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Przewalskin A: A New C_{23} Terpenoid with a 6/6/7 Carbon Ring Skeleton from *Salvia przewalskii* Maxim

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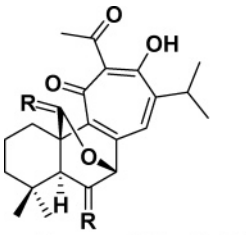
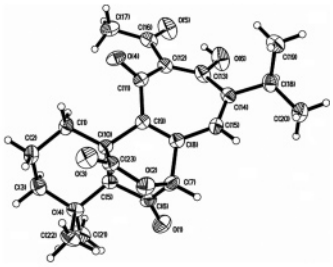
ABSTRACT



1. R = α - OH, β - H

2. R = O



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Przewalskin A (1), a novel C_{23} terpenoid with a 6/6/7 carbon ring skeleton, was isolated from *Salvia przewalskii*. Its structure was determined by comprehensive 1D NMR, 2D NMR, and MS spectroscopic analysis and subsequently confirmed by a single-crystal X-ray diffraction study of its PDC oxidation derivative (2). Compounds 1 and 2 showed modest anti-HIV-1 activity with EC_{50} = 41 and 89 μg/mL, respectively.

Salvia (including 700−1050 species) is the biggest genus in the economically and medicinally important Labiatae family and is widely distributed in the world.[1] Many species of this genus are used as folk medicine to treat a wide variety of ailments throughout the world.[2] As a part of our investigations on the chemical constituents of *Salvia* species, several new compounds were isolated and characterized.[3,4] *Salvia przewalskii* Maxim is a traditional Chinese herb used

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(1) Wu, C. Y.; Li, H. W. *Acta Bot. Yunn.* **1982**, *4*, 97−118.
(2) Kunming Institute of Botany, Chinese Academy of Sciences In *Flora Yunnannica*; Science Press: Beijing, 1977; Vol. 1, pp 661−662.
(3) (a) Xu, G.; Peng, L. Y.; Niu, X. M.; Zhao, Q. S.; Li, R. T.; Sun, H. D. *Helv. Chim. Acta* **2004**, *87*, 949−955. (b) Xu, G.; Peng, L. Y.; Li, X. L.; Zhao, Y.; Tu, L.; Zhao, Q. S.; Sun, H. D.; Lu, Y.; Mao, L.; Zheng, Q. T. *Helv. Chim. Acta* **2005**, *88*, 2370−2374. (c) Xu, G.; Peng, L. Y.; Zhao, Y.; Li, X. L.; Tu, L.; Zhao, Q. S.; Sun, H. D. *Chem. Pharm. Bull.* **2005**, *53*, 1575−1576.
(4) Xu, G.; Peng, L. Y.; Lu, L.; Weng, Z. Y.; Zhao, Y.; Li, X. L.; Zhao, Q. S.; Sun, H. D. *Planta Med.* **2006**, *72*, 82−86.

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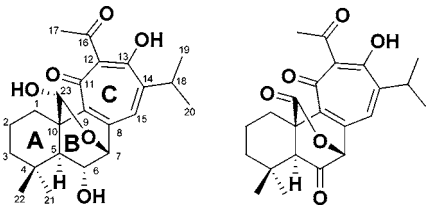
as the surrogate of *S. miltiorrhiza* (Danshen) for the treatment of various cardiovascular diseases.[2] Many bioactive abietane diterpenoids, especially *o*-quinones, have been reported as the main secondary metabolites of this plant.[5] In our search for new bioactive natural products, a novel C_{23} terpenoid (przewalskin A, **1**) was isolated from the acetone extract of this plant. C_{23} terpenoids with 23 carbons in the skeleton derived from normal diterpenoids are very rare in natural products. To the best of our knowledge, only 13 natural C_{23} terpenoids have been reported so far, of which 12 were isolated from *Salvia* plants.[4,6] The skeletal type displayed by przewalskin A was noticeable for its unusual 6/6/7 carbon ring skeleton with a unique seven-membered ring C substituted by an isopropyl and an acetyl group. This is the first report of a natural C_{23} terpenoid with a seven-membered ring C. The structure of **1** was established from its NMR and mass spectral data and confirmed by a single-crystal X-ray diffraction analysis of its PDC oxidation derivative (**2**). Described here are the isolation, structure elucidation, and plausible biogenetic pathway of **1**, together with the biological activities of **1** and **2**.



