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## Antibacterial constituents from *Pedicularis armata*

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A new neolignan glycoside named armaoside (**1**), together with six known compounds (**2–7**), have been isolated from the whole plant of *Pedicularis armata* Maxim. The structure of **1** was elucidated as *erythro*-(7*S*,8*R*)-1-(4- $\beta$ -D-glucopyranosyl-3-methoxyphenyl)-2-[3,5-dimethoxy-4-oxo-cinnamic aldehyde]-propane-1, 3-diol by spectroscopic and chemical methods. All compounds were assayed against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*.

**Keywords:** *Pedicularis armata*; Scrophulariaceae; Neolignan glycoside; Armaoside; Antibacterial activity

### 1. Introduction

The genus *Pedicularis* comprises about 329 species in China [1]. The roots and stems of many of them are used as Chinese folk medicine and as cardiac tonic for the treatment of collapse, exhaustion and senility [2]. In our research on *Pedicularis armata*, a new neolignan glycoside named armaoside (**1**), along with six known compounds, citrusin B (**2**) [3], euphroside (**3**) [4], mussaenoside (**4**) [5], geniposidic acid (**5**) [6], 8-epi-loganic acid (**6**) [7], and aucubin (**7**) [8,9], have been isolated from the ethanolic extract of *P. armata* Maxim. The structures of the known compounds were identified by comparing their corresponding properties (FAB-MS,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR) with the reported values in the literature. This paper reports the structure elucidation of the new compound (**1**) and antibacterial activity of compounds **1–7**. In addition, the absolute configuration of citrusin B (**2**) is reported for the first time.

### 2. Results and discussion

The *n*-BuOH-soluble fraction of the ethanolic extract of *Pedicularis armata* was successively chromatographed over silica gel column to afford compounds **1–7**. The structures of the known compounds **2–7** were identified by comparison of their spectral data with those reported in the literature (figure 1).

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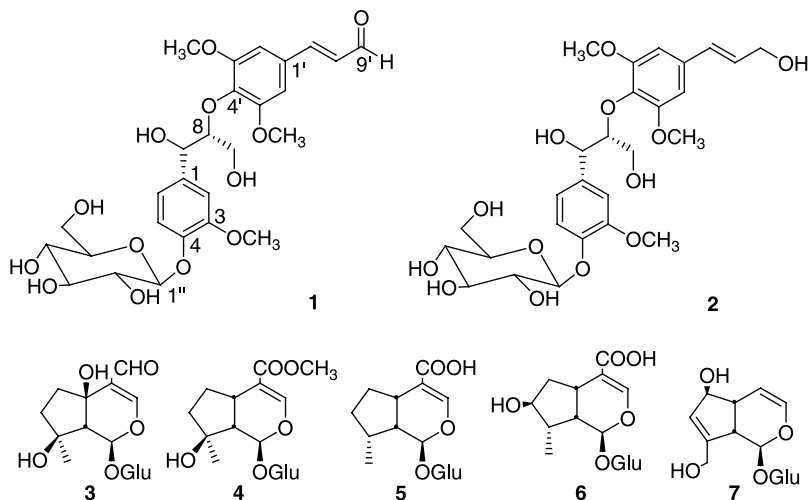


Figure 1. Structures of compounds 1–7.

