SHORT REPORT

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A new Diterpenoid from *Salvia przewalskii*

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Abstract: A new diterpenoid named ganxincastanic acid A (**1**), together with four known diterpenoids (**2**−**5**) and five known phenolic acids (**6**−**10**) were isolated from the roots and rhizomes of *Salvia przewalskii* Maxim., and their structures were established on the basis of MS and NMR spectral analyses.

Keywords: *Salvia przewalskii* Maxim.; diterpenoid; ganxincastanic acid A.

1. Plant Source

Salvia przewalskii Maxim., which is named Ganxishuweicao, Dazidanshen, Zidanshen or Gansudanshen in Chinese, is a herbaceous perennial plant of the genus *Salvia* (Lamiaceae) [1]. In Chinese Traditional Medicine, the roots and rhizomes of *S. przewalskii* as herbal remedies have been employed to remedy cardiovascular diseases by the local inhabitants for hundreds of years and widely to treat rheumatism in the west of Sichuan province [2-4]. And it is commercially available in the local herbal markets of Sichuan, Yunnan and Gansu provinces [4]. This plant is commonly used as the surrogate of *S. miltiorrhiza* called Danshen in Chinese due to its pharmacological potential for various cardiovascular disorders [5], antioxidant activity [6], aldose reductase inhibitory activity [7] and treatment of disseminated diseases intravascular coagulation, chronic hepatitis, cirrhosis and other diseases [2]. In order to investigate the chemical constituents of total phenolic acid extract of *S. przewalskii* (SPE), a new diterpenoid named ganxincastanic acid A (**1**) (Figure 1), along with nine known compounds (**2**−**10**), was isolated and identified.

The roots and rhizomes of *S. przewalskii* were collected from Linxia county of Gansu province, China in September 2013. It was authenticated by vice professor SUN Lianna (Department of Identification of Traditional Chinese Medicine, School of Pharmacy, Second Military Medical University of PLA in Shanghai). A voucher specimen (No.20130901) was deposited in the herbarium, Department of Pharmacy, Changzheng Hospital, Second Military Medical University.

2. Previous Studies

Diterpenoids and phenolic acids are the main chemical constituents of *S. przewalskii* [8]. In our previous study, four new diterpenoids named neo-przewaquinone A [9], tanshintriol A, tanshintriol B [10] and isoganxinonic acid A [11], and one new monoterpenoid glycoside named

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ganxinoside A [10] had been isolated from this plant. And we also found that SPE had pharmacological effects of not only reducing whole blood viscosity and increasing urine excretion [12], but also decreasing proteinuria, ameliorating foot process effacement and upregulating the levels of slit diaphragm proteins [13].

3. Present Study

The dried roots and rhizomes of *S. przewalskii* (5 kg) were chopped, then macerated for 48 h and percolated with 50% ethanol aqueous solution (75 L) at room temperature. After removal of ethanol, the extract (0.6 kg) was chromatographed over the macroporous adsorptive resin with water, 50%, 70% and 95% ethanol aqueous solution. The eluting solution of 50% ethanol was evaporated under reduced pressure to give SPE (90 g), which was subjected to silica gel column chromatography with the gradient solvent of $CHCl_3$-CH_3OH (30:1~1:1, *v/v*) to give five fractions (Fr. 1-5).

Fr. 2 (11 g) was repeatedly separated by silica gel column chromatography eluting with the gradient solvent of $CHCl_3$-CH_3OH (25:1, 20:1, 15:1, 10:1, *v/v*) and purified by Sephadex LH-20 gel column chromatography using $CHCl_3$-CH_3OH (1:1, *v/v*) as a eluent to obtain compound **2** (41 mg), **3** (48 mg), **4** (27 mg) and **5** (22 mg). Fr. 4 (4 g) was purified using semi-preparative HPLC eluting with CH_3OH-H_2O solvent system of decrease polarity (3:7, 4:6, *v/v*) to yield compound **1** (18 mg). Fr. 5 (49 g) was chromatographed on MCI gel column with the gradient solvent of CH_3OH-H_2O (1:9, 2:8, 3:7, 4:6, 5:5, *v/v*) to afford five sub-fractions (SFr. 5.1-5.5). SFr. 5.2 was repeatedly separated by Sephadex LH-20 gel and ODS C_{18} column chromatography eluting with the gradient solvent of CH_3OH-H_2O (2:8, 3:7, *v/v*) to obtain compound **10** (112 mg), **9** (62 mg) and **8** (980 mg). SFr. 5.3 was purified by ODS C_{18} column chromatography using CH_3OH-H_2O (3:7, *v/v*) as eluent to yield compound **7** (91 mg). SFr. 5.4 was submitted on the same column chromatography method with the eluent of CH_3OH-H_2O (4:6, *v/v*) to acquire compound **6** (85 mg).

