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Two New Phenolic Compounds from *Schizonepeta tenuifolia* (Benth.) Briq

Xu-Hua Huang[1], Jian-Hua Shao[1], Chun-Chao Zhao[1*], Jia Chen[1], Xiu-Hua Shan[2], Xiao-Qing Xu[1] and Wen-Ting Zhang[1]

[1]*College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225009, China*
[2]Second affiliated hospital of Baotou medical college, Baotou, Inner Mongolia 014030, *China*

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Abstract: Two new phenolic compounds, Schitenoside A (**1**) and Schitenoside B (**2**), have been isolated together with six known compounds: 3,4-dihydroxyphenethyl alcohol-4-*O*-*β*-D-glucopyranoside (**3**), 2-(3-hydroxy-4-methoxyphenyl) ethanol 1-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (**4**), benzyl 7-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (**5**), 2-hydroxybenzoic acid (**6**), m-hydroxybenzoic acid (**7**) and trans-caffeic acid (**8**), from the *Schizonepeta tenuifolia*. Their structures were elucidated by spectroscopic analysis. Compounds (**3**-**7**) were isolated from *Schizonepeta* genus for the first time. Compounds **1** and **2** showed a week antibacterial activity against four test strains, involving both Gram-positive and Gram-negative bacteria.

Keywords: Lamiaceae; *Schizonepeta tenuifolia*; phenolic compounds; antibacterial activity.

1. Introduction

Schizonepeta tenuifolia (Benth.) Briq. belongs to the Lamiaceae family, it's an annual herbaceous plant and is widespread in China [1]. *S. tenuifolia*, used as a source of Jingjie, has a pungent taste with a slightly "warm" property according to the theory of traditional Chinese medicine. Therefore, it has been well known for the treatment of common cold with headache, cough and fever [2]. Phytochemical investigations of *S. tenuifolia* have led to the isolation of several kinds of chemical constituents such as phenolic compounds and essential oils [3-5]. As part of our systematic study on the genus *Schizonepeta* of China Lamiaceae plants, we have investigated the constituents of *S. tenuifolia*.

From the *n*-butanol extract, two new phenolic compounds, Schitenoside A (**1**) and Schitenoside B (**2**), together with six known compounds, have been obtained. Herein, details of the isolation and structure elucidation of the chemical constituents **1**-**8** (Figure 1) are described, and the antibacterial activity of new compounds against four test strains was evaluated using the inhibition-zone assay.

2. Materials and Methods

2.1. General

NMR Spectra were performed on a Bruker AVANCE-600 spectrometer with tetramethylsilane (TMS) as an internal standard at 600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR. HR-MS spectra were obtained by electrospray ionization (ESI) on a Bruker MaXis ultra-high resolution (UHR) TOF apparatus. TLC was carried out on silica-gel plates (Yan-tai Institute of Chemical Technology). Column chromatography (CC) was performed on silica gel (200 – 300, 300 – 400 mesh; Qingdao

* Corresponding author: E-Mail: cczhao@yzu.edu.cn; Phone: 086-514-87971797; Fax: 086-514-87311374

Marine Chemical Factory), Sephadex LH-20 (40-70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-18 gel (40-63 μm; Merck, Darmstadt, Germany). All analytical grade solvents were produced by Sinopharm Chemical Reagent Co., Ltd.

2.2. Plant material

The whole plant of *S. tenuifolia* was collected in Guizhou Province, P. R. China, in October 2008, and identified by Prof. Qi-shi Sun, Shengyang Pharmaceutical University, China. A voucher specimen (No. SA080107) has been deposited at the College of Bioscience and Biotechnology, Yangzhou University, China.

