Two New Icetexane Diterpenoids from *Salvia przewalskii*

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Two new icetexane diterpenoids, przewalskin C, D (1, 2), together with sixteen known diterpenoids, were isolated from *Salvia przewalskii*. Eight of known ones (compounds 3, 12—18) were reported firstly from this plant. To the best of our knowledge, it's the first report of icetexane diterpenoids from this plant. The identification and structural elucidation of these compounds were based on 1D- and 2D-NMR spectral data analysis.

Key words *Salvia przewalskii*; icetexane diterpenoid; przewalskin C; przewalskin D

The Tibetian folk drug 'Hong Qin Jiao' is the dried roots of *Salvia przewalskii* MAXIM (Labiatae) which is widely distributed in the northwest of China.[1,2] Many bioactive abietane diterpenoids, especially naphthaquinones, have been reported as the main secondary metabolites of this plant.[3—6] In a continuation of our research work on the diterpenoids from *Salvia* species,[7] we examined the constituents of *S. przewalskii* MAXIM collected in Shanggelila of Yunnan province. Two new icetexane diterpenoids, named przewalskin C, D (**1**, **2**), were isolated together with sixteen known diterpenoids.

The Me_2CO fraction of *S. przewalskii* (whole parts) was subjected to silica gel chromatography to afford two new icetexane diterpenoids trivially named as przewalskin C, D (**1**, **2**) along with sixteen known ones: barbatusol (**3**),[8] tanshinone I (**4**),[9] cryptotanshinone (**5**),[10] tanshinone IIA (**6**),[11] ferruginol (**7**),[12] sugiol (**8**),[13] carnosol (**9**),[14] przewalskin (**10**),[5] 1,2-dihydrotanshinone (**11**),[15] salvicanol (**12**),[16] rosmadial (**13**),[10] pisiferin (**14**),[17] pisiferanol (**15**),[17] 8,11,13-icetexantrien-10,11,12-triol (**16**),[18] danshenspiroketallactone (**17**),[19] and epi-danshenspiroketallactone (**18**).[19] The identification and structural elucidation of these compounds were based on 1D- and 2D-NMR spectral data analysis.

Compound **1** was obtained as an amorphous powder. The molecular formula, $C_{21}H_{30}O_3$, was deduced from the positive high-resolution electrospray ionization mass spectrum (HR-ESI-MS) ($[M+Na]^+$ *m/z* 353.2084) and NMR spectra (Table 1). The IR spectrum displayed the absorption bands due to aromatic ring (1626 cm^{-1}) and hydroxyl groups (3440 cm^{-1}). The UV spectrum showed the absorption maximum at 240.2 nm (1.85) and 275.4 nm (1.71), indicative of a simple phenolic ring. The ^{13}C-NMR spectrum showed five methyls (including a methoxyl), four methylenes, five methines (including two olefinic ones and an oxygenated one), and seven quaternary carbons (including six olefinic ones). Considering the structures of the compounds previously isolated from this genus and the formula of **1**, along with the characteristic methine signals at δ_C 27.3 and 43.7 due to C-15 and C-5, respectively, a noticeable quaternary carbon signals at δ_C 31.7 (C-4), and four characteristic methyl signals at δ_C 22.6 (C-16, C-17), 27.6 (C-18), and 26.6 (C-19), compound **1** could be ascribed to be an icetexane or abietane diterpenoid.[15—18] Carefully analysis of the 1D-NMR spectra indicated that compound **1** could be ascribed to be an icetexane diterpenoid by the absence of the characteristic quaternary carbon (C-10) and methyl group (Me-20) for abietane diterpenoids.[8,16—18] A comparison of the ^{1}H- and ^{13}C-NMR spectral data of **1** with those of barbatusol (**3**) indicated that they were strikingly similar except for the presence of a methoxyl group in **1**.[8] The methoxyl group was located at C-7 on the basis of the heteronuclear multiple bond correlation (HMBC) correlations of δ_H 3.26 (3H, s, OMe)/C-7 (δ_C 82.5), together with correlations of δ_H 4.32 (1H, dd, J=8.3, 3.5 Hz, H-7)/C-5 (δ_C 43.7, d), C-6 (36.5, t), C-8 (122.3, s), C-9 (131.9, s), C-14 (116.0, d), and the methoxyl signal at δ_C 56.7 (Fig. 1).

The ROESY cross-peaks of H-5/H-18, H-5/H-7, and H-7/H-14 established the H-5 and H-7 were both in α-orientation. Accordingly, the structure of przewalskin C (**1**) was elucidated as 7β-methoxyicetexa-1(10),8,11,13-tetraene-11,12-diol.

