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Two new coumarin glycosides from Chimonanthus nitens

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Two new coumarin glycosides from *Chimonanthus nitens*

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Two new coumarin glycosides, namely nitensosides A–B (**1**–**2**), together with six known compounds, scopolin (**3**), 5,6,7-trimethoxycoumarin (**4**), D-calycanthine (**5**), calycanthoside (**6**), xeroboside (**7**), and scopoletin (**8**), were isolated from *Chimonanthus nitens*. The structures of the new compounds were elucidated by comprehensive analysis of IR, MS, and NMR spectroscopic data. Compounds **3**, **4**, **7**, and **8** showed moderate inhibitory activity against *Micrococcus luteus*.

Keywords: *Chimonanthus nitens*; coumarin glycosides; antimicrobial activities; nitensoside A; nitensoside B

1. Introduction

Chimonanthus nitens was one of the genus *Chimonanthus* (Family Calycanthaceae) endemic to China and has been widely distributed in southwestern of China [1], especially in the regions of ethnic minorities. In Guizhou province, the water decoction of the plant has mainly been used as Chinese *Miao Minzu* medicine[1] for the treatment of influenza, insolation, and chronic bronchitis for 500 years [2,3]. Previous phytochemical investgation discovered that the genus of *Chimonanthus* contained alkaloids, coumarins, flavonoids, sesquiterpenoids, and volatile oils [4–9], and the primary alkaloids in the species, D-calycanthine, had strong anticonvulsant activity [10,11] and chimonanthine was a very strong analgesic agent [12,13]. Continuous investigation on this *Miao Minzu* medicine has led to the isolation of eight compounds including two new coumarin glycosides, namely nitensosides A–B (**1**–**2**), along with six known compounds, scopolin (**3**) [14], 5,6,7-trimethoxycoumarin (**4**) [15], D-calycanthine (**5**) [16], calycanthoside (**6**) [17], xeroboside (**7**), and scopoletin (**8**) [14] (Figure 1). In this study, we report the isolation and structural elucidation of compounds **1** and **2**, as well as antimicrobial activities of compounds **1**–**8**.

2. Results and discussion

Nitensoside A (**1**) was obtained as white amorphous powder. On the basis of HR-ESI-MS peak at *m/z* 503.1381 $[M + H]^+$, the molecular formula was deduced as $C_{21}H_{26}O_{14}$. The IR spectrum exhibited absorption bands for hydroxy group (3448 cm^{-1}), carboxyl group (1704 cm^{-1}), and phenyl ring (1659 and 1573 cm^{-1}). The ^{13}C NMR, DEPT, and HMQC data (Table 1) showed 1 methoxyl group, 3 methylene groups, 11 methine groups, and 6 quaternary carbons. The ^1H NMR signals at δ 7.88 and 6.22 (each 1H, d, $J = 9.6$ Hz) were characteristic for H-3 and H-4 protons of coumarin. The HMBC correlations (Figure 2) from H-3 to C-2 and C-10; from H-4 to C-2, C-5, C-9, and C-10; and from H-5 to C-4, C-6, and C-7 indicated that the aglycone of **1** was 7,8-dihydroxy-6-methoxy coumarin.

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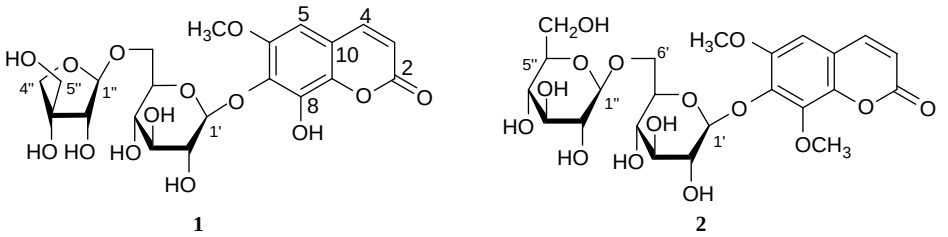

Figure 1. Chemical structures of compounds **1** and **2**.

The correlations between a singlet signal at δ_H 7.04 (1H, s, H-5) with H-4 and one methoxyl in the ROESY spectrum (Figure 2) further supported the above discussion. Two doublets at δ_H 4.96 (1H, d, $J = 7.6$ Hz) and 4.72 (1H, d, $J = 2.4$ Hz) were assigned to two anomeric protons. The acid hydrolysis of **1** afforded D-glucose and D-apiose, which were identified by comparing their high performance liquid chromatography (HPLC) retention times and optical rotations with authentic samples and the anomeric carbon signals of sugar moieties D-glucopyrano-

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2** in DMSO-d_6 (400 and 100 MHz, respectively).

