Two New Abietane Diterpenoids from *Salvia yunnanensis*

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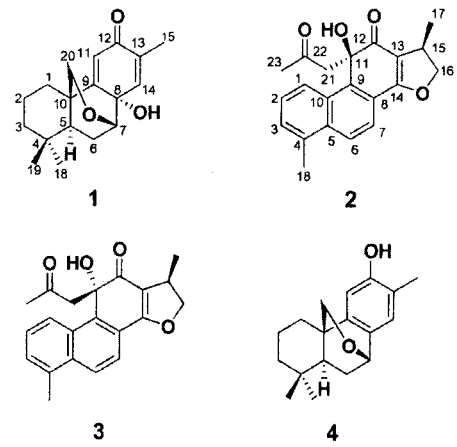
Abstract

Two new abietane diterpenoids, yunnannin A (**1**) and danshenol C (**2**), were isolated from *Salvia yunnanensis* together with ten known diterpenoids, danshenol A (**3**), przewalskin (**4**), tanshinone IIA, tanshinone I, crypotanshinone, 1,2-dihydrotanshinone, tanshinlactone, 5,6-dehydrosugiol, 12-hydroxy-6,7-seco-8,11,3-abietatriene-6,7-dial and phytol. Their structures were established based on spectroscopic data, chemical reactions and comparison with literature data. Compounds **1–3** were tested for their antitumor activity in T-24, QGY, K562, Me180 and BIU87 cell lines. Compound **3** showed inhibited growth of K562 (IC_{50} = 0.53 μg/mL), T-24 (IC_{50} = 7.94 μg/mL), QGY (IC_{50} = 4.65 μg/mL) and Me180 (IC_{50} = 6.89 μg/mL) cell lines while compound **2** was inactive. Compound **1** showed moderate inhibitory activity on QGY (IC_{50} = 16.75 μg/mL) and Me180 (IC_{50} = 5.84 μg/mL) cells.

Salvia miltiorrhiza Bunge (Labiatae) is a traditional Chinese herb used in the treatment of various cardiovascular diseases [1]. More than 30 abietane diterpenoids have been isolated from this plant [2], [3]. In the Southwest of China, *S. yunnanensis* C .H. Wright has been used as the surrogate of *S. miltiorrhiza* [4]. However, no chemical constituents have been reported from *S. yunnanensis*. In our continuous research analyzing the diterpenoids from *Salvia* species [5], we examined the constituents of *S. yunnanensis* collected in Eryuan county of the Yunnan province. Two new diterpenoids, named yunnannin A (**1**) and danshenol C (**2**), were isolated from this plant together with ten known diterpenoids, danshenol A (**3**) [6], przewalskin (**4**) [7], 12-hydroxy-6,7-seco-8,11,13-abietatriene-6,7-dial [8], tanshinlactone [9], phytol [10], 5,6-dehydrosugiol [11], 1,2-dihydrotanshinone [12], tanshinone I [13], crypotanshinone [10] and tanshinone IIA [15]. To the best of our knowledge, compound **2** was the third example of 21-carbon abietane-type diterpenoids with an additional CH_3-CO-CH_2 group at C-11 of the C-ring [6]. The major constituents of *S. yunnanensis*, tanshinone IIA, tanshinone I, and crypotanshinone were identical to the ones of *S. miltiorrhiza*, which supports the usage of *S. yunnanensis* as the surrogate of *S. miltiorrhiza*. We report here the isolation and structure elucidation of new compounds, as well as the cytotoxic activities against T-24, QGY, K562, Me180 and BIU87 cell lines of compounds **1–3**.