Compound **1** was obtained as white amorphous powder; its molecular formula  $C_{27}H_{34}O_{13}$  was established by HRESI-MS at  $m/z$  589.1893  $[M + Na]^+$ , and the IR spectrum showed absorption peaks at  $3397\text{ cm}^{-1}$  (hydroxyl),  $1707\text{ cm}^{-1}$  (a conjugated carbonyl group) and  $1667, 1582, 1505, 1461\text{ cm}^{-1}$  (aromatic ring). Its  $^1\text{H}$  NMR spectrum showed the presence of two *trans*-double bond protons at  $\delta$  7.60 (d, 1H,  $J = 15.6\text{ Hz}$ , H-7') and 6.76 (dd, 1H,  $J = 15.6, 8.0\text{ Hz}$ , H-8'), an aldehyde proton at  $\delta$  9.62 (d, 1H,  $J = 8.0\text{ Hz}$ , H-9'), a 1,3,4-trisubstituted aromatic ring at  $\delta$  7.08 (d, 1H,  $J = 1.6\text{ Hz}$ , H-2), 7.11 (d, 1H,  $J = 8.0\text{ Hz}$ , H-5) and 6.91 (dd, 1H,  $J = 8.0, 1.6\text{ Hz}$ , H-6), a 1,3,4,5-tetrasubstituted aromatic ring at  $\delta$  7.00 (brs, 2H, H-2', 6'), and an anomeric proton of glucose at  $\delta$  5.01 (d, 1H, 7.8 Hz, H-1''), which could be deduced on the basis of their chemical shifts, splitting patterns and coupling constants. The  $^{13}\text{C}$  NMR and DEPT spectra of **1** showed three methoxy groups ( $\delta$  55.6), an oxygenated methylene ( $\delta$  61.1, C-9), two oxygenated methines ( $\delta$  72.8, C-7; 86.1, C-8), and six oxygenated glycoside carbons ( $\delta$  101.8, CH, C-1''; 73.7 CH, C-2''; 76.6, CH, C-3''; 70.2, CH, C-4''; 77.0, CH, C-5''; 61.3,  $\text{CH}_2$ , C-6'') in the more upfield area. Further analysis of the  $^1\text{H}$ – $^1\text{H}$  COSY spectrum of compound **1** disclosed that H-8' correlated to H-7' and H-9', while H-8 ( $\delta$  4.40, m, 1H) correlated to H-7 ( $\delta$  4.94, d, 1H,  $J = 4.8\text{ Hz}$ ) and H-9 ( $\delta$  3.90, dd, 1H,  $J = 10.8, 4.2\text{ Hz}$ , H-9a;  $\delta$  3.63, dd,  $J = 10.8, 5.6\text{ Hz}$ , H-9b). On the basis of such preliminary information and in combination with the observed HMBC correlations from H-7 to C-1, C-2, and C-6, and from H-7' to C-1', C-2', and C-6', the presence of two C6–C3 units in the molecule of compound **1** was evident, which could be inferred that this compound is an 8-*O*-4' neolignan derivative. The location of the methoxyls and glucose were assigned on the basis of HMBC correlations from  $\delta_{\text{H}}$  3.85 (9H, s, 3-OMe, 3'-OMe, and 5'-OMe) to  $\delta_{\text{C}}$  149.3 (C-3) and 153.6 (C-3' and C-5'), and H-1'' to  $\delta_{\text{C}}$  146.7 (C-4). The coupling constant of H-1'' ( $J = 7.8\text{ Hz}$ ) suggested the glucose was in  $\beta$ -orientation, and the D-glucose was confirmed by PC and by its optical rotation ( $[\alpha]_{\text{D}}^{20} + 46$  ( $c$  0.08,  $\text{H}_2\text{O}$ )) after acid hydrolysis with HCl of compound **1**. In fact, the signal pattern was somewhat similar to that reported for citrusin B (**2**) [3], except for the presence of an aldehyde group in **1** instead of the hydroxymethyl group in **2**. This was consistent with the position of the signal for C-7' ( $\delta_{\text{C}}$  154.0) which is 23.8 ppm downfield compared to that of C-7' ( $\delta_{\text{C}}$  130.2) in citrusin B (**2**) (table 1). The coupling constant between H-7 and H-8 was 4.8 Hz, which suggested that the relative

Table 1.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data of **1** and **2** ( $\text{CD}_3\text{OD}$ , TMS,  $\delta_{\text{ppm}}$ ,  $J_{\text{Hz}}$ )<sup>†</sup>.

