ble. The robustness could be demonstrated for changes of pH between 6.8 and 7.2, molality of NaCl between 0.25 and 0.35 M and temperature between 20 and 30 °C.

The results show that this method is selective and sensitive and therefore suitable for analyzing alemtuzumab in aqueous solution. Also a physical and chemical stability of Alemtuzumab was shown for a time-period of at least 14 days.

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A novel sesquiterpene from the roots of *Vladimiria souliei*

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A novel sesquiterpene lactone, 1β, 14-peroxy-4α-hydroxy-5αH, 7αH,6βH-eudesm-11(13)-en-6, 12 olide (**1**), was isolated from the roots of *Vladimiria souliei*. The structure was elucidated by spectroscopic methods.

Vladimiria souliei (Franch.) Ling is a medicinal plant mainly distributed in Sichuan Province, China. Its roots, a traditional Chinese medicine, have been used for relieving pain and stomach diseases since ancient times (Editorial commission of Traditional Chinese Medicine 1999). Previous phytochemical investigations on this species resulted in the isolation of sesquiterpene lactones (Tan et al. 1990a), and ligans (Tan et al. 1990b). In the course of our study, a new sesquiterpene lactone was isolated from the roots of *Vladimiria souliei* (Franch.) Ling. The structure was elucidated by spectroscopic methods. In this paper, we present the isolation and structural elucidation of the new sesquiterpene lactone based on the spectral analysis.

Compound **1** was obtained as colorless oil. Its ESI-MS spectrum showed a quasi molecular ion peak at m/z: 303 $[M + Na]^+$. Its molecular formula was determined as $C_{15}H_{20}O_5$ from its HR-ESI-MS spectrum (m/z 303.1217 $[M + Na]^+$, Calcd for $C_{15}H_{20}O_5Na$, 303.1208).

The ^{1}H NMR showed the olefinic protons of the double bond at δ 6.12 (1 H, d, J = 3.1 Hz, H-13a), δ 5.46 (1 H, d, J = 3.1 Hz, H-13b) along with one methyl at δ 1.49 (3 H, s, H-15). In addition, four protons of oxygenated carbons at δ 4.49 (1 H, br. s, H-1), 3.63 (1 H, t, J = 10.8, 10.8 Hz, H-6), 4.19 (1 H, d, J = 6.2 Hz, H-14a), 5.00 (1 H, J = 6.2 Hz, H-14b) and other protons in the upfield region were also observed in the ^{1}H NMR spectrum of compound **1**. The ^{13}C NMR spectrum revealed 15 carbon

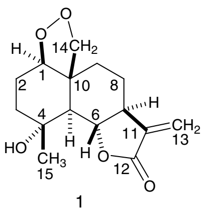

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Fig. 1: Chemical structure of compound **1**

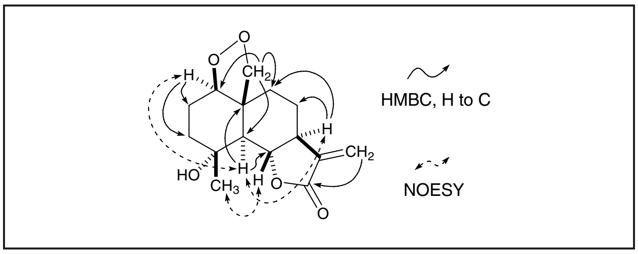

Fig. 2: The key HMBC and NOESY correlations of compound **1**

signals, including two olefinic carbons of the double bond [δ_C138.3 (C-11), δ_C118.3 (C-13)], four oxygenated carbon signals [δ_C 85.1 (C-1), δ_C 70.6 (C-4), δ_C 81.0 (C-6), δ_C 76.6 (C-14)], one methyl [δ_C 33.5 (C-15)], one ester carbonyl carbon signal [δ_C 170.0 (C-12)], and other carbon signals in the upfield. The other carbon signals in the upfield were further classified into methylenes [δ_C 26.6 (C-2), δ_C 32.9 (C-3), δ_C 22.3 (C-8), δ_C 37.5 (C-9)], methines [δ_C 50.7 (C-5), δ_C 48.6 (C-7), and one quaternary carbon at δ_C 42.0 (C-10)], which were confirmed by the HMQC and DEPT spectra. All the above spectral data indicated that compound **1** is a eudesmane sesquiterpene (Tan et al. 1990a; Blay et al 1993, Fig. 1).