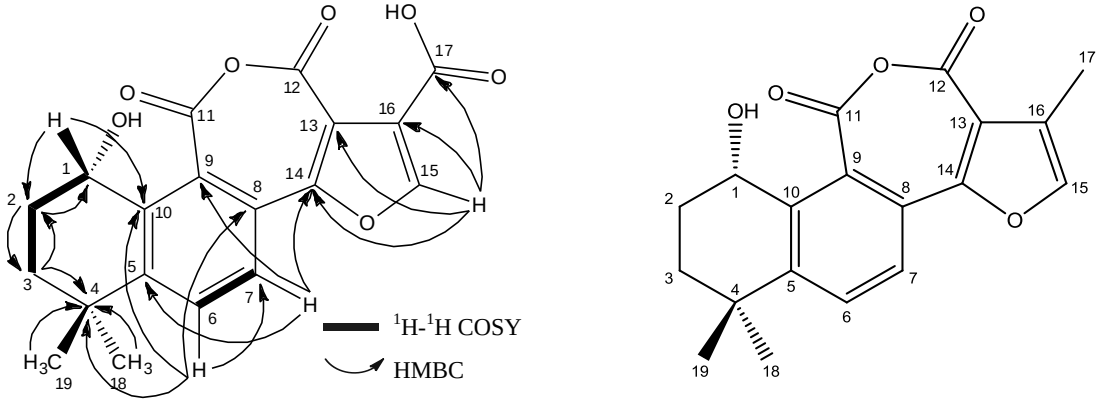
Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data for compound **1** (in CD_3OD, δ in ppm, *J* in Hz).

Positions	δ_H	δ_C & DEPT	HMBC (H-C)
1	5.38 (1H, *dd*, *J* = 5.4, 11.4)	77.6 (CH)	C2, C3, C5, C9, C10
2	1.55 (1Hα, *qd*, *J* = 4.8, 11.4) 2.31 (1Hβ, *m*)	26.2 (CH_2)	C1, C3, C10
3	1.89 (2H, *m*)	36.7 (CH_2)	C1, C2, C4, C5, C18, C19
4	-	34.8 (C)	-
5	-	144.1 (C)	-
6	7.71 (1H, *d*, *J* = 7.8)	130.5 (CH)	C4, C7, C8, C10
7	7.60 (1H, *d*, *J* = 7.8)	132.3 (CH)	C5, C9, C14
8	-	126.8 (C)	-
9	-	123.8 (C)	-
10	-	148.0 (C)	-
11	-	168.4 (C)	-
12	-	164.9 (C)	-
13	-	119.0 (C)	-
14	-	153.6 (C)	-
15	8.15 (1H, *s*)	148.5 (CH)	C13, C14, C16, C17
16	-	122.9 (C)	-
17	-	164.4 (C)	-
18	1.20 (3H, *s*)	31.7 (CH_3)	C3, C4, C5, C19
19	1.43 (3H, *s*)	30.9 (CH_3)	C3, C4, C5, C18

*Ganxincastanic acid A (**1**)*: Pale-yellow powder; $[\alpha]_D^{24} = -14.2$ (*c* 0.1, CH_3OH); UV(CH_3OH) λ_{max} nm (logε): 268 (4.32); IR(KBr) ν_{max} cm^{-1}: 3420, 3005, 2962, 2872, 1722, 1291 and 920. ^{1}H NMR and ^{13}C NMR data see Table 1. HRESI-MS *m/z*: 355.0838$[M-H]^-$ (calcd. $C_{19}H_{16}O_7$ for 356.0896). The spectra are given supoorting information file.

