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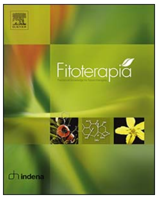

An unusual racemic C_{12}-norabietane diterpene and a new abietane diterpene alkaloid from *Salvia miltiorrhiza* Bunge

Xue-Ge Pan[a,b,1], Jun He[c,1], Xiao-Xue Wang[c], Jia Zhang[d], Bing-Zhi Ma[c], Wei-Ku Zhang[b,*], Jie-Kun Xu[a,*]

[a] *School of Life Sciences, Beijing University of Chinese Medicine, Beijing 100029, PR China*
[b] *Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing 100029, PR China*
[c] *Department of Pharmacy, China-Japan Friendship Hospital, Beijing 100029, PR China*
[d] *School of Chinese Medicine Sciences, Beijing University of Chinese Medicine, Beijing 100029, PR China*

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ABSTRACT

A rare C_{12}-norabietane diterpene racemate (**1**) and a new abietane diterpene alkaloid (**2**) were isolated from the roots of *Salvia miltiorrhiza* Bunge. Their structures were established by comprehensive spectroscopic analyses, and **1** was successfully resolved by chiral HPLC, demonstrating that **1** is racemic. The absolute configurations of **1a** [(+)-miltiorolide A], **1b** [(−)-miltiorolide A], and **2** were determined using TDDFT-ECD calculations. **1a** and **1b** are the first examples of enantiomeric C_{12}-norabietane diterpenes featuring an isobutylene with a tetrahydronaphthalene-butyrolactone ring system. The cytotoxic activities of the isolates (**1** and **2**) were evaluated against three human cancer cell lines BEL-7402, HT-29 and PANC-28. A plausible biogenetic pathway of **1** was also proposed.

1. Introduction

The roots of *Salvia miltiorrhiza* Bunge (Lamiaceae family), commonly known as "Danshen" or "Tanshen", are one of the most popular traditional herbal medicines in China. The roots show therapeutic potential for the treatment of cardiovascular and cerebrovascular diseases [1]. Diterpenes are considered to be the predominant bioactive constituents in the *S. miltiorrhiza*, and they exhibit diverse biological activities, such as antitumor, antibacterial, antioxidant and antidiabetic activities [2,3].

As we are currently interested in the bioactive and structurally unique natural diterpenes of this plant, we carried out a phytochemical study on the roots of *S. miltiorrhiza*. A pair of rare enantiomeric C_{12}-norabietane diterpenes and a new abietane diterpene alkaloid are reported herein (**1** and **2**) (Fig. 1). To the best of our knowledge, up to now, only six C_{12}-norabietane diterpenes have been characterized following isolation from natural sources [4,5]. Notably, this is the first report on the isolation of a C_{12}-norabietane diterpene racemate featuring an isobutylene with a tetrahydronaphthalene-butyrolactone ring system, which is a unique structure for C_{12}-norabietane diterpenes and further elucidates the biosynthesis of these compounds. Additionally, the cytotoxicities of **1** and **2** were evaluated against BEL-7402, HT-29 and PANC-28 cell lines.

2. Experimental

2.1. General procedures

Optical rotations and ECD spectra were recorded on a JASCO P-2000, and JASCO J-815 CD spectrometers (JASCO, Easton, MD, U.S.A.), respectively. The UV spectra were measured on a GENESYS 10SUV-VIS Spectrophotometer. IR spectra were obtained on a Nicolet iS5 spectrometer (Thermo Scientific, Waltham, MA, U.S.A.). HRESIMS spectra were performed on a Q-Exactive Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, U.S.A.). NMR experiments were performed on Bruker 400 and 600 MHz NMR instruments (Bruker-Biospin, Billerica, MA, U.S.A.). CD was recorded on a Chirascan (Applied Photophysics Ltd., Surrey, UK). Preparative HPLC was performed on a Shimadzu LC-6AR instrument with an SPD-20A detector or a Waters 515 instrument with a Waters 2487 UV detector using an YMC-Pack column (250 mm × 20 mm, 10 μm) or an ES Sonoma C18 column (250 mm × 20 mm, 10 μm). Column chromatography was carried out with macroporous resin (D101, Cangzhou Bon Adsorber Technology Company), silica gel (200–300 mesh, Qingdao Ocean Chemical

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[*] Corresponding authors.
E-mail addresses: cpuzwk@163.com (W.-K. Zhang), xjkbucm@163.com (J.-K. Xu).
[1] X.-G Pan and J. He contributed equally to this research.