1 **2** **3** **4**

Compound **1** was isolated as a colorless oil. The formula of **1** was established as $C_{18}H_{24}O_3$ based on HR-ESI-MS ([M + H]$^+$ *m/z* = 289.1802) and ^{13}C-NMR spectral data (Table **1**). The IR spectrum displayed the absorption bands due to a hydroxy group at 3444 cm^{-1} and a conjugated carbonyl group at 1671 cm^{-1}. Compound **1** can be ascribed to be an abietane type norditerpenoid according to the characteristic methine signal at δ_C = 38.1 due to C-5, two noticeable quaternary carbon signals at δ_C = 33.5 (C-4) and 40.2 (C-10) and two characteristic methyl carbon signals at δ_C = 32.6 (Me-18), 22.1 (Me-19) [6], [7], [14], [15]. Comparison of ^{1}H- and ^{13}C-NMR spectral data of **1** and **4** indicated that the skeletons of the two compounds were the same and that the structures were also very similar [8]. The major differences in the ^{13}C-NMR spectral data were the presence of a conjugated carbonyl group (δ_C = 188.3) and one oxygenated quaternary carbon (δ_C = 68.1) in **1** instead of two olefinic quaternary ones in **4**. The HMBC correlations between the oxygenated quaternary carbon at δ_C = 68.1 and H-6, H-7, H-11, and H-14 suggested that the quaternary carbon could be ascribed to C-8 (Fig. **1**). The conjugated carbonyl group was assignable to C-12 by its HMBC correlations with H-11, H-14, and Me-15. The HMBC correlations of the methyl signal at δ_H = 1.85 with C-12, C-13 and C-14 indicated that the methyl can be ascribed to Me-15. The remaining fragment was confirmed to be identical to that of **4** upon analysis of HMQC, HMBC and COSY spectra.

The stereochemistry of **1** was established on the basis of a ROESY experiment (Fig. **2**). ROESY correlations of H-14 with Me-15, H-11 with H-1α and H-1β, H-20β with Me-19, H-7 with H-14 and of H-5α with Me-18 were observed, which supported the α-configuration of H-5 and H-7. The correlation of H-20α with H-14 indicated the α-configuration of OH-8. Therefore, the structure of yunnannin A was established as shown in **1**.

Compound **2** was obtained as a red amorphous powder with a molecular ion peak at *m/z* = 336 in the EI-MS. It was deduced to have the molecular formula $C_{21}H_{20}O_4$ based on HR-ESI-MS ([M +

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Table 1 1D-NMR data for **1** and **2** ($CDCl_3$)[a]

	1		2	
	^{13}C	^{1}H [b]	^{13}C	^{1}H
1	29.6 t	1.67 (m, Hβ) 1.57 (m, Hα)	125.7 d	9.00 (d, 8.8)
2	18.6 t	1.56 (m, Hα) 1.43 (m, Hβ)	126.7 d	7.49 (dd, 8.8, 7.4)
3	40.6 t	1.50 (m, Hα) 1.12 (m, Hβ)	128.5 d	7.42 (d, 7.4)
4	33.5 s		135.2 s	
5	38.1 d	2.04 (m)	134.8 s	
6	25.3 t	2.34 (m, Hα) 1.90 (m, Hβ)	125.5 d	8.08 (d, 8.8)
7	74.4 d	4.09 (brs)	120.0 d	7.77 (d, 8.8)
8	68.1 s		120.4 s	
9	171.2 s		141.3 s	
10	40.2 s		131.1 s	
11	120.5 d	5.92 (s)	79.4 s	
12	188.3 s		196.1 s	
13	137.5 s		113.3 s	
14	145.6 d	6.73 (brs)	171.5 s	
15	15.5 q	1.85 (brs)	34.8 d	3.61 (m)
16			81.6 t	4.92 (dd, 4.4, 9.3) 4.42 (dd, 4.4, 9.3)
17			19.4 q	1.37 (d, 6.85)
18	32.6 q	0.93 (s)	20.1 q	2.71 (s)
19	22.1 q	1.08 (s)		
20	74.1 t	4.35 (d, 8.6, Hβ) 3.10 (d, 8.6, Hα)		
21			57.2 t	3.27 (d, 13.2) 3.04 (d, 13.2)
22			205.1 s	
23			31.9 q	1.97 (s)

[a] ^{1}H- and ^{13}C-NMR spectra were obtained at 100 MHz and 400 MHz, respectively.
[b] Coupling constants are presented in Hz.

H]$^+$ *m/z* = 337.1439) and NMR spectral data (Table **1**). Its IR spectrum indicated the presence of hydroxy (ν_{max} = 3453 cm^{-1}) and ketone carbonyl groups (ν_{max} = 1706 cm^{-1}). The ^{13}C-NMR spectra and the DEPT indicated the presence of three methyls, two methylenes (including an oxygenated one), six methines (including one sp^3 and five sp^2 carbons) and nine quaternary carbons (including two ketone carbonyl carbons and seven olefinic ones). In the EI-MS of **2**, the fragment ions at *m/z* = 293 and 279 corresponded to the loss of $[CH_3COCH_2]^+$ and $[CH_3CO]^+$ groups from the molecular ion, which implied the existence of an additional C_3 unit (CH_3COCH_2 group) [5]. The similarity of the 1D-NMR

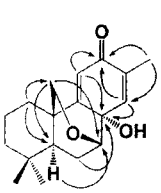

Fig. 1 Selected HMBC correlations of **1**.