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) for 24 h each time 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 IV 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−H_2O 8:2) to afford **1** (22.5 mg).

Compound **1** was obtained as yellowish amorphous powder. It was deduced to have a molecular formula of $C_{23}H_{30}O_6$ by HRESIMS (found $[M + Na]^+$ 425.1949; calcd 425.1940), indicating 9 degrees of unsaturation. IR absorptions at 3443, 1632, and 1590 cm^{-1} implied the existence of OH and carbonyl groups. The ^{13}C NMR and DEPT spectra (Table 1) indicated that **1** possessed two carbonyl groups

Table 1. ^1H and ^{13}C NMR Assignments of **1** and **2**

	1[a]		**2**[b]	
no.	δ_H (mult, *J*, Hz)	δ_C (mult)	δ_H (mult, *J*, Hz)	δ_C (mult)
1α	2.20 (m)	27.8 t	2.26 (dt,3.9,13.5)	26.0 t
1β	2.20 (m)		2.40 (overlap)	
2α	1.59 (m)	18.8 t	1.72 (br d, 13.9)	18.2 t
2β	1.59 (m)		2.00 (br q, 13.9)	
3α	1.30 (m)	41.7 t	1.41 (br d, 13.5)	40.6 t
3β	1.50 (m)		1.52 (br d, 13.5)	
4		34.3 s		36.1 s
5α	1.22 (d, 3.9)	55.4 d	2.15 (s)	51.7 d
6	4.05 (br d, 2.8)	70.1 d		200.6 s
7	4.54 (d, 4.4)	75.3 d	5.08 (s)	83.9 d
8		138.8 s		134.7 s
9		147.9 s		146.0 s
10		48.3 s		50.5 s
11		189.7 s		186.3 s
12		120.9 s		120.6 s
13		173.7 s		174.3 s
14		146.2 s		151.8 s
15	6.75 (s)	133.4 d	6.70 (s)	127.0 d
16		205.5 s		204.1 s
17	2.36 (3H, s)	28.5 q	2.43 (br s)	28.6 q
18	3.27 (sept, 6.9)	30.5 d	3.36 (sept, 6.6)	30.5 d
19	1.17 (3H, d, 6.9)	22.9 q	1.21 (d, 6.6)	22.5 q
20	1.12 (3H, d, 6.9)	22.7 q	1.14 (d, 6.6)	22.5 q
21	1.00 (3H, s)	34.0 q	1.28 (br s)	33.2 q
22	1.00 (3H, s)	23.2 q	0.90 (br s)	20.1 q
23	5.38 (d, 5.4)	90.9 d		170.5 s

[a] Data were recorded in acetone-d_6 on a Bruker AM-400 MHz. [b] Data were recorded in $CDCl_3$ on a Bruker AM-400 MHz.

(including an acetyl group), seven quaternary carbons (including five olefinic ones), six methines (including three oxygenated ones), three methylenes, and five methyls, which suggested that **1** had a C_{23} terpenoid skeleton. According to the characteristic signals for normal abietane diterpenoids at 34.3 (s, C-4), 55.4 (d, C-5), 48.3 (s, C-10), 30.5 (d, C-18), 22.9 (q, Me-19), 22.7 (q, Me-20), 34.0 (q, Me-21), and 23.2 (q, Me-22), compound **1** should derive from an abietane diterpenoid.[7] The HMBC spectrum (in acetone-d_6) of **1** displayed the following correlations: H-5 (δ_H 1.22, d, J = 3.9 Hz) with C-9, C-21, C-22, and C-23; H-6 (δ_H 4.05, br d, J = 2.8 Hz) with C-4 and C-8; H-7 (4.54, d, J = 4.4 Hz)

(5) (a) Xue, M.; Shi, Y. B.; Cui, Y.; Zhang, B.; Luo, Y. J.; Zhou, Z. T.; Xia, W. J.; Zhao, R. C. *Nat. Prod. Res. Dev.* **1999**, *12*, 27−32. (b) Wang, N.; Luo, H. W. *Zhongcaoyao* **1989**, *20*, 7−8. (c) Xu, X. M.; Xiao, Z. Y. *Zhongcaoyao* **1984**, *15*, 1−4. (d) Li, B.; Niu, F. D.; Lin, Z. W.; Zhang, H. J.; Wang, D. Z.; Sun, H. D. *Phytochemistry* **1991**, *30*, 3815−3817.

