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Przewalskin B, a Novel Diterpenoid with an Unprecedented Skeleton from *Salvia przewalskii* Maxim

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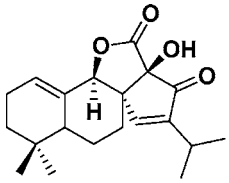
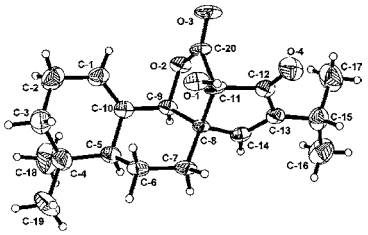
ABSTRACT





Przewalskin B (1), a novel diterpenoid possessing a unique skeleton, was isolated from a Chinese medicinal plant *Salvia przewalskii*. Its structure and relative stereochemistry were elucidated by extensive NMR analysis and a single-crystal X-ray study. A possible biosynthetic pathway for 1 was proposed. Compound 1 exhibited modest anti-HIV-1 activity with EC_{50} = 30 μg/mL.

The genus *Salvia* is a rich source of diterpenoids with structural diversity.[1] Many diterpenoids with interesting bioactivities, such as tanshinone IIA, salvicine, neotanshinlactone, and salvinorin A, have been reported from this genus.[2] *S. przewalskii* was a traditional medicinal plant used as the surrogate of *S. miltiorrhhiza* (Danshen) for the treatment of various cardiovascular diseases.[3] Aiming at identifying structurally interesting and bioactive metabolites from the *Salvia* species, we examined the acetone extract of *S. przewalskii* Maxim. A novel C_{23} terpenoid and two new icetexane diterpenoids have been isolated and reported in our earlier publications.[4] In this paper, we report an additional novel diterpenoid (przewalskin B, **1**) with an unprecedented skeleton from this plant. The skeleton of **1** was noticeable for its unique rearranged skeleton probably derived from a

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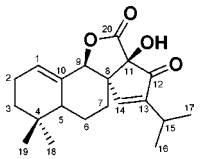
normal abietane diterpenoid. Compound **1** could be seen as the first diterpenoid with a five-membered spiro ring and α-hydroxy-β-ketone lactone moieties, although three spirolactone norditerpenoids have been reported.[5] Compound **1** exerted weak cytotoxicity against C8166 cells with CC_{50} = 100.74 μg/mL and showed anti-HIV-1_{IIIB} activity with EC_{50} = 30.32 μg/mL and SI (selectivity index) = 3.32. In addition, compound **1** showed no significant inhibitory activity toward the HL-60, K562, OVCA-2780, A549, and HepG-2 cell lines (IC_{50} > 100 μg/mL). The isolation, structural elucidation, and bioactivities of **1** are described in this paper.



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The whole plants of *S. przewalskii* were collected in Shanggelila in the Yunnan province, PRC, in August, 2003, and were identified by Prof. H. W. Li. The air-dried and powdered sample (10.3 kg) was extracted with acetone (3 × 30 L × 24 h) at room temperature and concentrated in a vacuum to give a crude extract which was subjected to column chromatography over DM-130 porous resin and eluted with MeOH−H_2O (1:1 and 9:1). The MeOH−H_2O (9:1) fraction (390 g) was subjected to column chromatography over silica gel, eluting with a gradient of EtOAc in petroleum ether, to yield seven fractions (I−VII). Fraction V was subjected to further column chromatography over silica gel, C-18, Sephadex LH-20, and semipreparative HPLC (Agilent 1100 HPLC system, Zorbax SB-C-18, Agilent, 9.4 × 250 mm, MeOH−MeCN−H_2O 7:1:2) to afford **1** (3.6 mg).

Przewalskin B (**1**) was isolated as a colorless needle.[6] Its molecular formula was determined as $C_{20}H_{26}O_4$ by HRESIMS for the $[M + Na]^+$ ion at *m/z* 353.1731. The IR spectrum of **1** showed the absorptions for hydroxy (3476 cm^{-1}), lactone (1767 cm^{-1}), conjugated ketone (1706 cm^{-1}), and olefinic (1618 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **1** (Table 1) showed 20 carbon resonances due to seven quaternary carbon (including one ketone, one lactone, an oxygenated one, and two olefinic ones), five tertiary carbon (including an oxygenated and two olefinic ones), four methylene, and four methyl groups.