2.3. Extraction and Isolation

The air-dried parts of the whole plant of *S. tenuifolia* (3.2 kg) were extracted with ethanol (95% v/v) three times, 2 h for each. The ethanol extract was concentrated under reduced pressure to yield a crude residue, which was then suspended in 8 liters of water and partitioned sequentially with petroleum ether, ethyl acetate and *n*-butanol to give three portions. The *n*-butanol portion (83 g) was subjected to the macroporous resin HPD 100 column. A successive elution of the column with H_2O and EtOH (95% v/v) yielded two corresponding portion after removing solvents. The portion (61 g) eluted by 95% EtOH was chromatographed on silica gel CC, eluting with the gradient $CHCl_3$—MeOH—H_2O (80:20:2–70:30:5–60:40:10), to provide eight fractions. Fr. 1 (12 g) was separated by Sephadex LH-20, eluting with MeOH, to afford three subfractions (Ea_1–Ea_3) on the basis of TLC analysis. Subsequent separation of Ea_1 (3 g) over ODS eluted with MeOH (35% v/v) gave compounds **4** (13 mg) and **5** (9 mg). Ea_2 (2 g) was isolated by ODS, eluting with MeOH—H_2O (20% v/v), to afford compound **6** (10 mg). Ea_3 (1 g) was also purified by ODS, eluting with MeOH—H_2O (25% v/v), to obtain compound **8** (3 mg). Fr. 2 (4 g) was subjected to silica gel CC, eluting with the gradient $CHCl_3$—MeOH (100:6–100:10–100:20–100:30–100:50), to give two subfractions (Eb_1–Eb_2), Eb_1 (1 g) was repeatedly chromatographed over Sephadex LH-20, eluting with MeOH, to get compounds **1** (1 mg), **2** (2 mg). Eb_2 (2 g) was repeatedly purified by Sephadex LH-20, eluting with $CHCl_3$—MeOH (2:1), to yield compounds **3** (3 mg). Fr. 4 (7 g) was separated by Sephadex LH-20, eluting with MeOH, to yield compound **7** (2 mg).

2.4. Antibacterial Assay

The antibacterial activity of compounds **1** and **2** against four test strains was evaluated using the inhibition-zone assay. Standard bacteria (Liaoning Province Institute of Drug Control, Liaoning, China) were grown in LB (Luria-Bertani) broth to an $OD_{600\ nm}$ of 0.8. A 10 μL aliquot of the bacteria was then taken and added to 8 mL fresh LB broth with 0.7% agar and poured over a 90 mm Petri dish containing 25 mL 1.5% agar in LB broth. A 10 μL of the solutions (2 mg/mL) of compounds 1 and 2 prepared in DMSO were added on filter papers with a diameter of 6.0 mm. After the agar had hardened, the filter papers were put onto its surface, and incubated overnight at 37 °C. The size of the clear zone formed on the agar showed the level of antibacterial activity of the examined sample. Ciprofloxacin was used as positive control.

Compounds 1 and 2 were dissolved in DMSO to a concentration of 2 mg/mL, then diluted with LB broth; on 96-well micro-plates, this solution was ½ serially diluted with the same broth, added with 0.1 mL/well of bacterial inoculum (10^6 bacteria/mL) and incubated at 37 °C for 24 h in ambient atmosphere. The MIC (the minimum inhibitory concentration) was defined as the lowest antimicrobial concentration that completely inhibited growth as detected by the naked eye [6, 7].

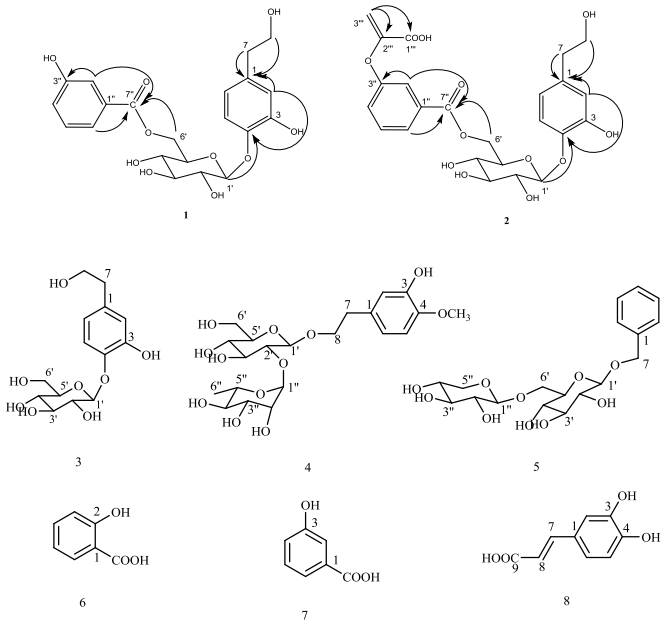

Figure 1. Key HMBC correlations of **1**, **2** and the structures of compounds **1**-**8**.