(6) (a) Luis, J. G.; Lahlou, E. H.; Andres, L. S.; Sood, G. H. N.; Ripoll, M. M. *Tetrahedron Lett.* **1996**, *37*, 4213−4216. (b) Luis, J. G.; Lahlou, E. H.; Andres, L. S. *Tetrahedron* **1996**, *52*, 12309−12312. (c) Miura, K.; Kikuzaki, H.; Nakatani, N. *Phytochemistry* **2001**, *58*, 1171−1175. (d) Tezuka, Y.; Kasimu, R.; Basnet, P.; Namba, T.; Kadota, S. *Chem. Pharm. Bull.* **1997**, *45*, 1306−1311. (e) Nagy, G.; Gunther, G.; Mathe, I.; Blunden, G.; Yang, M. H.; Carbb, T. A. *Biochem. Syst. Ecol.* **1998**, *26*, 797−799. (f) Gaspar-Marques, C.; Simoes, M. F.; Rodriguez, B. *J. Nat. Prod.* **2005**, *68*, 1408−1411.

(7) (a) Tada, M.; Hara, T.; Hara, C.; Chiba, K. *Phytochemistry* **1997**, *45*, 1475−1477. (b) Ulubelen, A.; Oksuz, S.; Topca, G.; Goren, A. C.; Voelter, W. *J. Nat. Prod.* **2001**, *64*, 549−551. (c) Nagy, G.; Gunter, G.; Mathe, I.; Blunden, G.; Yang, M. H.; Crabb, T. A. *Phytochemistry* **1999**, *51*, 809−812. (d) Esquivel, B.; Flores, M.; Hernandez-Ortega, S.; Toscano, R. A.; Ramamoorthy, T. P. *Phytochemistry* **1995**, *39*, 139−143. (e) Luis, J. G.; Andres, L. S. *Phytochemisty* **1993**, *33*, 635−638.

with C-5, C-9, and C-23; and the hemiketalic group proton at δ_H 5.38 (d, J = 5.4 Hz, H-23) with C-1, C-7, C-9, and C-10. The above evidence, along with two proton spin systems observed from the $^1H-^1H$ COSY spectrum, H_2-1/H_2-2/H_2-3 and H-5/H-6/H-7, showed the existence of fragment **1a** (Figure 1).

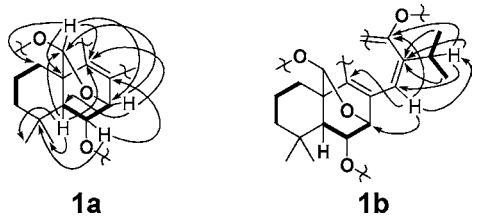


Figure 1. Structural fragments and key COSY (bold lines) and HMBC (arrows) correlations of **1** in acetone-d_6.

Furthermore, the HMBC correlations from Me-19 and Me-20 to C-14, coupled with the proton spin system Me-19/H-18/Me-20, revealed that an isopropyl group was substituted at C-14. The HMBC correlations of the unsaturated methine signal at δ_H 6.75 (1H, s, H-15) with C-7, C-9, C-13, and C-18 indicated that an unsaturated methine was located at C-15, which could be confirmed by the ROESY correlations of H-15 with H-7 and Me-20. In addition, the HMBC correlations of H-18 and H-15 with an O-bearing olefinic carbon at δ_C 173.7 (s, C-13) established the partial structure **1b** (Figure 1) having an oxy substituent at C-13.

Only four signals, an acetyl group (δ_C 205.5 and 28.5), a carbonyl group (δ_C 189.7), and an olefinic carbon at δ_C 120.9 remained. The carbonyl group at δ_C 189.7 was ascribed to C-11, and the acetyl group was assigned to C-12 (δ_C 120.9) on the basis of the HMBC correlation from the methyl at δ_H 2.36 (3H, s Me-17) to C-12 and the noticeable downfield chemical shift of C-13 at δ_C 173.7 which indicated two conjugated carbonyl groups at C-11 and C-16. Furthermore, the obvious HMBC correlations from H-7 to the carbonyl group at δ_C 189.7 confirmed that this carbonyl group could be ascribed to C-11. The evidence discussed above, coupled with fragment **1b**, gave the structure **1**. Because the HMBC correlation of H-7 to C-11 were correlated through four bonds, the structure of **1** elucidated by HMBC correlations remained uncertain. To unambiguously confirm the structure of **1**, an oxidation reaction by PDC was carried out and gave the derivative (**2**) of **1**. Compound **2** had the molecular formula $C_{23}H_{26}O_6$ as determined by analysis of ^1H and ^{13}C NMR spectral data, together with HRESIMS (found $[M + Na]^+$ 421.1619; calcd 4421.1627). It was found that **2** shared most structural features with **1** by detailed comparison of their ^1H and ^{13}C NMR spectral data (Table 1) and further analysis of the HMBC spectra of **2**. The only difference between compounds **1** and **2** was the appearance of two carbonyl groups at C-6 and C-23 in **2**, instead of two oxygenated methines in **1**. The single-crystal X-ray diffraction analysis of **2** (Figure 2) unambiguously confirmed the