	1		**2**	
No.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.
2		160.3		159.9
3	6.22 (d, 9.6)	112.4	6.37 (d, 9.6)	114.9
4	7.88 (d, 9.6)	144.8	7.92 (d, 9.6)	144.6
5	7.04 (s)	105.0	7.09 (s)	105.4
6		145.4		149.5
7		131.3		141.4
8		143.6		140.5
9		142.8		142.4
10		110.2		114.8
6-OMe	3.81 (3H, s)	56.1	3.87 (3H, s)	61.6
8-OMe			3.82 (3H, s)	56.5
Glucose-1′				
1′	4.96 (d, 7.6)	103.5	5.12 (d, 8.8)	101.9
2′	3.33 (m)	73.9	3.25 (m)	74.0
3′	3.25 (overlap)	76.3	3.29 (overlap)	76.8
4′	3.16 (m)	69.8	3.15 (m)	69.8
5′	3.23 (overlap)	76.0	2.71 (m)	76.3
6′	3.44 (overlap)	67.1	3.87 (overlap)	67.6
	3.72 (dd, 10.0, 2.0)		3.52 (dd, 11.6, 6.0)	
Apiose-2″			*Glucose-2″*	
1″	4.72 (d, 2.4)	109.2	3.95 (d, 8.0)	102.6
2″	3.62 (dd, 6.4, 2.4)	75.8	2.78 (m)	73.4
3″		78.9	2.91 (overlap)	76.6
4″	3.47 (d, 9.6)	73.3	2.95 (overlap)	69.7
	3.67 (d, 9.6)			
5″	3.24 (overlap)	63.3	2.92 (overlap)	76.6
6″			3.40 (dd, 11.2, 6.4)	60.9
			3.53 (dd, 11.2, 2.0)	

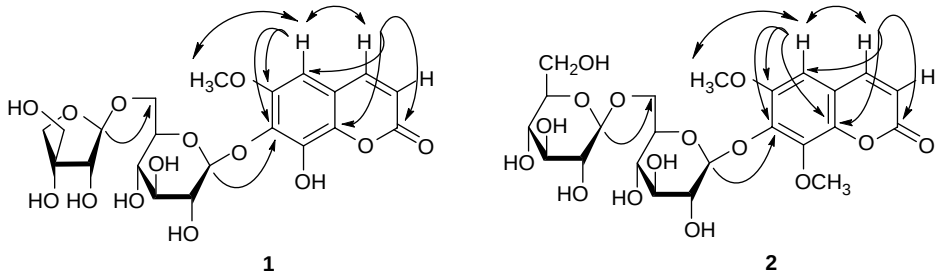

Figure 2. Key HMBC (→) and ROESY (↔) correlations of compounds **1** and **2**.

side and D-apiofuranoside resonated at δ_C 103.5 and 109.2, respectively, in agreement with the literature data [18,19]. In the HMBC spectrum, the correlations between H-1′ with C-7 and C-5′, and between H-1″ with C-6′ confirmed that the apiose was linked to the C-6′ of glucose and the diglycoside units were attached to the C-7 of aglycone. The β-linkages of the glucose and apiose were determined by their coupling constants [20]. Thus, the structure of compound **1** was elucidated as 7-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranosyl-8-hydroxy-6-methoxycoumarin.

Nitensoside B (**2**) was obtained as a white amorphous powder. The HR-ESI-MS showed an $[M + H]^+$ ion at *m/z* 547.1646, indicating the molecular formulas of $C_{23}H_{30}O_{15}$ and supported by 1D- and 2D-NMR. Its IR spectrum showed strong absorptions at ν_{max} 3442 cm^{-1} (hydroxy), 1724 cm^{-1} (carboxyl), and 1641 cm^{-1} (phenyl ring). The comparison of the ^1H and ^{13}C NMR spectral data (Table 1) of compounds **2** and **1** showed that they had similar coumarin skeletons except some differences at the sugar moieties and one substituted group. The above inferences were confirmed by the HMBC and ROESY spectra (Figure 2), and the 2D-NMR spectra showed that the aglycone of **1** was 7-hydroxy-6,8-dimethoxy coumarin. The acid hydrolysis of **2** afforded glucose and identified by comparing their HPLC retention times and optical rotations with authentic samples. The C-7 resonance signal at δ_C 141.4 correlated with the anomeric proton (H-1′) of $Glc_{1'}$, whereas the methylene carbon (C-6′) of $Glc_{1'}$ correlated with the anomeric proton (H-1″) of $Glc_{1''}$. The coupling constants of the two anomeric protons ($\delta_{H\text{-}1'}$ 5.12, d, $J = 8.8$ Hz and $\delta_{H\text{-}1''}$ 3.95, d, $J = 8.0$ Hz) indicated that both glucoses were confirmed to be β-conformation. The chirality of both of these glucose units was determined as D, and they thus formed a β-gentiobioside moiety, in agreement with literature data [21,22]. Thus, compound **2** was elucidated as 6,8-dimethoxy-7-hydroxycoumarin-7-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside.