Compound **2** was isolated as an amorphous powder. It has a molecular formula of $C_{20}H_{26}O_2$ based on the HR-ESI-MS peak at *m/z* 321.1839 ($[M+Na]^+$). The ^{13}C-NMR spectrum (Table 1) displayed 20 carbon signals in which contained four methyls, three methylenes, six methines (including four olefinic carbons), and seven quaternary carbons (including six olefinic ones). The same skeleton and ring C of **2** as those of **1** were inferred by the extensive comparison of the ^{13}C-NMR spectral data with those of **1**. Ten olefinic carbons can be found in the ^{13}C-NMR spectrum of **2**, which indicated the presence of two double-bonds except for the aromatic ring (ring C) in **2**. The olefinic proton at δ_H 6.34 (1H, s) could be assigned to H-20 by its HMBC correlations with C-1 (δ_C 129.4, d), C-5 (45.6, d) and C-9 (133.6, s), which suggested the presence of $\Delta^{10,20}$. The unsaturated proton at δ_H 6.29 (1H, d, J=9.6 Hz) showed HMBC correlations with C-5 (δ_C 45.6, d), C-9 (133.6, s) and C-10 (143.1, s), which indicated this

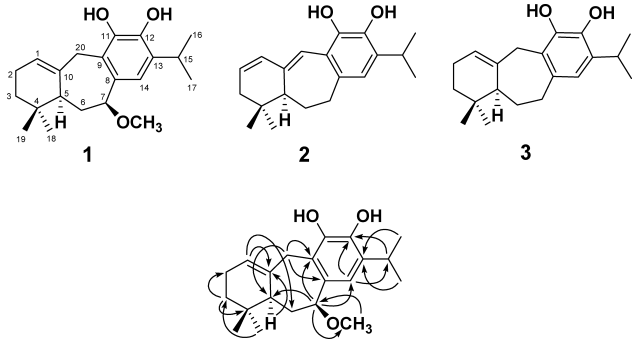

Fig. 1. Selected HMBC Correlations of **1**

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

	1		**2**	
	δ_C	δ_H	δ_C	δ_H
1	120.7 d	5.47 (br s)	129.4 d	6.29 (d, 9.6)
2	23.2 t	2.00 (m, H-2α)	127.8 d	5.75 (m)
		1.57 (m, H-2β)		
3	30.6 t	1.39 (m, H-3α)	37.0 t	2.03 (m)
		1.08 (m, H-3β)		1.81 (m)
4	31.7 s		30.9 s	
5	43.7 d	1.93 (m, H-5)	45.6 d	2.03 (m)
6	36.5 t	1.95 (m, H-6α)	34.0 t	2.09 (m, H-6α)
		1.57 (m, H-6β)		1.72 (m, H-6β)
7	82.5 d	4.32 (dd, 8.3, 3.5)	32.3 t	2.49 (m, H-7)
				2.38 (m, H-7)
8	122.3 s		122.4 s	
9	131.9 s		133.6 s	
10	137.7 s		143.1 s	
11	140.6 s		140.1 s	
12	140.2 s		139.4 s	
13	130.9 s		133.1 s	
14	116.0 d	6.64 (s)	117.9 d	6.58 (s)
15	27.3 d	3.07 (sept, 6.8)	27.3 d	3.19 (sept, 6.8)
16	22.6 q	1.24 (d, 6.8)	22.4 q	1.23 (d, 6.8)
17	22.6 q	1.23 (d, 6.8)	22.3 q	1.22 (d, 6.8)
18	27.6 q	0.86 (s)	28.8 q	0.84 (s)
19	26.6 q	0.84 (s)	26.0 q	0.83 (s)
20	33.2 t	3.55 (d, 15.8, H-20β)	120.0 d	6.34 (s)
		3.54 (d, 15.8, H-20α)		
OMe	56.7 q	3.26 (s)		

Spectra were measured at 100 MHz for ^{13}C and 400 MHz for ^{1}H in $CDCl_3$.

proton could be ascribed to H-1. Another olefinic proton at δ_H 5.75 (1H, m) which correlated with H-1 in the COSY experiment showed the HMBC correlations with C-3 (δ_C 37.0, t) and C-10 (143.1, s). So, this proton can be assigned to H-2. Accordingly, the structure of **2** was elucidated as icetexa-1,8,10(20),11,13-pentaene-11,12-diol, and named przewalskin D.