No.	$^1\text{H-NMR}$		$^{13}\text{C-NMR}$	
	<b>1</b>	<b>2</b>	<b>1</b>	<b>2</b>
1	–	–	130.2 (C)	133.6 (C)
2	7.08 (d, 1.6)	7.07 (brs)	111.3 (CH)	111.2 (CH)
3	–	–	149.3 (C)	149.3 (C)
4	–	–	146.7 (C)	146.0 (C)
5	7.11 (d, 8.0)	7.12 (d, 8.1)	116.4 (CH)	116.4 (CH)
6	6.91 (dd, 8.0, 1.6)	6.91 (dd, 8.1)	119.8 (CH)	119.6 (CH)
7	4.94 (d, 4.8) <sup>‡</sup>	4.94 (d, 5.4)	72.8 (CH)	72.6 (CH)
8	4.40 (m) <sup>‡</sup>	4.40 (m)	86.1 (CH)	86.1 (CH)
9a	3.90 (dd, 10.8, 4.2) <sup>‡</sup>	3.33–3.70	61.1 (CH <sub>2</sub> )	60.3 (CH <sub>2</sub> )
9b	3.63 (dd, 10.8, 5.6) <sup>‡</sup>	3.33–3.70		
1'	–	–	136.3 (C)	135.1 (C)
2'	7.00 (brs) <sup>¶</sup>	6.72 (brs) <sup>c</sup>	106.1 (CH) <sup>c</sup>	103.7 (CH) <sup>¶</sup>
3'	–	–	153.6 (C)	153.4 (C)
4'	–	–	138.7 (C)	136.3 (C)
5'	–	–	153.6 (C)	153.4 (C)
6'	7.00 (brs) <sup>¶</sup>	6.72 (brs) <sup>¶</sup>	106.1 (CH) <sup>¶</sup>	103.7 (CH) <sup>¶</sup>
7'	7.60 (d, 15.6)	6.54 (d, 15.9)	154.0 (CH)	130.2 (CH)
8'	6.76 (dd, 15.6, 8.0)	6.32 (dd, 15.9, 5.4)	127.9 (CH)	128.7 (CH)
9'	9.62 (d, 8.0)	4.23/4.21 (s)	194.9 (CH)	62.4 (CH <sub>2</sub> )
1''	5.01 (d, 7.8)	4.84 (d, 7.8)	101.8 (CH)	101.8 (CH)
2''	3.38–3.82	3.33–3.70	73.7 (CH)	73.7 (CH)
3''	3.38–3.82	3.33–3.70	76.6 (CH)	76.6 (CH)
4''	3.38–3.82	3.33–3.70	70.2 (CH)	70.2 (CH)
5''	3.38–3.82	3.33–3.70	77.0 (CH)	77.0 (CH)
6''	3.38–3.82	3.33–3.70	61.3 (CH <sub>2</sub> )	61.3 (CH <sub>2</sub> )
OMe	3.85 (s) <sup>¶</sup>	3.84/3.81(s) <sup>¶</sup>	55.6 (CH <sub>3</sub> ) <sup>¶</sup>	55.56 (CH <sub>3</sub> ) <sup>¶</sup>

<sup>†</sup> The  $^1\text{H}$  NMR of **1** and **2** were obtained at 400 MHz and 300 MHz, and the  $^{13}\text{C}$  NMR of **1** and **2** were obtained at 100 MHz and 75 MHz, respectively.

<sup>‡</sup> The signals were assigned by TOCSY 1D.

<sup>¶</sup> Overlapped signals.

configuration between two protons is *erythro* [10]. The absolute configuration *7S,8R* for compound **1** derived from the negative CD curve in the 210–250 nm range [11]. Thus compound **1** was structurally elucidated as *erythro*-(*7S,8R*)-1-(4-*O*- $\beta$ -D-glucopyranosyl-3-methoxyphenyl)-2-[3,5-dimethoxy-4-oxo-cinnamic aldehyde] propane-1,3-diol, which was named as armaoside.

Compound **2** was obtained as white amorphous powder, which was identified as citrusin B by comparing their corresponding properties (FAB-MS,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR) with the reported values in the literature [3]. The coupling constant of 4.8 Hz between H-7 and H-8 protons suggested a *erythro* relative configuration and negative CD curve in the 210–250 nm range agreed with a *7S,8R*-configuration in the case of **1**.

### 3. Experimental

#### 3.1 General experimental procedures

Optical rotations were measured on a Perkin–Elmer 341 Polarimeter. IR spectra were determined on a Nicolet 170SX FT-IR instrument in KBr pellets. HRESI-MS were measured on a Bruker APEX II instrument and FAB-MS on a VG-ZAB-HS mass spectrometer (at 70 eV).  $^1\text{H}$  NMR (400 MHz or 300 MHz),  $^{13}\text{C}$  NMR (100 MHz or 75 MHz), HMBC, TOCSY and  $^1\text{H}$ ,  $^1\text{H}$  NOESY spectra were taken with TMS as internal standard on a Varian

mercury-400 BB-NMR and mercury-300 BB-NMR spectrometer. Column chromatography was performed on silica gel (200–300 mesh, Marine Chemical factory, Qingdao, China) and TLC was conducted on silica GF254 (Marine Chemical factory, Qingdao).

