Immunosuppressive Diterpenes from *Veronicastrum sibiricum*

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Two new diterpenes, named sibiriquinone A (1) and B (2), along with four known diterpenes have been isolated from the aerial part of *Veronicastrum sibiricum*. Their structures were elucidated by spectroscopy. The isolated compounds showed significant immunosuppressive activities.

**Key words** *Veronicastrum sibiricum*; diterpene; immunosuppressive activity

*Veronicastrum sibiricum* (L.) Pennell. (Scrophulariaceae), a medicinal plant growing in North China, has been used for treatment of rheumatism, dysentery, and arthritis.1) Pharmacological research of extracts with *V. sibiricum* indicated that the CHCl₃-extract had strong anti-inflammatory activity.2) In our search for pharmacologically active compounds from crude drugs of plant origin, we have started work on the isolation of the active principles from the aerial part of *V. sibiricum*. This paper deals with the isolation and structure elucidation of two new and four known compounds from the petroleum ether-soluble fraction of *V. sibiricum*. An immunosuppressive bioassay was carried out with these noteworthy diterpenoids showing significant inhibitory effect on lymphocyte transformation.

The petroleum ether-soluble fraction of aerial part of *V. sibiricum* was separated by repeated silica gel column chromatography, Sephadex LH-20 and preparative HPLC to give compounds 1—6.

Sibiriquinone A (1) was obtained as red solid, and its HR-ESI-MS spectrum showed a molecular ion peak at m/z 280.1485, indicating a molecular formula of C₁₉H₂₀O₂. The IR spectrum of the compound showed a carbonyl bond (1722 cm⁻¹) and the UV spectrum revealed the presence of an aromatic ring (225, 275 nm). The ¹H-NMR data revealed the presence of five olefinic protons [δ_H 7.87 (1H, d, J = 10.2 Hz), 7.50 (1H, d, J = 7.8 Hz), 7.12 (1H, d, J = 7.8 Hz), 7.09 (1H, s), 6.33 (1H, m, H-2)], one isopropyl group [δ_H 3.02 (1H, sept, J = 6.9 Hz), 1.17 (6H, d, J = 6.9 Hz)], and two tertiary methyl groups [δ_H 1.29 (6H, s)]. The ¹³C-NMR spectrum of 1 revealed nineteen carbon signals, including two carbonyl carbons (δ_C 183.2, 181.5), five double bonds in the lower field region [δ_C 148.0 (s), 144.9 (s), 139.9 (d), 139.5 (s), 137.2 (s), 134.4 (d), 134.2 (s), 130.6 (d), 129.2 (d), 124.7 (d)]. In addition, signals of four methyl groups, one methine, one methane and a quaternary carbon signals were also observed. Based on the above information, compound 1 was assumed to be an abietane-diterpene, with a p-quinoone ring, similar to that of triquitoquinone H which was reported in the previous paper.3)

In the HMBC spectrum of 1, the methine proton signal δ_H 7.12 (H-7) correlated with the signals at δ_C 181.5 (C-14), 148.0 (C-5), 130.6 (C-6), and 139.9 (C-9), and the methyl proton signal at δ_H 1.29 (H-18 or 19) correlated with the signals at δ_C 38.0 (C-3), 34.0 (C-4), 148.0 (C-5). In turn, the proton signal at δ_H 7.87 (H-1) correlated with the carbon signals at δ_C 38.0 (C-3), 148.0 (C-5), 137.2 (C-10). Therefore, the B-ring of 1 was an aromatic ring, and remaining double bond was located at C-1(2). Thus, the structure of sibirinone A (1) was elucidated as 11,14-dioxo-abiet-1,5(10)6,8,12-pentaene (Fig. 1). Assignments of the ¹H- and ¹³C-NMR spectral data were made on the basis of the 2D NMR spectra (see Experimental and Table 1).

Sibiriquinone B (2), C₁₉H₂₂O₂ ([M]⁺ at m/z 282.1615, HR-ESI-MS), showed the presence of a ketone group in the IR spectrum (1728 cm⁻¹) and the UV absorptions (220, 260 nm) of an aromatic ring. The ¹H-NMR spectrum of 2 showed three olefinic protons [δ_H 7.59 (1H, d, J = 7.9 Hz), 7.11 (1H, d, J = 7.9 Hz), 7.07 (1H, s), one isopropyl group [δ_H 3.02 (1H, sept, J = 6.9 Hz, H-15), 1.16 (6H, d, J = 6.9 Hz), and two tertiary methyls [δ_H 1.30 (6H, s)]. Its ¹³C-NMR spectral data was similar to those of 1, except for C-1, C-2 and C-10 (Table 1). Compound 2 was assumed to be a 1,2-dihydro sibiriquinone A. In the HMBC spectrum, the proton signal at δ_H 3.17 (H-1) correlated with the signals at δ_C 19.0 (C-2), 37.8 (C-3), 149.6 (C-5), and 144.5 (C-10), and the methyl proton signal at δ_H 1.30 (H-18, 19) correlated with the signals at δ_C 37.8 (C-3) and 149.6 (C-5). Therefore, the structure of sibiriquinone B (2) was determined to be 11,14-dioxo-abiet-5(10),6,8,12-tetraene.