The structure of **1** was elucidated by the analysis of 2D NMR data and X-ray analysis. The following correlations can be found in the HMBC spectrum of **1** recorded in $CDCl_3$ (Figure 1): H-1 (δ_H 5.75, br s) with C-2, C-3, and C-5; Me-18 (δ_H 0.86, s, 3H) and Me-19 (0.91, s, each 3H) with C-3, C-4, and C-5, and Me-18 with C-19; H-6a (δ_H 1.73, m) with C-5, C-7, C-8, and C-10; H-7a (δ_H 1.80, m, 1H) and H-7b (δ_H 1.70, m, 1H) with C-5 and C-8; H-14 (δ_H 7.08, s) with C-7, C-8, C-12, C-13, and C-15; H-15 (δ_H 2.59, sept, J = 7.6 Hz) with C-12, C-13, C-14, C-16, and C-17; OH-11 (δ_H 3.79, br s) with C-8 and C-12. The correlations of Me-16 (δ_H 1.10, d, 7.6, 3H) and Me-17 (δ_H 1.05, d, 7.6, 3H) with C-13 and C-15 indicated the presence of an isopropyl moiety.

Table 1. ^1H and ^{13}C NMR Assignments of **1**

	in $CDCl_3$		in acetone-d_6	
no.	δ_H (mult, J, Hz)	δ_C (mult)	δ_H (mult, J, Hz)	δ_C (mult)
1	5.75, br s	121.0, d	5.65, br s	120.0, d
2α	2.03, m	22.3, t	2.04, m	23.0, t
2β	2.03, m		2.04, m	
3α	1.38, m	32.3, t	1.42, m	33.3, t
3β	1.20, m		1.22, m	
4		31.2, s		31.7, s
5α	1.52, m	44.9, d	1.71, m	45.3, d
6a	1.73, m	25.1, t	1.83, m	25.7, t
6b	1.52, m		1.63, m	
7a	1.80, m	29.3, t	1.85, m	29.7, t
7b	1.70, m		1.78, m	
8		54.2, s		54.9, s
9	4.76, s	82.1, d	4.96, br s	82.3, d
10		135.9, s		138.2, s
11		82.1, s		83.5, s
12		200.4, s		201.0, s
13		149.0, s		149.2, s
14	7.08, s	158.3, d	7.44, s	159.4, d
15	2.59, sept, 7.6	25.2, d	2.54, sept, 7.1	25.9, d
16	1.10, d, 7.6	20.5, q	1.10, d, 7.1	20.8, q
17	1.05, d, 7.6	20.4, q	1.04, d, 7.1	20.7, q
18	0.86, s	26.3, q	0.88, s	26.4, q
19	0.91, s	27.5, q	0.93, s	27.9, q
20		173.1, s		173.7, s
OH-11	3.79, br s			

In the HMBC spectrum of **1** recorded in $CDCl_3$, H-9 showed weak correlation with only C-20; in addition, the HMBC correlations of H-1 with C-9, H-9 with C-11, and H-14 with C-9 and C-11 could not be assigned due to the overlap of C-9 and C-11. Fortunately, in the HMBC spectrum of **1** recorded in acetone-d_6, the HMBC correlations of H-9/C-1, C-8, C-10, C-11, C-14, and C-20 could be observed. The HMBC correlations of H-1/C-9, H-9/C-11, and H-14/C-9 and C-11 could be assigned due to good resolution between C-9 and C-11.

On the basis of the above HMBC correlations, together with H-1/H_2-2/H_2-3 and H-5/H_2-6/H_2-7 proton spin systems

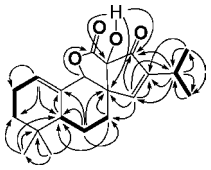

Figure 1. Key COSY and HMBC correlations of **1** in $CDCl_3$.

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(6) Przewalskin B (**1**). Colorless crystal; $[\alpha]_D^{18.0}$ −25.7° (*c* 0.49, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (1.20) nm; IR (KBr) ν_{max} 3490, 3476, 2961, 2922, 1767, 1737, 1705, 1618, 1454 cm^{-1}; NMR, see Table 1; EIMS *m/z* (rel. int.) 330 ($[M]^+$, 28), 312 (26), 286 (28), 243 (100), 238 (43), 211 (42), 187 (58), 169 (38), 117 (44), 91 (89), 79 (66), 77 (63); HR-ESIMS found 353.1731, calcd for $C_{20}H_{26}O_4Na$ 353.1729.

obtained from the $^1H-^1H$ COSY spectrum recorded in $CDCl_3$, the basic structure of **1** was determined.

