Sesquiterpenes, Nortriterpenes and Other Constituents from 
Ligularia tongolensis

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Two new eremophilane-type sesquiterpenes, 3β-(2’-methylbutanoyloxy)-8βH-eremophil-7(11)-ene-12,8α-(14,6α)-diolide (1) and 8βH-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,3β,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) plants1–7 we have studied the roots of Ligularia tongolensis (FRANCH) HANDMAZZ 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 6,9 9,12 10,13 11,14 12,15 and 13,16 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, [α]D20 +125.5° (CHCl3), 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, C29H48O3, 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 [δH 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); δC 175.5 (C), 41.3 (CH), 26.7 (CH2), 11.4 (CH3), 16.4 (CH3)], and the other 15 carbon signals suggested that 1 was an eremophilendiolide-type sesquiterpenes and its NMR data were similar to those of the known compound, 3β-angeloyloxy-8βH-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 moiety at C-3 was deduced from the heteronuclear multiple bond correlation (HMBC) spectrum, in which H-3 (δH 5.47) gave a long-range coupling with C-1’ (δC 175.5). Stereochemically, Me-14 and Me-15 are biogenetically β-orientated.10 The positive nuclear Overhauser effect spectroscopy (NOEs) between Me-15 and H-10 (5.30%) showed a cis-fused eremiphile. The coupling pattern observed for H-3 (δH 5.47 q, 3.0 Hz) implied that H-3 was an equatorial proton and should be β-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)-8βH-eremophil-7(11)-ene-12,8α(14,6α)-diolide.

The molecular formula of compound 2, C18H26O3, was determined by the HR-SI-MS, 13C-NMR and DEPT (distortion-less 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 δH 6.85, C-3 at δC 136.9 and C-4 at δC 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 8βH-eremophil-3,7(11)-dien-12,8α (14,6α)-diolide.

The molecular formula C29H48O5 for compound 7 was determined by the high resolution electrospray ionization mass spectrometry (HR-ESI-MS) and the data of 13C-NMR and DEPT. Its IR spectrum showed strong hydroxyl bands at...
7-methyls, 8-methylenes, 8-methines and 6 quaternary, among which, two sp² 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 δC 129.6 (CH) and 140.5 (C) in the ¹³C-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 CH₂-28 attached to C-17 and the appearance of one methine C-17 (δC 39.3, CH) instead of one quaternary carbon (δC 33—47, C) in the known urs-12-ene derivatives.²⁰,²¹ A broad singlet at δH 2.50 in the ¹H-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 ¹H-¹H correlation spectroscopy (¹H–¹H COSY). So 7 was deduced to have a 28-norurs-12-ene skeleton. Except for 19-hydroxyl, the other two oxygenated methines in the ¹³C-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-2α and β Me-24 (4.8%), and between H-2α and β Me-25 (6.7%), indicating that these three proton systems were on the β side of the A-ring. NOEs between H-3α 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 musanocapric and musanergic 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 C₃₃H₄₈O₃ was shown by accurate mass measurement at HR-ESI-MS. The IR spectrum of 8 showed hydroxyl bands at 3388 cm⁻¹ and a greater band at 1687 cm⁻¹, which was very similar to that of 7. The ¹H-, ¹³C-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 δH 67.5 for C-2 and δ 84.8 for C-3, but compound 7 was at δH 69.8 for C-2 and δ 84.8 for C-3. Furthermore, the ¹H-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 system²³ 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 NOE 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-2ax showed NOEs with the following spin systems: β Me-25 (9.0%), Me-24 (5.1%) and β H-3β (8.9%). Finally, H-3α 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 sulforhodamine B (SRB) method the anti-tumor activities of compounds 1, 2, 3, 7 and 8 against human he-
patoma (SMCC-7721), human embryo liver (L-02) and human leukemia (HL-60) cell lines were studied in comparison with standard 10-hydroxycamptothecine. The IC50 values of all the five tested compounds are weak with their values much more than 200 μg/ml, except for compound 8 whose IC50 value is 120.68 in HL-60 cell lines. The data reported in Table 3. Cytotoxicity (IC50 μg/ml) of Compounds 1, 2, 3 and 8.

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<th>Compound</th>
<th>SMMC-7721</th>
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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 CHCl3–MeOH (30:1, 15:1, 10:1, 7:1, 5:1) to give 10 frs: fr. 1 (1 mg), fr. 2 (1 mg), fr. 3 (1 mg), fr. 4 (1 mg), fr. 5 (1 mg), fr. 6 (1 mg), fr. 7 (1 mg), fr. 8 (1 mg), fr. 9 (1 mg), fr. 10 (1 mg). Fr. 3-2 (4 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 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)—MeOH (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 CHCl3–MeOH (10:1, 15:1, 20:1, 25:1, 30:1) to give 5 frs: fr. 4-1 (1 g), fr. 4-2 (1 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 CHCl3–MeOH (15:1, 10:1, 7:1, 5:1) to give 13 (200 mg).

1340 Vol. 53, No. 10

Table 3: Cytotoxicity (IC50 μg/ml) of Compounds 1, 2, 3 and 8.

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 obtained on a Varian Mercury-300BB NMR spectrometer with TMS as the internal standard. Electron impact ionization mass spectrometry (EI-MS) data were obtained on a Nicolet NEXUS 670 FT-IR instrument (neat). One-dimensional (1D) and two-dimensional (2D) NMR spectra were measured on a Perkin-Elmer 241 polarimeter. IR spectra were measured on a Nicolet NEXUS 670 FT-IR instrument (neat)

References