Additionally, nine known compounds were identified as tanshinone I (**2**) [14], cryptotanshinone (**3**) [14], dihydrotanshinone I (**4**) [14], tanshinone IIB (**5**) [14], protocatechuic acid (**6**) [15], caffeic acid (**7**) [16], rosmarinic acid (**8**) [17], lithospermic acid (**9**) [18] and salvianolic acid B (**10**) [19] by the comparisons of their ^{1}H and ^{13}C NMR spectral data with those reported values in the literature, respectively.

Compound **1** was obtained as a pale-yellow powder. The HRESI-MS gave the molecular formula to be $C_{19}H_{16}O_7$ (*m/z* 355.0838[M−H]$^-$, calcd. $C_{19}H_{16}O_7$ for 356.0896). The maximum absorption wavelength at 268 nm of the UV spectrum showed the existence of long conjugation structure. Meanwhile, the IR spectrum showed the presence of hydroxyl (3005 cm^{-1}), carboxyl or carbonyl functional groups (1722 cm^{-1}). The ^{1}H NMR spectrum of **1** gave two methyl signals [δ 1.20 (3H, *s*), 1.43 (3H, *s*)], a methylene signal [δ 1.89 (2H, *m*)], a splitting methylene signal [δ 1.55 (1H, *qd*, *J* = 4.8, 11.4 Hz), 2.31 (1H, *m*)], a methine with an oxygen attached [δ 5.38 (1H, *dd*, *J* = 5.4, 11.4)], three aromatic proton signals [δ 7.60 (1H, *d*, *J* = 7.8 Hz), 7.71 (1H, *d*, *J* = 7.8 Hz), 8.15 (1H, *s*)]. The ^{13}C NMR and DEPT spectra of **1** provided nineteen carbon signals, including two methyls at δ 30.9, 31.7, two methylenes at δ 26.2, 36.7, a methine with an oxygen attached at δ 77.6, three aromatic methines at δ 130.5, 132.3, 148.5, a saturated quaternary carbon at δ 34.8, seven aromatic quaternary carbons at δ 119.0, 122.9, 123.8, 126.8, 144.1, 148.0, 153.6 and three carboxyl or carbonyl carbons at δ 164.4, 164.9, 168.4. The ^{13}C NMR spectrum of **1** was closely similar to that of a known diterpenoid named castanol A (**1a**) [20] (As shown in Figure 1). The only difference was the replacement of a methyl at C-17 in **1a** by a carboxyl in **1**. The HMBC correlations between H-15 (δ 8.15) and C-17 (δ 164.4) further confirmed that the carboxyl at C-17 was present. The HMBC correlations between H-3 (δ 5.38) and C-4 (δ 34.8), C-18 (δ 31.7), C-19 (δ 30.9), between H-18 (δ 1.20), H-19 (δ 1.43) and C-3 (δ 36.7), C-4 (δ 34.8), C-5(δ 144.1) corroborated that the linkage position of the methyls at C-18 and C19 was located at C-4. The HMBC correlations between H-1 (δ 5.38) and C-2 (δ 26.2), C-10 (δ 148.0) affirmed that the linkage position of the hydroxyl was located at C-1. As the ^{1}H NMR splitting patterns of H-1 [δ 5.38 (1H, *dd*, *J* = 5.4, 11.4Hz)] in **1** and that of H-1 [δ 5.20 (1H, *dd*, *J* = 5.1, 11.5Hz)] in **1a** were very similar, the stereo configurations of the chiral C-1 in **1** and in **1a** was identical. From these evidences, the structure of compound **1** was established as Figure 1.



1 **1a**

Figure 1. Structure of compounds **1** and **1a**, and the key correlations in ^{1}H-^{1}H COSY and HMBC of compound **1**

Noticeably, diterpenoids with a carbon skeleton of a furo[3,2-c]naphth[2,1-e]oxepine-10,12-dione like compound **1** only had been reported from *Salvia przewalskii* Maxim. [21], *S. castanea* Diels f. *pubescens* [20], *S. yunnanens*is C.H.Wright [21], *S. miltiorrhiza* Bunge [22] and *S. miltiorrhiza* f. *alba* C. Y. Wu et H. W. Li [23]. From one side, it could suggest a close relationship between *S. przewalskii* and the other four *Salvia* plants of *S. castanea*, *S. yunnanens*is, *S. miltiorrhiza* Bunge and *S. miltiorrhiza* f. *alba*.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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