In the ROESY spectrum of **1** recorded in $CDCl_3$, the ROESY correlations of Me-19/H-2β and H-3β confirmed the β-orientation of Me-19. The correlation between H-9 and H-14 suggested that they were in the same orientation. The correlations of H-15/Me-16 and Me-17 were also observed, which confirmed the presence of an isopropyl group (Figure 2).

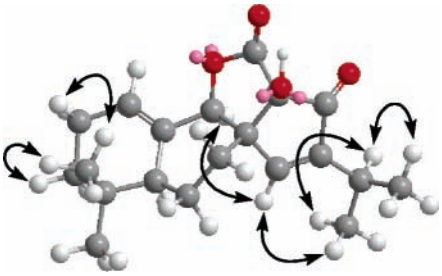

Figure 2. Key ROESY correlations of **1**.

However, the ROESY spectra could not provide more sufficient information to elucidate the stereochemistry of C-8, C-9, and C-11. After many attempts with different solvents, a single crystal of **1** was finally obtained from MeOH−H_2O (95:5) solvents. A single-crystal X-ray study of **1** clarified the β-orientation of OH-11 and C-11 and the α-orientation of H-9 and C-14 and unambiguously confirmed the structure of **1** (Figure 3).[7]

(7) Crystallographic data for **1**: $C_{20}H_{26}O_4$, $M = 330.41$, orthorhombic, space group $P2_12_12_1$, $a = 6.751$ Å, $b = 11.662$ Å, $c = 23.577$ Å, $V = 1856.2(15)$ Å ^{3}, $Z = 4$, $D_c = 1.182$ g/cm^3, crystal dimensions $0.15 \times 0.10 \times 0.10$ mm were used for measurements on an ENRAF-NONIUSVCAD 4 with a graphite monochromator (ω-2θ scans, $2\theta_{max} = 28°$), Mo Kα radiation. The total number of reflections measured was 9396, of which 4025 were unique and were 1983 observed, $I > 2\sigma(I)$. Final indices: $R_f = 0.0752$, $R_w = 0.1421$ ($w = 1/\sigma|F|^2$) for observed reflections, and R1 = 0.1436, wR2 = 0.1671 for all reflections. The crystal structure (**1**) was solved by direct methods using SHELX-97 (Sheldrich, G. M. University of Gottingen: Gottingen, Germany, 1990) and expanded using difference Fourier techniques, refined by SHELX-97 (Sheldrich, G. M. 1997). Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 626690). Copies of these data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax (+44) 1223−336-033; or deposit@ccdc.cam.ac.uk).

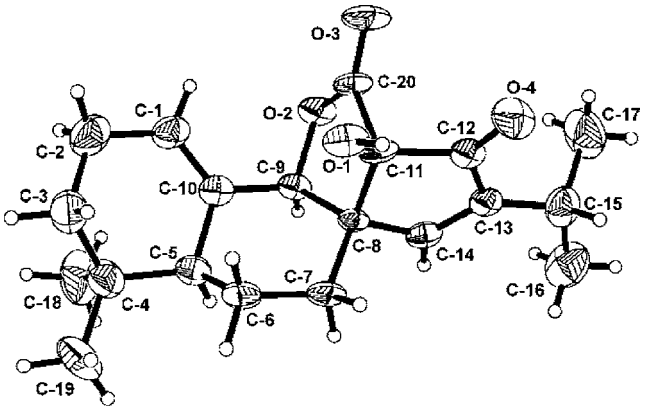

Figure 3. X-ray crystallographic structure of **1**.

Compound **1** was tested for cytotoxicity toward the human promyyelocytic leukemia cells (HL-60), human leukemia cells (K562), ovarian cancer cells (OVCA 2780), human lung cancer cells (A549), and human hepatoma cells (HepG2) by the MTT method,[8] and *cis*-platin (DDP) was used as a positive control in this experiment. However, it showed no significant inhibitory activity with an IC_{50} value greater than 100 μg/mL for the five cell lines. Compound **1** was also tested for cytotoxic activity against C8166 cells (CC_{50}) and for the cytopathic effects against HIV-1_{IIIB} (EC_{50}), using AZT as a positive control ($EC_{50} = 0.0034$ μg/mL and $CC_{50} >$ 200 μg/mL).[9] It exerted cytotoxicity against C8166 cells with $CC_{50} = 100.74$ μg/mL and showed anti-HIV-1_{IIIB} activity with $EC_{50} = 30.32$ μg/mL and SI (selectivity index) = 3.32.

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Supporting Information Available: 1D and 2D NMR spectra and X-ray crystallographic data (CIF file) of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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