### 3.2 Plant material

*Pedicularis armata* was collected in the Gansu province of China, in August 1999. It was identified by Professor Guo-Liang Zhang of the Department of Biology, Lanzhou University. A voucher specimen (No. 9908PA) is deposited in the herbarium of our institute.

**3.2.1 Extraction and isolation.** The air-dried whole plant of *Pedicularis armata* (1.8 kg) was powdered and extracted three times (7 days per time) at room temperature with 95% ethanol. The extract was concentrated under reduced pressure to yield residue (168 g); the residue was diluted with hot water and the water-insoluble material was removed by filtration through Celite. The filtrate was extracted with EtOAc and n-BuOH. The n-BuOH portion (144 g) was chromatographed over a silica gel column (eluted with EtOAc/MeOH from 30:1 to 1:1), and five fractions were collected according to TLC analysis. Fraction 2 (16 g) was purified over a silica gel column (eluted with CHCl<sub>3</sub>/MeOH 20:1 to 1:1) to obtain **5** (58 mg). Fraction 3 (11 g) was subjected to a silica gel column (eluted with CHCl<sub>3</sub>/MeOH from 20:1 to 1:1) to obtain **2** (10 mg), **3** (28 mg), **4** (17 mg) and **5** (9 mg). Fraction 4 (13 g) was applied to a silica gel column (eluted with CHCl<sub>3</sub>/MeOH from 20:1 to 1:1) to afford **7** (12 mg) and **1** (7 mg).

### 3.3 Antibacterial assays

The antibacterial screening was carried out by employing the cup-plate method. Chloramphenicol was used as a positive control. Three strains of bacteria, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*, were cultured in beef broth and incubated at 37°C for 24 h. After dilution of beef broth, the three bacteria were cultured in agar medium dishes, respectively; six cups (8 × 10 mm) were put onto the dishes, and each tested compound (0.2 ml of 100 µg/ml) was respectively added into the cups under aseptic conditions. Then the dishes were cultured at 37°C for 24 h. The zone of inhibition of the growth of bacteria, which were produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the antibacterial activity. Each test was performed twice. The preliminary results indicated that most of the compounds were active against these bacteria, and Compounds **6** and **7** exhibited strong antibacterial activities against *E. coli* and *S. aureus* respectively (table 2).

### 3.4 Armaoside (1)

White amorphous powder,  $[\alpha]_D^{20} - 15$  (c 0.35, CH<sub>3</sub>OH); IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3397, 2922, 2851, 1740, 1707, 1667, 1582, 1505, 1461, 1423, 1363, 1335, 1266, 1246, 1224, 1127, 1075, 1042, 918, 819, 736, 694, 655, 406; UV 206.6 nm (1.371); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) and <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 Hz), see table 1; HRESI-MS  $m/z$  589.1893 (calcd for C<sub>27</sub>H<sub>34</sub>O<sub>13</sub>Na, 589.1892); CD in MeOH:  $[\theta]_{224} = -795$ ,  $[\theta]_{231} = -875$ .

Table 2. Antibacterial activity of compounds 1–7.

Compound	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. aureus</i>
1	++	++	++
2	++	+	++
3	+	++	++
4	+	++	++
5	–	++	++
6	+	+++	++
7	++	++	+++
H <sub>2</sub> O	–	–	–
Chloramphenicol	+++	+++	+++

Zone diameter of growth inhibition: <10 mm (–), 10–12 mm (+), 13–15 mm (++) and 16–20 mm (+++).

### 3.5 *Cirusin B* (2)

White amorphous powder,  $[\alpha]_D^{20} - 43.6$  (*c* 1.72, CH<sub>3</sub>OH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz) and <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz), see table 1; FAB-MS *m/z* 575.2 (M + Li)<sup>+</sup>, 591.2 (M + Na)<sup>+</sup>;  $[\theta]_{225} = -10127$ .

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