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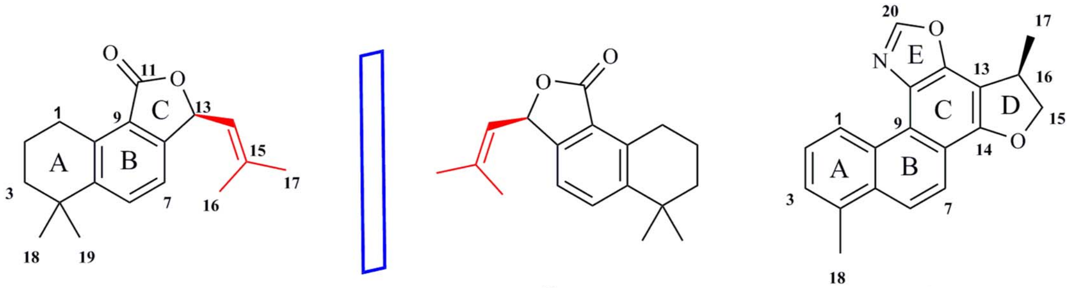


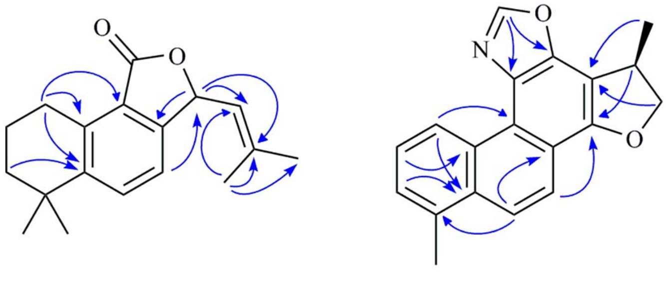
1a **1b** **2**

Fig. 1. Structures of compounds **1** and **2**.

Table 1
^{1}H and ^{13}C NMR data of **1** and **2** (CDCl$_3$, δ in ppm, J in Hz).

No.	**1**[a]		**2**[b]	
	δ_H	δ_C	δ_H	δ_C
1	3.24 (m)	26.1	10.17 (d, 8.4)	126.4
2	1.85 (m)	18.7	7.67 (dd, 6.6, 8.4)	126.7
3	1.70 (m)	38.7	7.51 (d, 6.6)	128.3
4		34.2		134.5
5		147.4		131.7
6	7.61 (d, 8.4)	133.0	8.02 (d, 9.0)	122.2
7	7.10 (d, 8.4)	119.5	8.07 (d, 9.0)	120.3
8		148.5		116.5
9		123.0		123.6
10		137.9		130.1
11		171.3		131.2
12				145.8
13	6.01 (d, 9.2)	76.9		111.2
14	5.10 (d, 9.2)	121.0		156.1
15		141.0	5.05 (t, 9.0) 4.47 (dd, 7.2, 9.0)	80.0
16	1.82 (d, 1.2)	18.9	4.15 (m)	36.7
17	1.95 (d, 1.2)	26.1	1.61 (d, 6.6)	19.4
18	1.31 (s)	32.1	2.81 (s)	20.5
19	1.31 (s)	32.0		
20			8.24 (s)	149.6

[a] Recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C.
[b] Recorded at 600 MHz for ^{1}H and 125 MHz for ^{13}C.



1 **2**

Fig. 2. Key HMBC correlations of **1** and **2**.