In this paper, we have reported 18 diterpenoids. Among them, przewalskin C, D were two new icetexane diterpenoids, and eight of the known ones (compounds **3**, **12**—**18**) were all the first time to be reported from *S. przewalskii*. Icetexane diterpenoids, which can be seen as rearranged abietane diterpenoids, were the first time to be reported from *S. przewalskii* although some have been isolated from the genus *Salvia*.[16,18]

Experimental

General Procedure Optical rotations were measured with a HORIBA SEPA-300 High Sensitive Polarimeter. UV spectra were obtained on a UV 2401 PC spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. ^{1}H- and ^{13}C-NMR experiments were performed on a Bruker AM-400 spectrometer, while 2D NMR spectra were recorded by using a Bruker DRX-500 NMR instrument. EI-MS were obtained on a VG Auto Spec-3000 spectrometer (70 eV). ESI-MS and HR-ESI-MS were taken on an API Qstar Pulsar instrument. Column chromatography was performed on silica gel and silica gel H (200—300 mesh, 10—40 μm, Qingdao Marine Chemical Inc. China). Lichroprep RP-18 (43—63 μm, Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.) were also used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material The plant sample of *S. przewalskii* (whole plant) were collected in shanggelila of Yunnan province in August, 2002, and was identified by Prof. Xi-Wen Li of the Kunming Institute of Botany, the Chinese Academy of Sciences, P. R. China. A voucher specimen, 2000216, was deposited in Kunming Institute of Botany.

Extraction and Isolation The dried and powdered (11.9 kg) *S. przewalskii* were extracted with Me_2CO (40 l) for three times (3 d each time) at room temperature. The solvent was removed under vacuum. The gummy residue (310 g) was subjected to column chromatography over DM-130 porous resin and eluted with MeOH–H_2O (5 : 5, 9 : 1). The residue of MeOH–H_2O (9 : 1) fraction was partitioned between H_2O and EtOAc. The EtOAc part was subjected to column chromatography over silica gel. Mixtures of petroleum ether–Me_2CO of increasing polarity were used as eluents. Six fractions were collected and combined by monitoring with TLC. Compounds **4** (7 g), **5** (27 g), and **6** (24 g) was crystallized from the second, third, and forth fraction, respectively. The first fraction was chromatographed on a silica gel column eluted with petroleum ether–$CHCl_3$–EtOAc (9 : 0.7 : 0.3) to afford **7** (78 mg) and **14** (12 mg). The second fraction was chromatographed over silica gel (cyclohexane–EtOAc, 85 : 15) and Sephadex LH-20 (CH_3Cl–MeOH, 1 : 1) to give **8** (15 mg), **9** (288 mg), **13** (18 mg), **3** (450 mg), and **15** (58 mg). Fraction 3 was repeatedly chromatographed over silica gel (CH_3Cl–EtOAc, 9 : 1), Lichroprep RP-18 eluted by MeOH–H_2O (from 7 : 3 to 1 : 0), and Sephadex LH-20 (CH_3Cl–MeOH, 1 : 1) to give **10** (230 mg), **12** (84 mg), **16** (66 mg), **17** (6 mg), and **18** (2 mg). Compounds **1** (4 mg), **2** (3 mg), and **11** (12 mg) were purified employing silica gel (CH_3Cl–EtOAc, 4 : 1) and HPLC by MeOH–MeCN–H_2O (70 : 5 : 25) from the fifth fraction.

Przewalskin C (**1**): A white amorphous powder, $[\alpha]_D^{21.6}$: −192.08° (c=0.05, CH_3Cl). UV λ_{max} (MeOH) nm (log ε): 240.2 (1.85), 275.4 (1.71). IR (KBr) cm^{-1}: 3440, 2958, 2924, 1627, 1444, 1384, 1363, 1296, 1109, 1092, 1006, 963. ^{1}H- and ^{13}C-NMR: Table 1. EI-MS *m/z*: 330 ($[M]^+$, 6), 298 (70), 283 (12), 255 (15), 242 (100), 227 (34), 199 (18), 181 (14), 165 (12), 95 (5). Positive HR-ESI-MS *m/z*: 353.2084 (Calcd for $[M+Na]^+$, $C_{21}H_{30}O_3Na$: 353.2092).

Przewalskin D (**2**): A white amorphous powder, $[\alpha]_D^{21.7}$: +6.70° (c=0.29, CH_3Cl). UV λ_{max} (MeOH) nm (log ε): 239.2 (1.42), 288.2 (1.52). IR (KBr) cm^{-1}: 3426, 2958, 2925, 1627, 1439, 1365, 1295, 1221, 1163, 1110, 1059, 1030, 1005. ^{1}H- and ^{13}C-NMR: Table 1. EI-MS *m/z*: 298 ($[M]^+$, 100), 283 (12), 255 (14), 241 (9), 195 (18), 149 (50). Positive HR-ESI-MS *m/z*: 321.1839 (Calcd for $[M+Na]^+$, $C_{20}H_{26}O_2Na$: 321.1830).

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