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was obtained as yellow amorphous powder. The molecular formula was determined as $C_{21}H_{24}O_{10}$ by HR-ESI MS at *m/z* 459.1270 $[M+Na]^+$ (calcd. for $C_{21}H_{24}O_{10}Na$, 459.1267). It showed a positive reaction to Molisch reagent. The sugar moiety was determined as D-glucose by TLC analysis and measuring the optical rotation of the acid hydrolysis solution of **1**, $[\alpha]_D^{20}$+42.9° (*c*=0.04, H_2O).The ^1H NMR spectrum indicated the presence of a 1,3-disubstituted benzene ring [δ_H 7.40 (1H, br.d, *J* = 7.7 Hz, H-6"), δ_H 7.38 (1H, br.s, H-2"), δ_H 7.32 (1H, br.dd, *J* = 7.7, 8.0 Hz, H-5"), δ_H 7.05 (1H, br.d, *J* = 8.0 Hz, H-4")], a 1,3,4-trisubstituted benzene ring [δ_H 6.86 (1H, br.s, H-2), δ_H 6.68 (2H, m, H-5, 6)], a hydroxyethyl protons [δ_H 2.48 (1H, t, *J* = 7.0 Hz, H-7a), δ_H 2.49 (1H, t, *J* = 7.0 Hz, H-7b), δ_H 3.46 (2H, t, *J* = 7.0 Hz, H-8)], and signals characteristic of a glucopyranosyl residue. The coupling constant of the signal at δ_H 4.74 (1H, d, *J* =7.1 Hz) assignable to an anomeric proton of the glucose indicated a *β*-configuration for the glucosidic linkage. Its structure was determined by 1 D (^1H, ^{13}C) and 2 D (HSQC and HMBC) NMR (Table 1, Figure 1) experiments. The 1D NMR spectroscopic data of **1** showed a quite similar pattern with those of **3**, 3,4-dihydroxyphenethyl alcohol-4-O-*β*-D-glucopyranoside [8]. The only difference between **1** and **3** was an extra m-hydroxybenzoyl group [δ_C: 130.8 (C-1"), 115.6 (C-2"), 157.5 (C-3"), 120.3 (C-4"), 129.7 (C-5"), 119.7 (C-6"), 165.6 (C-7")] in **1**. In the ^{13}C NMR spectrum (Table 1), the downfield shift of a methylene carbon (C-6' at δ_C 64.1), along with the upfield shift of a neighboring carbon (C-5' at δ_C 73.8) of a glucose suggested that the m-hydroxybenzoyl group was linked with the glucosyl group at C-6'. In the HMBC spectrum, the relevant correlations between the C-7" (δ_C 165.6) and the H-6' signal, and between C-4 (δ_C 144.9) and H-1' (δ_H 4.74), revealed that the m-hydroxybenzoyl group was located at C-6' and *β*-glucopyranosyl moiety was located at C-4. These spectral data established that **1** was a new compound, given a trivial name, Schitenoside A.

Compound **2** was obtained as yellow amorphous powder. The molecular formula was determined as $C_{24}H_{26}O_{12}$ by ESI-MS at *m/z* 529.1317 $[M+Na]^+$ (calcd. for $C_{24}H_{26}O_{12}Na$, 529.1322). It showed a positive reaction to Molisch reagent and Bromocresol green. The sugar moiety was determined as D-glucose by TLC analysis and measuring the optical rotation of the acid hydrolysis solution of **1**, $[\alpha]_D^{20}$+42.9° (*c*=0.04, H_2O). The ^1H NMR and ^{13}C NMR spectroscopic data (Table 1) of **2** showed a quite similar pattern with those of **1** and **3**, The only difference between **2** and **3** was an extra 3"-(1'"-carboxyvinyloxy) benzoyl group, a 1,3-disubstituted benzene ring [δ_H 7.54 (1H, br.d, *J* = 7.7 Hz, H-6"), δ_H 7.41 (1H, d, *J* = 2.1 Hz, H-2"), δ_H 7.40 (1H, br.dd, *J* = 7.7, 8.2 Hz, H-5"), δ_H 7.12 (1H, dd, *J* =

2.1, 8.2 Hz, H-4"); δ_C: 130.6 (C-1"), 116.9 (C-2"), 158.1 (C-3"), 121.7(C-4"), 129.5 (C-5"), 121.9 (C-6")], a 2-substituted acrylic acid protons [δ_H 5.41 (1H, br.s, H-3a'"), δ_H 4.74 (1H, br.s, H-3b'"); δ_C: 164.0 (C-1'"),157.2 (C-2'"), 102.1 (C-3'")], and δ_C 165.4 (C-7") in **2**. Additionally, a 1,3,4-trisubstituted benzene ring [δ_H 6.82 (1H, d, *J* = 1.1 Hz, H-2), δ_H 6.68 (1H, d, *J* = 8.1 Hz, H-5), δ_H 6.65 (1H, dd, *J* = 1.1, 8.1 Hz, H-6); δ_C: 130.5 (C-1), 117.3 (C-2), 145.0 (C-3), 144.9 (C-4), 115.6 (C-5'), 123.2 (C-6)], and a hydroxyethyl protons [δ_H 2.43 (2H, t, *J* = 6.8 Hz, H-7), δ_H 3.40 (2H, t, *J* = 6.8 Hz, H-8); δ_C: 38.3 (C-7), 62.3 (C-8)]. Furthermore, the downfield shift of the δ_C 64.4 (C-6') and the HMBC correlation (Figure 1) between H-6'/C-7" suggested that the 3"-(1'"-carboxyvinyloxy) benzoyl group was coupled with the glucosyl group at C-6'. The relevant correlation between the C-4 (δ_C 144.9) and H-1', revealed that the β-glucopyranosyl moiety was located at C-4. Thus the compound **2** was determined to be Schitenoside B.

