **7** and **8** against human he-

Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1** and **2** in $CDCl_3$[a]

Position	**1**[b]		**2**	
	δ_C	δ_H	δ_C	δ_H
1	21.0 t	1.49 m/2.10 m	21.8 t	1.74 m/ 2.04 m
2	25.1 t	1.58 m	22.0 t	2.23 m/2.33 m
3	64.4 d	5.47 (q, 3.0)	136.9 d	6.85 (dd, 3.6, 3.0)
4	42.5 d	2.41 (d, 3.0)	129.6 s	—
5	44.2 s	—	44.0 s	—
6	82.8 d	4.98 br s	81.8 d	5.14 br s
7	154.0 s	—	155.9 s	—
8	77.4 d	4.72 (dd, 11.1, 3.9)	77.4 d	4.68 (dd, 9.3, 4.2)
9	32.1 t	1.54 m/2.36 m	33.0 t	1.08 m/2.23 m
10	34.8 d	2.17 m	33.6 d	2.15 m
11	126.2 s	—	125.4 s	—
12	171.1 s	—	173.4 s	—
13	9.3 q	1.99 br s	9.4 q	2.00 br s
14	173.1 s	—	168.3 s	—
15	23.2 q	1.54 s	27.0 q	1.42 s

a) Chemical shifts (δ) in ppm relative to TMS; coupling constants (J in Hz) are given in parentheses. *b*) 2′-Methylbutanoyloxy: δ_C 175.5 (s, C-1′), 41.3 (d, C-2′), 26.7 (t, C-3′), 11.4 (q, C-4′), 16.4 (q, C-5′); δ_H 2.38 (m, H-2′), 1.48/1.66 (m, H_2-3′), 0.92 (dd, 7.5, 7.2, H_3-4′), 1.14 (d, 7.2, H_3-5′).

Table 2. ^{1}H- and ^{13}C-NMR Data for Compounds **7** and **8** in CD_3OD[a]

Position	**7**		**8**	
	δ_C	δ_H	δ_C	δ_H
1	48.4 t	0.93 dd (12.6, 10.5)/ 1.94 dd (12.6, 4.2)	42.8 t	1.26 m/1.59 m
2	69.8 d	3.62 ddd (10.5, 9.6, 4.2)	67.5 d	3.92 m
3	84.8 d	2.91 d (9.6)	80.4 d	3.30 br s
4	40.8 s	—	39.8 s	—
5	57.0 d	1.22 m	49.9 d	1.23 m
6	20.0 t	1.65 m/1.32 m	19.6 t	1.52 m/1.30 m
7	34.3 t	1.50 m/1.30 m	34.1 t	1.48 m/1.33 m
8	41.4 s	—	41.6 s	—
9	48.7 d	1.75 m	49.0 d	1.66 m
10	39.5 s	—	39.7 s	—
11	25.0 t	2.00 m	25.0 t	1.99 m
12	129.6 d	5.29 br s	129.7 d	5.29 br s
13	140.5 s	—	140.4 s	—
14	43.0 s	—	43.1 s	—
15	29.9 t	1.51 m/1.02 m	29.9 t	1.49 m/1.01 m
16	27.3 t	2.56 m/1.82 m	27.4 t	2.57 m/1.85 m
17	39.3 d	1.50 m	39.7 d	1.50 m
18	55.4 d	2.50 br s	55.4 d	2.50 br s
19	73.9 s	—	73.9 s	—
20	43.4 d	1.42 m	43.4 d	1.41 m
21	27.6 t	1.31 m/0.99 m	27.6 t	1.30 m/0.99 m
22	26.9 t	1.51 m/1.30 m	27.0 t	1.50 m/1.31 m
23	29.6 q	1.01 s	29.6 q	0.99 s
24	17.3 q	0.81 s	22.8 q	0.87 s
25	16.9 q	1.00 s	17.3 q	0.99 s
26	17.8 q	0.80 s	17.8 q	0.78 s
27	25.0 q	1.33 s	25.2 q	1.35 s
29	31.1 q	1.19 s	31.0 q	1.19 s
30	20.0 q	0.92 d (6.9)	19.6 q	0.92 d (6.6)

a) Chemical shifts (δ) in ppm relative to TMS; coupling constants (J in Hz) are given in parentheses.