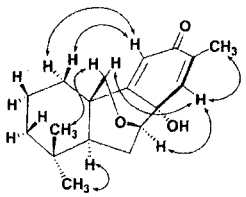

Fig. 2 Selected ROESY correlations of **1**.

spectral characteristics of compounds **2** and **3** indicated that **2** was a stereoisomer of **3** differing in the stereochemistry at C-11 or C-15 [5]. This was supported by the HMBC correlations (Fig. **3**).

The ROESY spectra of **2** and **3** were very similar to each other. Therefore, it is difficult to distinguish the configuration of **2** from **3** by the ROESY experiment. To solve this problem, we carried out the retro-aldol reactions of **2** and **3** according to the method reported by Tezuka et al. [6]. After treatment with $Eu(DPM)_3$, both **2** and **3** were converted into dihydrotanshinone, which indicated that the configurations of C-11 were different for **2** and **3**. Furthermore, the optical rotation value of **2** was at +43.86° while that of **3** was −47.14°, which confirmed the relative configuration difference at C-11 in **2** and **3**. Accordingly, the structure of danshenol C can be represented as shown in **2**.

Compounds **1**–**3** were tested for their cytotoxic activities against T-24 (urinary carcinoma), QGY (hepatocellular carcinoma), K562 (chronic myelogenous leukemia), Me180 (cervix epidermoid carcinoma) and BIU87 (transitional urinary bladder cell carcinoma) cell lines using the improved MTT method as reported previously [16], [17]. It is interesting that the cytotoxic activities of **2** and **3**, being stereoisomers, differed significantly. Compound **3** was active in K562 (IC_{50} = 0.53 μg/mL), T-24 (IC_{50} = 7.94 μg/mL), QGY (IC_{50} = 4.65 μg/mL), and Me180 (IC_{50} = 6.89 μg/mL) cell lines (Table **2**). However, compound **2** was only weakly active in Me180 (IC_{50} = 10.90 μg/mL) and K562 (IC_{50} > 30 μg/mL) cell lines. Compound **1** showed moderate inhibitory activity in QGY and Me180 cell lines with IC_{50} values of 16.75 and 5.84 (μg/mL), respectively.

Materials and Methods

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 using a Bruker DRX-500 NMR instrument. EI-MS and ESI-MS were taken on VG Auto Spec-3000 and API Qstar Pulsar instruments, respectively. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18, 9.4 mm × 25 cm column, a flow rate of 3 mL/min and detection with a UV detector at 272 nm. Silica gel for TLC and column chromatography was obtained from Qingdao Marine Chemical Inc., China.

The plant sample of *Salvia yunnanensis* was collected in Eyuan of the Yunnan province in July, 2000 and was identified by Prof. Xi-Wen Li of the Kunming Institute of Botany, Chinese Academy of

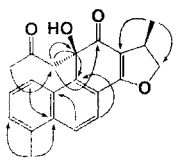

Fig. 3 Selected HMBC correlations of **2**.

Sciences. A voucher specimen (No. 2000215) was deposited in the Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