Table 1. ^{1}H- (600 MHz) and ^{13}C- (150 MHz) spectral data of **1-3** in DMSO (δ in ppm and *J* in Hz).[a]

Position	**1**		**2**		**3**
	δ_H	δ_C	δ_H	δ_C	δ_C
1		130.5		130.5	130.3
2	6.86 (br.s)	117.0	6.82 (d *J* = 1.1)	117.3	117.6
3		144.9		145.0	145.0
4		144.9		144.9	145.0
5	6.68 (m)	115.7	6.68 (d *J* = 8.1)	115.6	115.5
6	6.68 (m)	123.2	6.65 (dd *J* = 1.1, 8.1)	123.2	123.2
7	2.48 (t *J* = 7.0); 2.49 (t *J* = 7.0)	38.2	2.43 (t *J* = 6.8)	38.3	38.5
8	3.46 (t *J* = 7.0)	62.2	3.40 (t *J* = 6.8)	62.3	62.4
1'	4.74 (d *J* = 7.1)	102.2	4.74 (d *J* = 7.1) [c]	102.2	102.5
2'	3.33-3.46 [b]	73.2	3.34-3.46 [b]	73.3	73.4
3'	3.33-3.46 [b]	75.5	3.34-3.46 [b]	75.5	77.2
4'	3.31 (m)	69.9	3.28 (m)	70.1	69.9
5'	3.73 (m)	73.8	3.73 (m)	73.9	75.9
6'	4.31 (dd *J* = 6.5, 10.7) 4.56 (br.d *J* = 10.7)	64.1	4.31 (dd *J* = 7.0, 11.2) 4.59 (br.d *J* = 11.2)	64.4	60.8
1"		130.8		130.6	
2"	7.38 (br.s)	115.6	7.41 (d *J* = 2.1)	116.9	
3"		157.5		158.1	
4"	7.05 (br.d *J* = 8.0)	120.3	7.12 (dd *J* = 2.1, 8.2)	121.7	
5"	7.32 (br.dd *J* = 7.7, 8.0)	129.7	7.40 (br.dd *J* = 7.7, 8.2)	129.5	
6"	7.40 (br.d *J* = 7.7)	119.7	7.54 (br.d *J* = 7.7)	121.9	
7"		165.6		165.4	
1'"				164.0	
2'"				157.2	
3'"			4.74 (br.s) [c] 5.41 (br.s)	102.1	

[a] Assignments were based on 2D NMR including HSQC and HMBC. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses; [b, c] Overlapped.

3.2. Antibacterial activity

We found that compounds **1** and **2** showed moderate activity against the four test strains, involving both Gram-positive and Gram-negative bacteria.

Table 2. The results of antibacterial activity of compounds **1** and **2**[a].

Microorganisms	Diameter of inhibition zone (mm)			MIC(μg/mL)	
	1 2 mg/mL	**2** 2 mg/mL	Ciprofloxacin 0.5 mg/mL	**1**	**2**
Staphylococcus aureus [CMCC (B) 26003]	13.8	11.1	34.0	125	62.5
Salmonella typhimurium [CMCC (B) 50094]	11.5	12.0	33.5	62.5	62.5
Escherichia coli [CMCC (B) 44102]	12.3	9.5	30.0	62.5	250
Pseudomonas aeruginosa [CMCC (B) 10104]	12.0	9.6	32.1	62.5	250

[a] The diameter of the filter paper is 6.0 mm.

Compounds **4**-**8**, by comparison with the published data, were identified as 2-(3-hydroxy-4-methoxyphenyl) ethanol 1-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (**4**) [9], benzyl 7-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (**5**) [10], 2-hydroxybenzoic acid (**6**) [11], m-hydroxybenzoic acid (**7**) [12], and trans-caffeic acid (**8**) [13], respectively.

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Supporting Information

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

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