In the HMBC spectrum (Fig. 2), the long rang correlations of H-1 and C-10/C-9/C-2/C-3/C-5, H-6 and C-4/C-5/C-10/C-7/C-8, H-14 and C-1/C-10/C-9, were observed. Based on these long range correlations, the carbon signals at δ 85.1, 81.0, 76.6 were attributed to C-1, C-6, C-14, respectively. Another oxygenated carbon signal at δ 70.6 was assigned to C-4 considering the correlations of H-2/H-3/H-5/H-6/H-15 and C-4. By the further analysis of the HMQC, HMBC and ^{1}H-^{1}H COSY spectra, all the proton and carbon signals were assigned unambiguously. However, the values of chemical shift of C-1(δ 85.1) and C-14 (δ 76.6) seemed a little different from those of common oxygenated carbons, which suggested the presence of a peroxy ring at C-1and C-14. The relative stereochemistry of compound **1** was elucidated by NOESY spectrum (Fig. 2) and the comparison of coupling constants with those reported in the literature. Therefore, compound **1** was elucidated as 1β,14β-peroxy-4α-hydroxy-5α*H*,7α*H*, 6β*H*-eudesm-11(13)-en-6,12 olide.

The cytotoxicity of was evaluated against A375-S2 cell line using MTT assay, which showed weak activity with an IC_{50} value >100 μM.

Experimental

1. Apparatus

The IR spectrum was measured on a Bio-Rad Fourier transform infrared (FTIR) spectrometer with KBr discs. The ESI-MS spectrum was performed on an LCQ-Advantage mass spectrometer made by Finnigan Company of America. HR-ESI-MS spectrum was obtained using an Ionspec 7.0T FTICR MS. The NMR spectra were recorded on an AV600 instrument (600 MHz for ^{1}H and 150 MHz for ^{13}C) made by Bruker Company of Switzerland with TMS as internal standard. HPLC separations were performed on a YMC-pack PREP-ODS column (20 × 250 mm) equipped with UV230$^+$ spectrophotometric detector and a Elite P230p series pumping system. Silica gel (200-300 meshes) and silica gel GF_{254} (Qingdao Marine Chemical Group Co. Ltd.) were used for column chromatography and TLC, respectively.

2. Plant material

The roots of *Vladimiria souliei* (Franch.) Ling were collected from Sichuan province, China, in July 2006. A voucher specimen (No. 20060703) is deposited at laboratory of the Research Department of Natural Medicine, college of Pharmaceutical Sciences, Nankai University, China.

3. Extraction and isolation

The roots (8.0 kg) of *Vladimiria souliei* (Franch.) Ling were extracted with methanol three times under reflux. Removing the solvent, the methanol extract was suspended in distilled water and successively partitioned three times by petroleum ether (P. E.) solvent to give 210.0 g residues. The petroleum ether soluble part was fractionated by silica gel column chromatography using P. E.-acetone (100 : 0 – 0 : 100) as eluent to obtain eight fractions. Fraction 6 was subjected to column chromatography over Sephadex LH-20 and further purified by PHPLC to afford compound **1**.

4. Characterization of compound 1

1β,14-Peroxy-4α-hydroxy-5αH,7αH,6βH-eudesm-11(13)-en-6,12 olide (**1**): $[\alpha]_D^{26}$: + 15.1 (c 0.05, MeOH); R_f: 0.45 (petroleum ether-acetone, 3 : 1); IR(KBr): 3560, 1760, 1662 cm^{-1}; ESI-MS: m/z 303 $[M + Na]^+$; HR-ESI-MS m/z 303.1217 $[M + Na]^+$, Calcd for $C_{15}H_{20}O_5Na$, 303.1208; ^{1}H NMR (600 MHz, $CDCl_3$): 4.49 (1 H, br. s, H-1), 1.86 (1 H, m, H-2), 1.47 (1 H, m, H-2), 2.51 (1 H, m, H-3) 1.87 (1 H, m, H-3), 1.63 (1 H, m, H-5), 3.63 (1 H, t, J = 10.8 Hz, H-6), 2.56 (1 H, m, H-7), 2.55 (1 H, m, H-8), 1.44 (1 H, m, H-8), 2.42 (1 H, m, H-9), 1.59 (1 H, m, H-9), 5.46 (1 H, d, J = 3.1 Hz, H-13b), 6.12 (1 H, d, J = 3.1 Hz, H-13a), 5.00 (1 H, d, J = 6.2 Hz, H-14b), 4.19 (1 H, d, J = 6.2 Hz, H-14a), 1.49 (3 H, s, H-15); ^{13}C NMR (150 MHz, $CDCl_3$): 85.1 (C-1), 26.6 (C-2), 32.9 (C-3), 70.6 (C-4), 50.7 (C-5), 81.0 (C-6), 48.6 (C-7), 22.3 (C-8), 37.5 (C-9), 42.0 (C-10), 138.3 (C-11), 170.0 (C-12), 118.3 (C-13), 76.6 (C-14), 33.5 (C-15).

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