Company), Sephadex LH-20 (18–110 μm, Pharmacia Fine Chemicals, Uppsala, Sweden), and YMC-Pack ODS-A (250 mm × 20 mm, 5 μm, Kyoto, Japan). HPLC-DAD analysis was set up on Agilent 1100 series system (Agilent Technologies, CA, U.S.A.) with a Sonoma C18 (2) column (250 mm × 4.6 mm, 5 μm). Thin-layer chromatography (TLC) was performed using Silica gel GF254 (Qingdao Marine Chemical Company).

2.2. Plant material

The roots of *Salvia miltiorrhiza* Bunge were collected in October 2009 in Zhumadian, Henan Province, China. The plant materials were identified by Prof. Lin Ma of Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences. A voucher specimen (No. 20091020) was deposited at the Institute of Clinical Medical Sciences, China-Japan Friendship Hospital, Beijing, China.

2.3. Extraction and isolation

The roots of *S. miltiorrhiza* Bunge (50.0 kg) were extracted with 95% EtOH at reflux to afford 4.4 kg of crude extract, which was suspended in H_2O and successively extracted with petroleum ether, EtOAc, and n-BuOH. The petroleum ether solution was concentrated to give a residue (416.7 g), which was subjected to column silica gel (200–300 mesh) column chromatography (6 cm × 76 cm) and eluted with a gradient of petroleum ether-EtOAc (100:0; 100:5; 100:10; 9:1; 6:1; 4:1) to obtain seven fractions A–J. Fr.B (121.4 g) was further subjected to silica gel (200–300 mesh) column chromatography and six fraction (Fr. B1– B6) were obtained. Fr.B3 (4.5 g) was submitted to ODS eluting with CH_3OH-H_2O (5%–100%) gradiently to yield nine fractions (Fr.B3-A–B3-I). Fr.B3-C(105 mg) was submitted to Sephadex LH-20 column eluting with $CHCl_3$-CH_3OH (1:1) and then was further separated via preparative HPLC, with the detection wavelength of 230 nm, and a flow-rate of 5 mL/min, and eluted with CH_3CN-H_2O (70:30) to provide **1** (8.8 mg, t_R 80 min). Similarly, Fr.B3-F (30 mg) was purified by Sephadex LH-20 column eluting with $CHCl_3$-CH_3OH (1:1) and separated via preparative HPLC, with the detection wavelength of 254 nm, and a flow-rate of 5 mL/min, and eluted with MeOH-H_2O (80:20) to afford **2** (5.4 mg, t_R 130 min).

2.3.1. Miltiorolide A (1)

White amorphous powder, [α]23 D + 0.0 (*c* 1.00, MeOH); UV (MeOH) λ_{max}(log ε): 208 (2.08), 288 (0.17) nm; IR ν_{max}: 2969, 2941, 2868, 2381, 1756, 1521, 1484, 1297, 1082 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1. HRESIMS: *m/z* 271.1685 [M + H]$^+$ (calcd for $C_{18}H_{23}O_2$, 271.1693). ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 244 (+ 7.38), ((+)-**miltiorolide A**, **1a**); λ_{max} ($\Delta\varepsilon$) 240 (− 6.83), ((−)-**miltiorolide A**, **1b**).

2.3.2. Hendersine B (2)

Pale yellow needles, [α]23 D + 13.0 (*c* 1.00, MeOH); UV (MeOH) λ_{max}(log ε): 223 (1.41), 250(1.02), 288(1.04), 349(0.19), 367(0.22) nm; IR ν_{max}: 3371, 2942, 2831, 1866, 1609, 1507, 1088 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1. HRESIMS: *m/z* 290.1168 [M + H]$^+$ (calcd for $C_{19}H_{16}NO_2$, 290.1176). ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 246 (+ 2.41).

2.4. Biological activities

Cultured the human lung adenocarcinoma cell line BEL-7402 and human pancreatic cancer cell line PANC-28 in RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere with 5% CO_2 in air at 37 °C. Human fibrosarcoma cell line HT-29 were