Two New Nortriterpenes from *Ligularia tongolensis*

Yi Feng Han, Kun Gao,* and Zhong Jian Jia*
State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, P. R. China

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Two new nortriterpenes having a ursane type structure were isolated from the roots of Chinese *Ligularia tongolensis*, and their structures were elucidated by interpretation of spectral data. Their relative stereochemistries were also clarified by detailed analysis of proton–proton coupling constants and by NOE experiments.

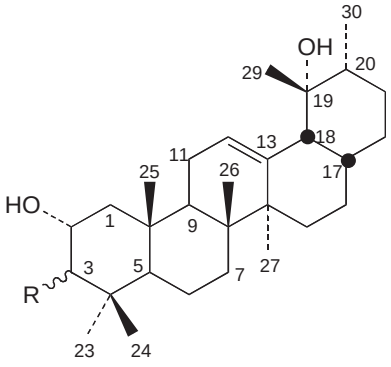
Ligularia tongolensis is widely distributed in northwestern China and has been used as a folk medicine to reduce phlegm, relieve cough, cure pulmonary tuberculosis, urinary track blockages, common cold, and pharyngitis.[1] Many species of *Ligularia* have been studied by our research group, and eremophilenolides are their major constituents.[2] However, from *Ligularia tongolensis*, which has never been studied preciously, we obtained except for a series of known eremophilenolides two novel triterpenes with a nor-carbon ursane skeleton, which was a rare structure of natural products. In this communication, we report the isolation and structural elucidation of the two new compounds.

Compounds **1** and **2** were isolated from the petroleum ether (60–90 °C)–Et_2O–MeOH (1:1:1) extract of the air-dried root (1.5 kg). The extract (80 g) was separated by repetitive column chromatography (SiO_2) to give **1** (4 mg) and **2** (3 mg) (Scheme 1).

Compound **1**, $[\alpha]_D^{20}$ −14 ° (*c* 0.125; CH_3OH), was obtained as an amorphous powder. The molecular formula of **1** was determined to be $C_{29}H_{48}O_3$ (6 unsaturations) by positive HRESI-MS (*m*/*z* 467.3488, calcd for $C_{29}H_{48}NaO_3$ $[M + Na]^+$ 467.3496). Its IR spectrum showed the absorption bands for hydroxyl groups (3334 cm^{-1}) and a double bond (1687 cm^{-1}). The ^1H NMR, ^{13}C NMR and DEPT spectra of **1** showed the presence of seven methyls, eight methylenes, eight methines, and six quaternarys, among which were two sp^2 carbon atoms of a carbon–carbon double bond, two methines bearing an oxygen and a quaternary carbon bearing an oxygen, indicating a nor-methyl pentacyclic triterpene structure with a double bond and three hydroxyls. Furthermore, the pair of characteristic double bond signals at δ 129.6 (CH) and 140.5 (C) in the ^{13}C NMR spectrum suggested a urs-12-ene skeleton.[3] However, the most significant difference between **1** and 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 39.3, CH) instead of one quarternary carbon (δ 33–47, C) in the known urs-12-ene derivatives.[4,5] A broad singlet at δ 2.50 in the ^1H NMR spectrum should be 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 ^1H–^1H COSY. So **1** was deduced to be a 28-norurs-skeleton.

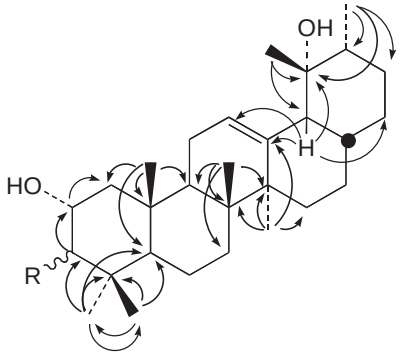
Except for 19-hydroxyl, other two oxygenated methines in the ^{13}C NMR spectrum were attributed to C-2, C-3 by analysis of HMBC data (Figure 1). The coupling constants between H-2 (δ 3.62, ddd, J = 10.5, 9.6, 4.2 Hz) and H-3 (δ 2.91, d, J = 9.6 Hz) confirmed that H-2 and H-3 were both axial with α and β-configuration, respectively. This was confirmed by the NOE experiment. Strong NOEs were observed between H-2_{ax} and β-Me-24 (4.8%), β-Me-25 (6.7%), indicating that these three proton systems are on the β-side of the A-ring. NOEs between H-3_{ax} and α-Me-23 (5.0%), indicating 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 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 acid.[6,7] Finally, NOEs were detected between β-Me-25 and β-Me-26, as expected. Thus, the structure of **1** was elucidated as 2α, 3β, 19α-trihydroxy-28-norurs-12-ene.