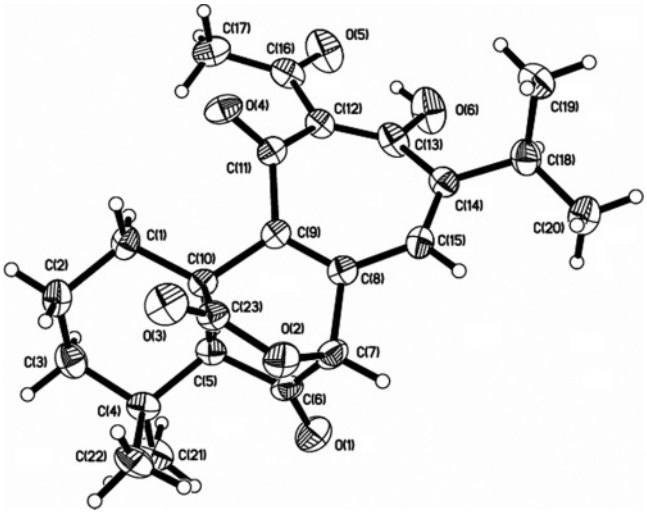


Figure 2. X-ray crystallographic structure of **2**.

expected unique skeleton and structure of both **1** and **2**.[8]

The stereochemistry of **1** was established on the basis of a ROESY experiment (Figure 3). Biogenetically, Me-22 and C-23 were β-oriented whereas H-5 and Me-21 were both in the α-orientation, which could be confirmed by ROESY correlations of Me-22/H-23 and Me-21/H-5. Then, H-7 could be determined as α-orientation due to the ether bridge between C-7 and C-23. The signals of Me-21 and Me-22 of **1** in ^1H NMR spectra overlapped each other in acetone-d_6 and $CDCl_3$ but displayed good separation with other signals in C_5D_5N. In the ROESY spectrum (in C_5D_5N) of **1**, the correlations of H-5 with Me-21 rather than Me-22 indicated that H-5 and Me-21 were α-oriented.

The ROESY correlations of H-23 with Me-22 suggested that Me-22 was in β-orientation, and H-23 was in the same side as Me-22. In addition, the ROESY correlations of H-7 with H-15 and H-15 with H-18, Me-19, and Me-20 further confirmed the stereochemistry of **1**.

Because acetone was used for the extraction of the plant material, compound **1** could have been an artifact product

(8) Crystallographic data for **2**: $C_{23}H_{26}O_6$, M = 398.17, orthorhombic, space group $P2_12_12_1$, a = 6.072 (4) Å, b = 15.035 (4) Å, c = 22.423 (6) Å, α = 90.00 (2)°, β= 90.00 (4)°, γ = 90.00 (4)°, V = 2047.1(15) Å^3, Z = 4, d = 1.293 g/cm^3, crystal dimensions 0.50 × 0.40 × 0.25 mm were used for measurements on an ENRAF−NONIUSVCAD 4 with a graphite monochromator (ω-2θ scans, $2\theta_{max}$ = 34°), Mo Kα radiation. The total number of reflections measured was 2257, of which 2233 were unique and 1176 observed, $I > 2\sigma(I)$. Final indices: R_f = 0.0508, R_w = 0.1281 ($w = 1/\sigma|F|^2$) for observed reflections, and R_1 = 0.1322, wR_2 = 0.1625 for all reflections (2233). The crystal structure (**2**) 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 **2** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 288652). 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, U.K.; fax (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