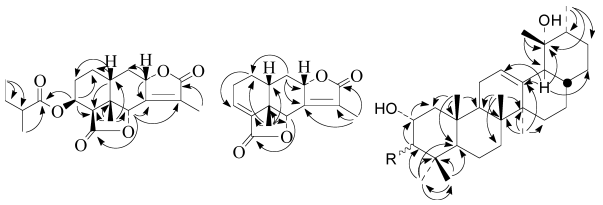

Fig. 2. Important HMBC Correlations of **1**, **2**, **7** and **8**

Table 3. Cytotoxocity (IC_{50}, μg/ml) of Compounds **1**, **2**, **3**, **7** and **8**

Compound	SMMC-7721	L-02	HL-60
1	≫200	≫200	≫200
2	399.13	≫200	447.87
3	≫200	≫200	392.72
7	≫200	≫200	≫200
8	356.31	≫200	120.68
10-Hydroxycampto-thecine	0.0157	0.0006	0.0084

patoma (SMMC-7721), human embryo liver (L-02) and human leukemia (HL-60) cell lines were studied in comparison with standard 10-hydroxycamptothecine. The IC_{50} against the three cell lines were listed in Table 3. The cytotoxicities of all the five tested compounds are weak with their IC_{50} values much more than 200 μg/ml, except for compound **8** whose IC_{50} value is 120.68 in HL-60 cell lines. The data comparison of compounds **7** and **8** showed that the active group of urs-skeleton compounds could be due to 3α-hydroxyl group.

Experimental

General Experimental Procedures Melting points were determined on a Kofler melting point apparatus and were uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were measured on a Nicolet NEXUS 670 FT-IR instrument (neat). One-dimensional (1D) and two-dimensional (2D) NMR spectra were measured on a Varian Mercury-300BB NMR spectrometer with TMS as the internal standard. Electron impact ionization mass spectrometry (EI-MS) data were obtained on an HP-5988 AGCMS spectrometer. HR-ESI-MS were recorded on a Bruker APEX II. UV spectrum was measured using a Shimadzu UV-260 spectrophotometer. Silica gel (200—300 mesh) used for CC, and silica GF_{254} (10—40 μ) for TLC were supplied by the Qingdao Marine Chemical Factory Qingdao, P. R. China. TLC spots were detected under a UV lamp or by heating after being spraying with 5% H_2SO_4 in EtOH (v/v).

Plant Material The roots of *Ligularia tongolensis* were collected at a high-altitude place in Muli County, Sichuan Province, P. R. China, in September 2003 and identified by Prof. Guoliang Zhang. A voucher specimen (No. 030910) was deposited at the College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation The air-dried roots of *L. tongolensis* (1.5 kg) was pulverized and extracted at room temperature with a solvent mixture of petroleum ether (60—90 °C)–Et_2O–MeOH (1 : 1 : 1) (5×7 d×3). The solutions were combined and evaporated to dryness (85 g) and the extract was subjected to repeated chromatography on a silica gel column (200—300 mesh, 800 g), eluted with a gradient of petroleum ether (60—90 °C)–Me_2CO (1 : 0, 30 : 1, 15 : 1, 10 : 1, 7 : 1, 5 : 1, 3 : 1, 1 : 1, 0 : 1). According to differences in composition indicated by TLC, 6 crude fractions [Fr. 1 (1 : 0—30 : 1, 20 g), Fr. 2 (15 : 1—10 : 1, 8.5 g), Fr. 3 (10 : 1—7 : 1, 8 g), Fr. 4 (7 : 1—5 : 1, 5 g), Fr. 5 (3 : 1—1 : 1, 22 g), Fr. 6 (0 : 1, 10 g)] were obtained. Fr. 1 (20 g) was chromatographed on a silica gel column and eluted with petroleum ether (60—90 °C)–EtOAc (30 : 1) to give **11** (500 mg) and **12** (30 mg). Fr. 2 (8.5 g) was chromatographed on a silica gel column and eluted with petroleum ether (60—90 °C)–EtOAc (10 : 1) to give 3 frs: fr. 2-1 (3.5 g), fr. 2-2 (2 g) and fr. 2-3 (3 g). Fr. 2-2 (2 g) was chromatographed on a silica gel column and eluted with petroleum ether (60—90 °C)–Me_2CO (15 : 1) to give **4** (1 mg). Fr. 3 (8 g) was chromatographed on a silica gel column and eluted with petroleum ether (60—90 °C)–EtOAc (5 : 1) to give 3 frs: fr. 3-1 (2 g), fr. 3-2 (4 g) and fr. 3-3 (2 g). Fr. 3-1 (2 g) was chromatographed on a silica gel column and eluted with a gradient of $CHCl_3$–Me_2CO (30 : 1, 15 : 1, 10 : 1), then purified by recrystallization to give **1** (5 mg), **2** (10 mg) and **3** (4 mg). Fr. 3-3 (2 g) was chromatographed on a silica gel column and eluted with $CHCl_3$–Me_2CO (10 : 1) to give **5** (5 mg), **6** (10 mg) and **9** (20 mg). Fr. 4 (5 g) was chromatographed on a silica gel column and eluted with a gradient of $CHCl_3$–MeOH (30 : 1, 15 : 1, 10 : 1) to give 5 frs: fr. 4-1 (1 g), fr. 4-2 (1.5 g), fr. 4-3 (1 g), fr. 4-4 (1 g) and fr. 4-5 (0.5 g). Fr 4-1 (1 g) was chromatographed on a silica gel column and eluted with $CHCl_3$–MeOH (30 : 1) to give **8** (3 mg). Fr 4-2 (1.5 g) was chromatographed on a silica gel column and eluted with $CHCl_3$–MeOH (15 : 1) to give 4 frs: fr. 4-2-1 (100 mg), fr. 4-2-2 (400 mg), fr. 4-2-3 (500 mg) and fr. 4-2-4 (500 mg). Fr 4-2-1 (100 mg) was further chromatographed using preparative TLC (silica GF_{254}, 10—40 μ, 1 mm) and EtOAc–EtOH–H_2O (30 : 1 : 0.5) to give **7** (3 mg, *Rf*=0.60) and **10** (5 mg, *Rf*=0.45). Fr. 5 (22 g) was chromatographed on a silica gel column and eluted with a gradient of $CHCl_3$–MeOH (15 : 1, 10 : 1, 7 : 1, 5 : 1) to give **13** (200 mg).

