Immunosuppressive Diterpenes from *Veronicastrum sibiricum*

Wenyuan GAO,[a] Rong ZHANG,[a] Wei JIA,[b] Jun ZHANG,[c] Yoshihisa TAKAISHI,[d] and Hongquan DUAN*,[a]

[a] *The College of Pharmaceuticals and Biotechnology, Tianjin University; Tianjin 300072, China:* [b] *The School of Pharmacy, Shanghai Jiao Tong University; Shanghai, 200030, China:* [c] *The School of Pharmacy, Tianjin Medical University; Tianjin 300070, China: and* [d] *Faculty of Pharmaceutical Sciences, University of Tokushima; 1–78 Shomachi, Tokushima 770–8505, Japan.* Received July 25, 2003; accepted September 4, 2003

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 $CHCl_3$-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**.

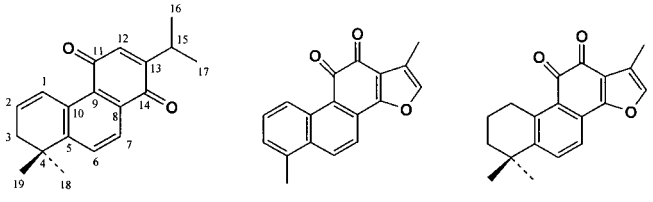
Sibiriquinone A (**1**) was obtained as red solid, and its HR-EI-MS spectrum showed a molecular ion peak at *m/z* 280.1485, indicating a molecular formula of $C_{19}H_{20}O_2$. The IR spectrum of the compound showed carbonyl bond (1722 cm^{-1}) and the UV spectrum revealed the presence of an aromatic ring (225, 275 nm). The ^{1}H-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 ^{13}C-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 methylene 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*-quinone ring, similar to that of tritoquinone 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-abieta-1,5(10),6,8,12-pentaene (Fig. 1). Assignments of the ^{1}H- and ^{13}C-NMR spectral data were made on the basis of the 2D NMR spectra (see Experimental and Table 1).

Sibiriquinone B (**2**), $C_{19}H_{22}O_2$ ($[M]^+$ at *m/z* 282.1615, HR-EI-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 ^{1}H-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 [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 ^{13}C-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-abieta-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)] cryptotanshinone (**6**),[7)] respectively.

In a search for immunosuppressive substances, we examined the immuno-inhibitory effect of these diterpenes on lymphocyte transformation[8,9)] (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



1 **3** **5**

2 1,2-dihydro **4** 15,17-dihydro **6** 15,17-dihydro

Fig. 1

* To whom correspondence should be addressed. e-mail: duan@tju.edu.cn

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

Position	**1**		**2**	
	H (*J*: Hz)	C	H (*J*: Hz)	C
1	7.87 (d, 10.2)	124.7	3.17 (t, 6.4)	29.7
2	6.33 (m)	134.4	1.80 (m)	19.0
3	2.28 (dd, 4.5, 1.8)	38.0	1.65 (m)	37.8
4	—	34.0	—	34.5
5	—	148.0	—	149.6
6	7.50 (d, 7.8)	130.6	7.59 (d, 7.9)	133.7
7	7.12 (d, 7.8)	129.2	7.11 (d, 7.9)	127.9
8	—	134.2	—	133.4
9	—	139.5	—	139.8
10	—	137.2	—	144.5
11	—	183.2	—	182.4
12	7.09 (s)	139.9	7.07 (s)	139.0
13	—	144.9	—	145.0
14	—	181.5	—	181.5
15	3.02 (sept., 6.9)	26.9	3.02 (sept., 6.9)	26.9
16	1.17 (d, 6.9)	21.5	1.16 (d, 6.9)	21.5
17	1.17 (d, 6.9)	21.5	1.16 (d, 6.9)	21.5
18	1.29 (s)	28.3	1.30 (s)	31.7
19	1.29 (s)	28.3	1.30 (s)	31.7

Table 2. Inhibitory Effects of Compounds **1**—**6** and P.e.extract

Compounds	Inhibition (%)		
	80 μg/ml	20 μg/ml	5 μg/ml
P.e.extract	35	3	−63
1	41	32	−12
2	52	35	12
3	37	22	6
4	49	28	−28
5	46	35	−24
6	42	36	17

$OD_{dexamethasone}$=63% (50 μg/ml).

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. ^{1}H-NMR, 300 MHz; ^{13}C-NMR, 75 MHz, both with tetramethylsilane as an internal standard. MS data were obtained on a MACKMS 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 Fourier Transform spectrometer (PERKIN-ELMER), UV spectra were obtained on a $UVIKON_{XS}$ recording 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%, 15 l each) at 50 °C for 6 h. The EtOH extracts were concd. *in vacuo* to give a residue (0.25 kg), which was partitioned between petroleum ether and H_2O. The petroleum ether layer was concd. to give a residue (24.3 g), which was chromatographed on a silica-gel (800 g) column (80×850 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 ($CHCl_3$–MeOH, 99 : 1, 95 : 5) 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, CH_3CN) to give **5** (27 mg). Fraction 9 (0.7 g) was chromatographed on silica gel ($CHCl_3$–*n*-hexane, 9 : 1) to give seven fractions. (fr. 9.1—9.7). Fraction 9.6 was separated by HPLC (ODS, CH_3CN) to give **1** (53 mg) and **2** (8 mg). Fraction 15 (1.8 g) was chromatographed on a silica-gel column ($CHCl_3$; $CHCl_3$–MeOH, 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 CH_3CN and dissolved portion was separated by HPLC (ODS, CH_3CN) to give **4** (7 mg) and **6** (17 mg).

Sibiriquinone A (**1**): Red solid, $[\alpha]_D^{25}$+16.6° (c=0.2, MeOH). UV λ_{max}^{MeOH} nm (log ε): 225 (4.25), 275 (3.24), 464 (3.52). IR ν_{max}^{KBr} cm^{-1}: 2962, 2927, 1722, 1662, 1626, 1465, 1428, 1258, 1232, 1147, 944, 755. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see Table 1. EI-MS: *m*/*z* 280 $[M]^+$ (5), 252 (54), 237 $[M-CH(CH_3)_2]^+$ (100), 222 (12), 179 (22), 149 (90), 71 (12), 57 (13), 41 (12). HR-EI-MS *m*/*z*: 280.1485. $C_{19}H_{20}O_2$ required 280.1463.

Sibiriquinone B (**2**): Red solid, $[\alpha]_D^{25}$+7.8° (c=0.2, MeOH). UV λ_{max}^{MeOH} nm (log ε): 220 (4.17), 260 (3.99), 450 (3.22). IR ν_{max}^{KBr} cm^{-1}: 2962, 2932, 1728, 1660, 1563, 1462, 1390, 1260, 1143, 938, 756. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see Table 1. EI-MS: *m*/*z* 282 (13), 255 (53), 239 $[M-CH(CH_3)_2]^+$ (100), 224 (14), 165 (12), 115 (4), 89 (4), 41 (6). HR-EI-MS *m*/*z*: 282.1615. $C_{19}H_{22}O_2$ required 282.1620.

Procedure of Bioassay The samples were prepared by dissolving the extract and compounds from *V. sibiricum* with dimethyl sulfoxide, followed by dilution of solutions into different concentrations with Hank's solution. Then, they were mixed with 1 ml of lymphocytes (5×10^6/ml) and incubated (37 °C, 5% CO_2) for 72 h, concanavalin A (Con A) being used as a control group. The OD values of the samples were measured at 490 nm.

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