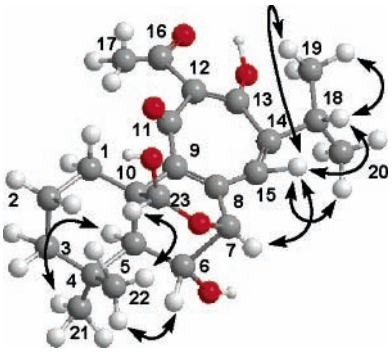
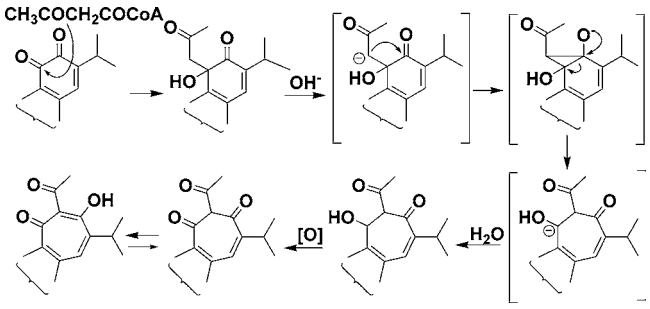


Figure 3. Key NOE correlations for **1** in C_5D_5N.

arising from a molecule of acetone and an *o*-quinone. To determine whether **1** is an artifact, another sample of *S. przewalskii* (with the same origin of the plant material used for the initial extraction) was extracted with $CHCl_3$. Compound **1** was also identified from the extract, which supported **1** as a natural metabolite of *S. przewalskii*.

From the biogenetic view, compound **1** may be derived from a normal *o*-quinone. The formation of **1** could be explained through a pathway (Scheme 1) including the

Scheme 1. Plausible Biogenetic Pathway of **1**



condensation of a normal *o*-quinone diterpenoid with acetoacetyl-CoA followed by an intramolecular aldol reaction and an oxidation reaction.

Considering some abietane quinones are known to have antitumor activity,[4,9] the antitumor activities of compounds **1** and **2** were evaluated with the HL-60, K562, OVCA-2780, A549, and HepG-2 cell lines; however, neither **1** nor **2** showed significant inhibitory activity (IC_{50} > 40 μg/mL for the five cell lines).

As some 1,3-diketone compounds exhibit obviously anti-HIV-1 activities,[10,11] compounds **1** and **2** were tested for their cytotoxic activity against C8166 cells (CC_{50}), using the MTT method as reported previously,[12] and for the cytopathic effects against HIV-1 (EC_{50}) (Table 2).[13] Compound **1** exerted cytotoxicity against C8166 cells with the CC_{50} = 89.13 μg/mL and showed anti-HIV-1 activity with EC_{50} = 40.74 μg/mL and SI (selectivity index) = 2.19.

Table 2. Summary of Cytotoxicity and Anti-HIV-1 Activity of Compounds **1** and **2**

	cytotoxicity CC50 (μg/mL)	anti-HIV-1 EC_{50} (μg/mL)	selectivity index CC_{50}/EC_{50}
1	89.13	40.74	2.19
2	158.49	89.13	1.78

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

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(9) Wu, W. L.; Chang, W. L.; Chen, C. F. *Am. J. Chin. Med.* **1991**, *19*, 207−216.
(10) Paris, G. C. G.; Zhang, X.; Marchand, C.; Neamati, N.; Cowansage, K.; Svarovskaia, E. S.; Pathak, V. K.; Tang, Y.; Nicklaus, M.; Pommier, Y.; Burke, T. R. *J. Med. Chem.* **2002**, *45*, 3184−3194.
(11) Wai, J.; Egbertson, M. S.; Payne, L. S.; Fisher, T. E.; Embrey, M. W.; Tran, L. O.; Melamed, J. P.; Langford, H. M.; Guare, J. P.; Zhuang, L.; Grey, V. E.; Vacca, J. P.; Holloway, M. K.; Naylor-Olsen, A. M.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Schleif, W. A.; Gabryelski, L. J.; Young, S. D. *J. Med. Chem.* **2000**, *43*, 4923−4926.
(12) Zheng, Y. T.; Ben, K. L.; Zhang, W. F.; Wang, J. H. *Immunopharmarcol. Immunotoxicol.* **1995**, *17*, 69−79.
(13) Zheng, Y. T.; Ben, K. L.; Jin, S. W. *Acta Pharm. Sin.* **1999**, *20*, 239−243.