Compond **2**, $[\alpha]_D^{20}$ +8 ° (*c* 0.125; CH_3OH), obtained also as an amorphous powder, revealed the presence of hydroxyl groups (3388 cm^{-1}), trisubstituted double bond (1678 cm^{-1}) in its IR spectrum. The HRSI-MS spectrum showed peaks at *m*/*z* 427.3591 (calcd for $C_{29}H_{47}O_2$ $[M - H_2O + H]^+$ 427.3571),



1 R = β OH
2 R = α OH

Scheme 1.



Key HMBC of **1**, **2**
→ H to C

Figure 1.

Table 1. ^{13}C NMR and DEPT data for compounds **1** and **2** (75.5 MHz, CD_3OD)

C	1	2
1	48.4 t	42.8 t
2	69.8 d	67.5 d
3	84.8 d	80.4 d
4	40.8 s	39.8 s
5	57.0 d	49.9 d
6	20.0 t	19.6 t
7	34.3 t	34.1 t
8	41.4 s	41.6 s
9	48.7 d	49.0 d
10	39.5 s	39.7 s
11	25.0 t	25.0 t
12	129.6 d	129.7 d
13	140.5 s	140.4 s
14	43.0 s	43.1 s
15	29.9 t	29.9 t
16	27.3 t	27.4 t
17	39.3 d	39.7 d
18	55.4 d	55.4 d
19	73.9 s	73.9 s
20	43.4 d	43.4 d
21	27.6 t	27.6 t
22	26.9 t	27.0 t
23	29.6 q	29.6 q
24	17.3 q	22.8 q
25	16.9 q	17.3 q
26	17.8 q	17.8 q
27	25.0 q	25.2 q
29	31.1 q	31.0 q
30	20.0 q	19.6 q

which suggested the molecular formula $C_{29}H_{48}O_3$ for **2**, and this suggestion was further confirmed by the ^{13}C NMR and DEPT data (Table 1). Its structure could be elucidated by comparison with the spectral data of **1**.

Comparison of the ^{13}C NMR and DEPT spectral data (Table 1) of **2** with **1**, showed that their structures were similar, except that the resonance signals of the hydroxylated carbons C-2 and C-3 of **2** were shifted upfield at δ 67.5 for C-2 and δ 80.4 for C-3, but compound **1** was at δ 69.8 for C-2 and δ 84.8 for C-3. Furthermore, the ^{1}H NMR spectrum of **2** showed signals at δ 3.92 (brd, $J = 10.5$ Hz, H-2β), δ 3.32 (brs, H-3β), which suggested the α-configuration for the two hydroxyl groups on ring A. Compounds reported with a 2α,3α-diol system[7] had the same chemical shifts for C-2 and C-3 as those of compound **2**. This also confirmed the configuration of 2α,3α-diol for compound **2**.

The relative stereochemistry was further confirmed by NOE difference measurements. The H-18 proton which is axial with respect to the E-ring but equatorial on the D-ring, gave a strong NOE with H-12 (12.9%), 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 **2** is 2α,3α,19α-trihydroxy-28-norurs-12-ene.

Table 2. ^{1}H NMR data for compounds **1** and **2** (300 MHz, CD_3OD, TMS as int. standard)

H	1	2
1ax	0.93 dd (12.6, 10.5)	1.26 m
1eq	1.94 dd (12.6, 4.2)	1.59 m
2	3.62 ddd (10.5, 9.6, 4.2)	3.92 brd (10.5)
3	2.91 d (9.6)	3.30 brs
5	1.22 m	1.23 m
6ax	1.65 m	1.52 m
6eq	1.32 m	1.30 m
7ax	1.50 m	1.48 m
7eq	1.30 m	1.33 m
9	1.75 m	1.66 m
11ax	2.00 m	1.99 m
11eq	2.00 m	1.99 m
12	5.29 brs	5.29 brs
15ax	1.51 m	1.49 m
15eq	1.02 m	1.01 m
16ax	2.56 m	2.57 m
16eq	1.82 m	1.85 m
17	1.50 m	1.50 m
18	2.50 brs	2.50 brs
20	1.42 m	1.41 m
21ax	1.31 m	1.30 m
21eq	0.99 m	0.99 m
22ax	1.51 m	1.50 m
22eq	1.30 m	1.31 m
23	1.01 s	0.99 s
24	0.81 s	0.87 s
25	1.00 s	0.99 s
26	0.80 s	0.78 s
27	1.33 s	1.35 s
29	1.19 s	1.19 s
30	0.92 d (6.9)	0.92 d (6.6)

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