SHORT REPORT

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A New Iridoid Glycoside Isolated from *Valeriana officinalis* L.

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Abstract: A new iridoid glycoside, (5*S*,7*S*,8*S*,9*R*)-7- hydroxy-$\Delta^{4,11}$-dihyronepeta-1,3-diol-8-*O*-*β*-D-glucopyranosyl (**1**), along with **5** known compounds, dioscoridin A (**2**), jatamanin J (**3**), longiflorone (**4**), apigenin-8-*O*-*β*-D-glucopyranoside (**5**), isosakuranetin-5-*O*-rutinoside (**6**), were isolated from the *Valeriana officinalis* L. Their structures were determined by extensive analysis using various spectroscopic techniques. Moreover, the cytotoxic activity assay toward three human tumor cell (A549, HCT116 and SW620) lines were evaluated by the MTT method in vitro for compounds **1**-**2**, using cisplatin as positive control. Experimental results showed that these compounds displayed weak cytotoxicity in the human cancer cell lines. Notably, compounds **2**, **3** and **6** were firstly isolated from this plant, compound **4** was isolated from the genus *Valeriana* for the first time, compound **5** was isolated for the first time from Valerianaceae family.

Key words: Iridoids; *Valeriana officinalis* L.; Valerianaceae; cytotoxicity.

1. Plant Source

In the present study, *Valeriana officinalis* L. were collected from the Taibai region of the Qinba Mountains, Shaanxi Province, China, in June 2018, and authenticated by Professor Wei Wang (Shaanxi University of Chinese Medicine). A voucher specimen (herbarium No. 20180901) was deposited at the Shaanxi University of Chinese Medicine, Xianyang, China.

2. Previous Studies

Valeriana officinalis L. is the dried root and rhizome of *Valeriana officinalis*, a genus of *Valeriana* in the family Sapotaceae. *V. officinalis* is not only widely used in the treatment of restlessness, palpitations, insomnia and other neurosis, but also more used in the treatment of anxiety, gastrointestinal pain and other diseases [1]. Plants of the genus *Valeriana* (Valerianaceae), comprising approximately 200 species, are widely distributed in Eurasia, South America and central North America, and 17 species are distributed in China [2]. Previous studies revealed that *Valeriana* species produced iridoids,

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sesquiterpenoids, lignans, flavonoids, alkaloids and fatty acids, among which

iridoids, sesquiterpenoids and diterpenes predominated [3]. Among these components, pharmacological investigations on the genus *Valeriana* showed that iridoids have a neuroprotective effect [4] and lignans are good for the treatment of Alzheimer's disease [5].

As part of our ongoing search for biological constituents from the *Valeriana officinalis* L., a new iridoid glycoside (5*S*,7*S*,8*S*,9*R*)-7-hydroxy-$\Delta^{4,11}$-dihyronepeta-1,3-diol-8-*O*-*β*-D-glucopyranosyl (**1**) and five known compounds, dioscoridin A (**2**) [6], jatamanin J (**3**) [7], longiflorone (**4**) [8], apigenin-8-*O*-*β*-D-glucopyranoside (**5**) [9], isosakuranetin-5-*O*-rutinoside (**6**) [10], were procured (Figure 1). In this study, we described the structure identification of new compound, as well as the cytotoxic evaluation of two compounds **1**-**2**.

3. Present Study

The *V. officinalis* (10 kg) were ground into crude powder, then extracted three times with 80% EtOH under reflux. The extracts were combined and concentrated under reduced pressure, and then, the crude extract was suspended in water and successively extracted with petroleum ether and n-BuOH.

After extraction with petroleum ether and n-BuOH, the remaining aqueous parts were subjected to macroporous resin column chromatography (CC), eluting with a gradient system of EtOH-H_2O (0:100 to 90:10) to give 6 fractions (Fr.1-6).