By comparing spectral data, the known compounds 3—6 were identified as tanshinone I (3),4) dihydrotanshinone (4),5) tanshinone IIA (5),6) and cryptotanshinone (6),7) respectively.

In a search for immunosuppressive substances, we examined the immuno-inhibitory effect of these diterpenes on lymphocyte transformation (Table 2). The values of inhibition percent of compounds 1—6 revealed a significant distinction to the concanavalin A (Con A) control group (p<0.01, n=6), and showed inhibitory effect on lymphocyte

![Fig. 1](image-url)
transformation by comparing with a reference compound (dexamethasone). The immunosuppressive activity of these four known compounds has been determined for the first time.

**Experimental**

**General Experimental Procedures** NMR experiments were run on a Bruker AVANCE 300 instrument. 1H-NMR, 300 MHz; 13C-NMR, 75 MHz, both with tetramethylsilane as an internal standard. MS data were obtained on a MACKMU Auospec-utima ETOF instrument. Chromatography column, Silica-gel 60 (Qingdao Haiyang Chemical Co., Ltd) and Sephadex LH-20 (Amersham Pharmacia Biotech); HPLC, JASCO Gulliver Series, PU-1580 (pump), RI1530 and UV1575 (detector). Column type, ODS (Hibar RT 250-25, LiChrosorb, RP-18); IR spectra were recorded on a 1710 Infrared pump), RI1530 and UV1575 (detector). Column type, ODS (Hibar RT 250-25, LiChrosorb, RP-18); IR spectra were recorded on a 1710 Infrared spectrometer (BIO-TEK). Optical rotation was measured with a MC 241 digital polarimeter (PERKIN-ELMER).

**Plant Material** The aerial part of *Veronicastrum sibiricum* (L.) PENNELL, was collected in Aug. 2001 in North China, and identified by Prof. Liang-Xin Wang. A voucher specimen (D20010801) was deposited at the College of Pharmaceuticals and Biotechnology, Tianjin University, China.

**Extraction and Isolation** The dried aerial part (2.0 kg) of *V. sibiricum* was crushed and extracted 3 times with EtOH (95%), 151 each at 50°C for 6 h. The EtOH extracts were concd. to give a residue (0.25 kg), which was partitioned between petroleum ether and H2O. The petroleum ether layer was concd. to give a residue (24.5 g), which was chromatographed on a silica-gel (800 g) column (80%:50 mm). The column was eluted with solvents of increasing polarity [petroleum ether–EtOAc (7:1, 5:1, 3:1, 1:1, 1:3), EtOAc, EtOAc–MeOH (19:1, 10:1) to give 21 fractions (fr. 1—21). Combined fractions 11 and 12 (1.8 g) were chromatographed on a silica gel column (CHCl3–MeOH, 99:1, 98:2) to give six fractions (fr. 11.1—11.6). Fraction 11.3 (250 mg) was chromatographed on Sephadex LH-20 (MeOH) to give five fractions (fr. 11.3.1—11.3.5). Fraction 11.3.5 was separated by HPLC (ODS, CH3CN) to give 5 (27 mg). Fraction 9 (0.7 g) was chromatographed on silica gel (CHCl3–n-hexane, 9:1) to give seven fractions. (fr. 9.1—9.7). Fraction 9.6 was separated by HPLC (ODS, CH3CN) to give 1 (53 mg) and 2 (8 mg). Fraction 15 (1.8 g) was chromatographed on a silica-gel column (CHCl3–CH2Cl2, 98:2) to give four fractions (fr. 15.1—15.4). Fraction 15.1 was crystallized using MeOH to give 3 (91 mg). Fraction 15.2 was dissolved with CH3CN and dissolved portion was separated by HPLC (ODS, CH3CN) to give 4 (7 mg) and 6 (17 mg).

**Table 1.** 1H- and 13C-NMR Spectral Data of Compounds 1 and 2

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<td>H (J / Hz)</td>
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**Table 2.** Inhibitory Effects of Compounds 1—6 and Pe. extract

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OD490nm=63% (50 µg/ml).

**References**