3β-(2′-Methylbutanoyloxy)-8βH-eremophil-7(11)-en-12,8α(14,6α)-diolide (**1**): Colorless plates, mp 190—191 °C. $[\alpha]_D^{20}$ +125.5° (*c*=0.40, $CHCl_3$). IR (KBr) cm^{-1}: 1800, 1767, 1714, 1670. EI-MS *m/z*: 362 $[M]^+$ (2), 260 (29), 231 (25), 83 (100). HR-SI-MS *m/z*: 363.1803 (Calcd for $[C_{20}H_{26}O_6+H]^+$ 363.1802). ^{1}H-NMR: see Table 2. ^{13}C-NMR: see Table 1.

8βH-Eremophil-3,7(11)-dien-12,8α(14,6α)-diolide (**2**): Colorless plates, mp 230—231 °C. $[\alpha]_D^{20}$ +28.0° (*c*=0.20, $CHCl_3$). IR (KBr) cm^{-1}: 1732, 1675, 1425. UV λ_{max} ($CHCl_3$) nm (log ε): 240 (0.209). EI-MS *m/z*: 260 $[M]^+$ (2), 231 (2), 163 (15), 84 (88). HR-SI-MS *m/z*: 261.1122 (Calcd for $[C_{15}H_{16}O_4+H]^+$ 261.1121. ^{1}H-NMR: see Table 2. ^{13}C-NMR: see Table 1.

2α,3β,19α-Trihydroxy-28-norurs-12-ene (**7**): An amorphous powder, $[\alpha]_D^{20}$ −14° (*c*=0.125; CH_3OH). IR (KBr) cm^{-1}: 3334, 1687. HR-ESI-MS *m/z*: 467.3488 (Calcd for $C_{29}H_{48}NaO_3$ $[M+Na]^+$ 467.3496). ^{1}H-NMR: see Table 2. ^{13}C-NMR: see Table 1.

2α,3α,19α-Trihydroxy-28-norurs-12-ene (**8**): An amorphous powder, $[\alpha]_D^{20}$ +8° (*c*=0.125; CH_3OH). IR (KBr) cm^{-1}: 3388, 1678. HR-SI-MS *m/z*: 427.3591 (Calcd for $C_{29}H_{47}O_2$ $[M-H_2O+H]^+$ 427.3571). ^{1}H-NMR: see Table 2. ^{13}C-NMR: see Table 1.

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