Fr. 1 (19.32 g) was subjected to CC on silica gel, eluting with CH_2Cl_2-MeOH (80:1 to 0:100) to give 5 sub-fractions (Fr. 1-1–Fr. 1-5). Subfraction Fr. 1-2 (200.6 mg) was subjected to Sephadex LH-20 column chromatography and eluted with CH_2Cl_2-CH_3OH (50:50) to give compounds **2** (9 mg) and **6** (14 mg). Then, Fr. 1-3 (179.2 mg) was purified by semi-preparative high-performance liquid chromatography with MeOH–H_2O (4:96) as the mobile phase to obtain compounds **1** (t_R = 28 min, 7 mg), **3** (t_R = 35 min, 19 mg) and **4** (t_R = 56 min, 11 mg) with a detecting wavelength of 249 nm. Fr. 1-4 (196.3 mg) was purified by semi-preparative high-performance liquid chromatography with MeOH–H_2O (13:87) as the mobile phase to obtain compound **5** (t_R = 15 min, 20 mg) with a detecting wavelength of 254 nm (see Figure 1).

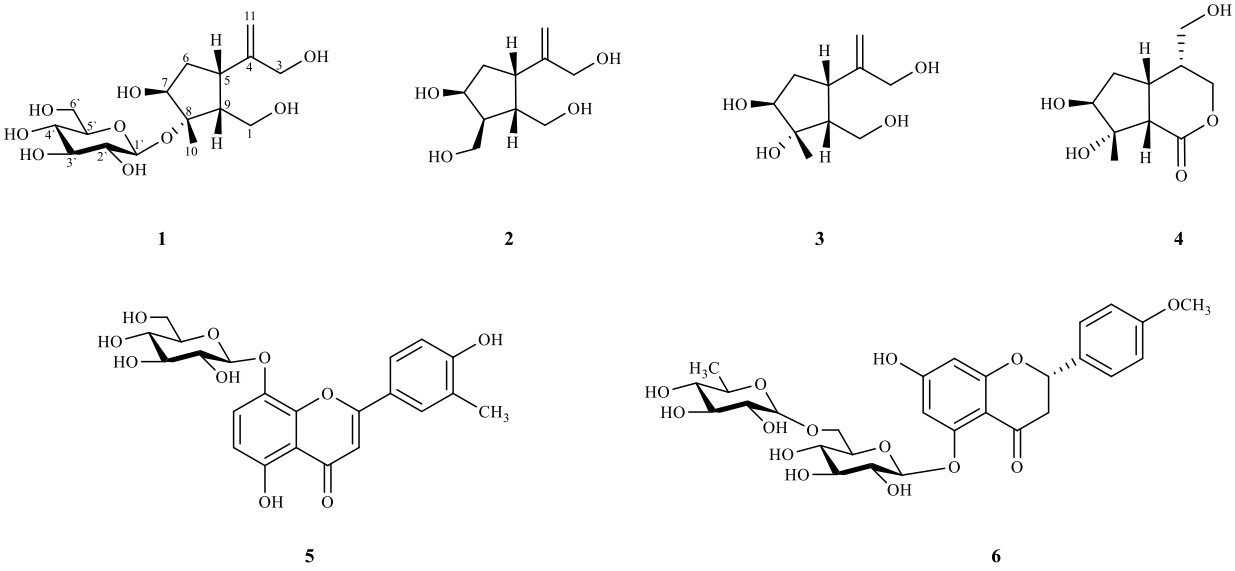

Figure 1. Structures of compounds **1**-**6**

Compound **1** was isolated as a colorless oily solid. Its molecular formula was determined as $C_{16}H_{28}O_9$ from the HR-ESI-MS at *m/z* 387.1619 $[M + Na]^+$ (calculated $C_{16}H_{28}O_9Na$, 387.1631). The IR