The dried and powdered (4.7 kg) *S. yunnanensis* was extracted with Me_2CO (3×25 L) at room temperature. The solvent was removed under vacuum. The gummy residue (200 g) was subjected to column chromatography (Ø 12×150 cm) over DM-130 porous resin and eluted with MeOH-H_2O (50% and 90%, each 10 L). The residue of the 90% MeOH-H_2O fraction was partitioned between H_2O (2.5 L) and EtOAc (2.5 L). The EtOAc part (65 g) was subjected to silica gel column chromatography (Ø 9×120 cm). Mixtures of petroleum-ether/EtOAc (1:0, 9:1, 8:2, 7:3, 6:4, 5:5, and 0:1, each 5 L) of increasing polarity were used as eluents. Seven fractions were collected, analyzed by TLC and combined. Tanshinone IIA (1.65 g) was crystallized from the second fraction (7 g). The remaining part of the second fraction was further subjected to silica gel column chromatography (Ø 7×90 cm) and was eluted with petroleum-ether:$CHCl_3$:EtOAc (75:20:5, 4 L) to give phytol (44 mg) and tanshinone I (258 mg). Crypotanshinone (100 mg) was crystallized from the third fraction. Tanshinlactone (5 mg), the mixture of **2** and **3** (20 mg) and 1,2-dihydrotanshinone (2 mg) were isolated from the third fraction (15 g) by silica gel column chromatography (Ø 7×120 cm) using petroleum-ether: $CHCl_3$:EtOAc (70:25:5, 10 L) as eluents. Compounds **2** (8 mg) and **3** (8 mg) were separated by semi-preparative HPLC using 85% MeOH-H_2O as eluent. Compound **1** (6 mg) and 12-hydroxy-6,7-seco-8,11,13-abietatriene-6,7-dial (6 mg) were isolated using an RP-18 column (Ø 7×80 cm) (MeOH:H_2O, 8:2, 10 L) from the fourth fraction (14 g). The fifth fraction (9 g) was repeatedly chromotographed over RP-18 (Ø 7×80 cm) using MeOH:H_2O (8:2, 8 L) to give the crude 5,6-dehydrosugiol and przewalskin, which were subjected to Sephadex LH-20 (Ø 2×150 cm) (CH_3OH:CH_3Cl, 1:1, 0.5 L) to give pure 5,6-dehydrosugiol (7 mg) and przewalskin (50 mg). Compounds **1–3** were developed on silica gel TLC (petroleum-ether: EtOAc, 8:2) with *Rf* values of 0.64, 0.55, and 0.50, respectively.

Table **2** Effect of compounds **1–3** on the growth of five human tumor lines

	IC50 (μg/mL)				
Compound	*T-24*	*QGY*	*K562*	*Me180*	*BIU87*
1	> 100	16.75	> 30	5.84	50.03
2	ND[a]	ND	> 30	10.90	> 100
3	7.94	4.65	0.53	6.89	44.37
cis-platin[b]	2.28	2.14	4.0	0.78	2.93

[a] ND: not determined, [b] Positive control.

Eu(DPM)$_3$ treatment of **2** *and* **3**: Eu(DPM)$_3$ (10 mg) was added to the $CHCl_3$ solution (0.6 mL) of **2** (2 mg) or **3** (2 mg) and incubated at room temperature overnight. The solution was detected by TLC (silica gel) developed in several solvent systems and compared to dihydrotanshinone. Both **2** and **3** were converted to the same compound, dihydrotanshinone (1.5 mg).

Yunnannin A (**1**): colorless oil; $[\alpha]_D^{19.1}$: +25.19° (*c* 0.60, MeOH); UV (MeOH): λ_{max} (log ε) = 297.2 (2.97), 237.0 (3.82), 204.0 (3.94) nm; IR (KBr): ν_{max} = 3444, 2931, 1671, 1636, 1367, 1162, 1034, 856 cm^{-1}; ^{1}H- and ^{13}C-NMR data: see Table **1**; EI-MS: *m/z* = 288 $[M]^+$ (22), 273 (95), 272 (80), 255 (37), 215 (38), 201 (41), 151 (56), 138 (100), 137 (81), 109 (25), 95 (13); positive HR-ESI-MS: *m/z* = 289.1802 $[M + H]^+$ (calcd. for $C_{18}H_{25}O_3$: 289.1804).

Danshenol C (**2**): red amorphous powder; $[\alpha]_D^{19.1}$: +43.86° (*c* 0.20, $CHCl_3$); UV (MeOH): λ_{max} (log ε) = 339 (3.09), 299 (3.54), 287 (3.54), 276 (3.58), 260 (3.56), 234.2 (4.04), 205.2 (4.25) nm; IR (KBr): ν_{max} = 3453, 2927, 1706, 1629, 1340, 1184, 780 cm^{-1}; ^{1}H- and ^{13}C-NMR: see Table **1**; EI-MS: *m/z* = 336 $[M]^+$ (67), 293 (17), 279 (100), 261 (17), 250 (76), 235 (94), 179 (30), 139 (14); positive HR-ESI-MS: *m/z* = 337.1439 $[M + H]^+$ (calcd. for $C_{21}H_{21}O_4$: 337.1440).

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