spectrum displayed for hydroxy (3366 cm^{-1}) and ether bonds (1027 cm^{-1}). This formula suggested 3 units of unsaturation in the molecule. The ^{1}H NMR spectrum (Table. 1) showed three protons of methines groups at δ_H 3.02 (1H, dd, *J* = 9.2, 9.2 Hz, H-5), 4.09 (1H, dd, *J* = 3.8, 3.8 Hz, H-7) and 2.20 (1H, dd, *J* = 8.8, 5.6 Hz, H-9); the geminal protons at δ_H 5.07 (1H, s, H-11a) and 5.23 (1H, s, H-11b), as two singlets and the coupling constants of approximately 0 Hz, characteristic of alkene hydrogen signals, ascribable to the aglycone proton signals of this iridoid glycoside. The ^{13}C NMR spectrum (Table. 1) exhibited 16 carbon signals, 10 of which belonged to the aglycone carbons. The presence of a terminal olefinic bond was inferred through a quaternary carbon signal at δ_C 150.2 (C-4), as well as a methylene carbon signal at δ_C 112.3 (C-11), which showed correlations with two olefinic proton signals at δ_H 5.07 (H-11a) and 5.23 (H-11b) in the HSQC spectrum; two hydroxymethyl carbon signals at δ_C 60.4 and 67.1 were attributed to C-1 and C-3, which showed correlations with proton signals at δ_H 3.46 (1H, dd, *J* = 11.2, 6.1 Hz, H-1a) and 3.83 (1H, dd, *J* = 11.2, 7.8 Hz, H-1b), δ_H 4.02 (1H, s, H-3a) and 4.04 (1H, s, H-3b) in the HSQC spectrum; the oxygenated quaternary carbon of C-8 was deduced by the resonance shifted to δ_C 89.8 after glycosylation. All above data suggested the aglycone of **1** was similar to Jatamanin J [7], except for C-7 - C-9, which resulted from glycosylation shifts. The above analysis was finally verified by of the 2D NMR data. The HSQC experiment allowed for the assignments of the proton and protonated carbon resonances in the NMR spectra of **1**. The ^{1}H-^{1}H COSY correlations (Figure 2) from H-5/H-6/H-9, from H-6/H-7/H-5 and from H-9/H-1/H-5, key HMBC correlations (Figure 2) from H-11 to C-4/C-3/C-5, from H-7 to C-8/C-5/Me-10/C-9, from Me-10 to C-8/C-7/C-9 and from H-9 to C-8/C-1/C-5 were observed. Accordingly, the structure of the aglycone moiety was identified as (5*S*,7*S*,8*S*,9*R*)-7-hydroxy-$\Delta^{4,11}$-dihyronepeta-1,3-diol. In addition, a characteristic of a glucosyl moiety (δ_C 99.2, 75.2, 77.7, 71.8, 78.5, 62.9) was observed. Coupling constant of the anomeric proton [δ_H 4.57 (1H, d, *J* = 7.8 Hz, H-1')] indicated *β*-configuration, while enzymatic hydrolysis of **1** confirmed the presence of D-glucose moiety by TLC comparison and optical rotation determination with an authentic sugar sample [11]. The HMBC correlation signals of δ_H 4.57/δ_C 89.8 indicated the glucosyl group was linked to C-8. The relative configuration of **1** was established by the NOESY experiment. The NOESY correlations (Figure 2) of H-9 with H-5 and H-10, H-7 with H-6a, H-5 with H-6b showed the cis-configuration of H-5 and H-9, the presence of 7*β*-OH and the location of *β*-D-glucopyranosyl unit on the *α*-position of cyclopentan moiety. The relative configuration of **1** was the same as that of Jatamanin J [7] and detailed comparison of the ^{1}H NMR coupling constants of **1** with Jatamanin J. In the CD spectrum, the negative cotton effects (CEs) in 200-210 nm (Figure 3) disclosed the 5*S*, 7*S*, 8*S*, 9*R* absolute configuration of **1**. To further determine its absolute configurations, the ECD curves (Figure 3) were simulated of **1** [(5*S*,7*S*,8*S*,9*R*)-1]. The experimental and calculated ECD curves of (5*S*,7*S*,8*S*,9*R*)-**1** matched well. Therefore, the structure of **1** was designated as (5*S*,7*S*,8*S*,9*R*)-7- hydroxy-$\Delta^{4,11}$-dihyronepeta-1,3-diol-8-*O*-*β*-D-glucopyranosyl.

Table 1. ^{1}H and ^{13}C NMR data for compound **1**.

Position	δ_C	δ_H	Position	δ_C	δ_H
1	60.4, CH_2	H-1a, 3.46, dd (11.2, 6.1)	10	19.8, CH_3	1.45, s
		H-1b, 3.83, dd (11.2, 7.8)	11	112.3, CH_2	H-11a, 5.07, s
3	67.1, CH_2	H-3a, 4.02, s			H-11b, 5.23, s
		H-3b 4.04, s	1'	99.2, CH	4.57, d (7.8)
4	150.2, C	-	2'	75.2, CH	3.17, dd (9.1, 7.7)
5	41.3, CH	3.02, dd (9.2, 9.2)	3'	77.7, CH	3.26, m
6	36.3, CH_2	H-6a, 1.62, ddd (13.0, 9.5, 3.5)	4'	71.8, CH	3.27, m
		H-6b, 2.36, ddd (13.0, 10.0, 3.6)	5'	78.5, CH	3.31, m
7	78.2, CH	4.09, dd (3.8, 3.8)	6'	62.9, CH_2	H-6'a, 3.62, dd (12.0, 5.6)
8	89.8, C	-			H-6'b, 3.83, dd (12.0, 1.6)
9	54.1, CH	2.20, dd (8.8, 5.6)			

*400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR in CD_3OD in ppm, *J* in Hz.

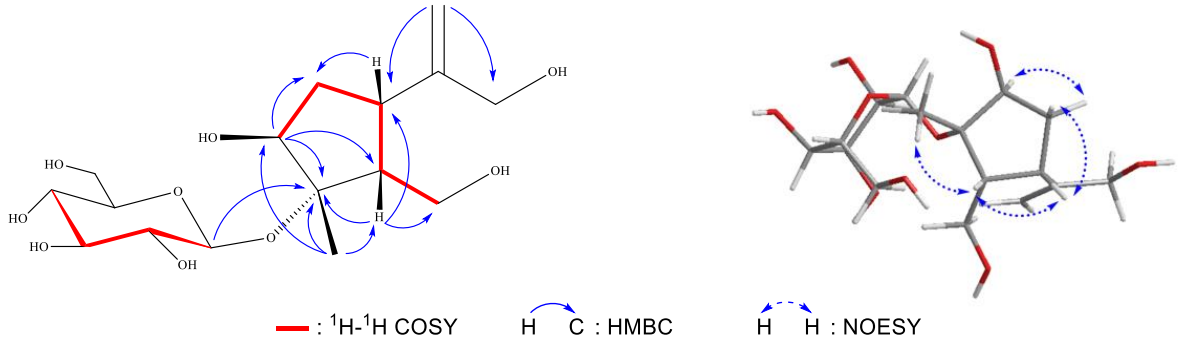

Figure 2. Key ^{1}H - ^{1}H COSY, HMBC and NOESY relevant of compound **1**

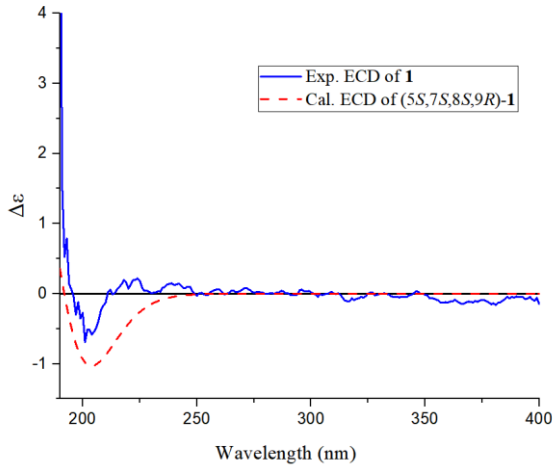

Figure 3. Experimental and calculated ECD spectra of **1**

Moreover, the cytotoxic activity assay toward three human tumor cell (A549, HCT116 and SW620) lines were measured by the MTT method in vitro for compounds **1**-**2**, using cisplatin as positive control. Experimental results (Table. 2) showed that these compounds displayed weak cytotoxicity in the human cancer cell lines.

Table 2. Cytotoxic effects of compounds **1**－**2** on A549, HCT116 and SW620 cancer cell lines. (IC_{50}, μM) [a]

Compounds	A549	HCT116	SW620
Cisplatin	32.06 ± 1.31	43.54 ± 3.30	32.47 ± 3.39
1	>100	>80	>100
2	>100	>80	>100

[a]IC_{50} values are means from three independent experiments (average ± SD) in which each compound concentration was tested in three replicate wells; Cisplatin as positive control

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Supporting Information

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