3. Experimental

3.1 General experimental procedures

Melting points were determined by XT-4 microscopic melting point apparatus and are uncorrected (Beijing Taike Instrument Company, Beijing, China). Optical rotations were measured on an Auto Pol I automatic polarimeter (Rudolph Research, Flanders, NJ, USA) at room temperature. IR spectra were recorded on Brucker Vector 22 spectrophotometer (Bruker Corporation, Brük, Germany). NMR spectra were recorded by Varian Inova 400 spectrometer (Varian Company, Palo Alto, CA, USA). The HR-ESI-MS were obtained on an API QSTAR Pulsar mass spectrometer (Bruker Corporation). HPLC was performed on a Agilent 1100 system consisting of an G1311A quatpump, an

G1313A ALS, G1314A VWD, G1322A Degasser, and a Hypersil NH_2 column, (250 mm × 4.6 mm, 5 μm), all operated by Agilent 1100 analysis software (Agilent Technologies, Palo Alto, CA, USA). All microorganisms were purchased directly from China General Microbiological Culture Collection Center. Column chromatography was carried out using silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Amersham Pharmacia Biotech, Stockholm, Sweden), and D-101 macroporous adsorption resin (Tianjin Pesticide of Company Limited, Tianjin, China). Thin layer chromatography was carried out on silica gel GF254 (Qingdao Marine Chemical Factory).

3.2 Plant material

The roots of *C. nitens* were collected in the Guiyang, Guizhou province, China, in May 2008. The plant material was identified by Prof. De-yuan Chen (Guiyang College of Traditional Chinese Medicine, Department of Chinese Medicine, Guiyang, China) and the specimen (No. GZCN08005) has been deposited at The Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang, China.

3.3 Extraction and isolation

The air-dried and powdered roots of *C. nitens* (8.0 kg) were extracted twice with water. After evaporation of the water, the crude extract (1.5 kg) was chromatographed on D-101 macroporous adsorption resin column, eluted with water and EtOH. The 75% EtOH elution portion (440 g) was chromatographed on a silica gel column eluted with a $CHCl_3$/MeH gradient (100% → 50% $CHCl_3$, v/v) to afford fractions A–E.

Fraction A (53 g) was chromatographed on a silica gel column eluted with a petroleum/acetone gradient (100% → 10% petroleum ether, v/v) to yield seven subfractions, A_1–A_7. Subfraction A_3 (10.8 g) was again eluted with a petroleum/acetone gradient (80% → 50% petroleum ether, v/v) at silica gel column to yield **6** (55 mg) and **8** (2.3 g). Fraction B (90 g) was subjected to a silica gel column and eluted with $CHCl_3$/MeOH (90% → 60% $CHCl_3$, v/v) to give three subfractions, B_1–B_3. Subfractions B_1 and B_2 were, respectively, chromatographed on a Sephadex LH-20 column eluting with $CHCl_3$/MeOH (1:1, v/v), the former yielded **3** (440 mg) and **4** (2.1 g), while the latter yielded **5** (8.1 g) and **7** (154 mg). Fraction C (60 g) was subjected to a silica gel column and eluted with $CHCl_3$/MeOH (90% → 10% $CHCl_3$, v/v) to afford five subfractions B_1–B_5. Subfraction B_3 was chromatographed on a Sephadex LH-20 column eluting with MeOH to yield **1** (41 mg) and **2** (25 mg).

3.3.1 Nitensoside A (*1*)

White amorphous powder (MeOH); m.p. 118–120°C; $[\alpha]_D^{25}$ − 43.5 (c 0.57, DMSO); IR (KBr) ν_{max}: 3448, 2926, 2856, 1704, 1573, and 1077 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS *m/z* 503.1381 $[M + H]^+$ (calcd for $C_{21}H_{27}O_{14}$, 503.1401).

3.3.2 Nitensoside B (*2*)

White amorphous powder (MeOH); m.p. 168–170°C; $[\alpha]_D^{25}$ 11. 1 (c 0.36, DMSO); IR (KBr) ν_{max}: 3442, 2924, 2852, 1724, 1641, and 1406 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MSP *m/z* 547.1646 $[M + H]^+$ (calcd for $C_{23}H_{31}O_{15}$, 547.1663).

3.3.3 Acidic hydrolysis of nitensoside A (*1*) and nitensoside B (*2*)

Compounds **1** and **2** (each 10 mg) were dissolved in 2 N of ethanolic H_2SO_4, respectively, and then refluxed for 2 h.

The reaction mixture was extracted with 5EtOAc (3×5 ml). The water layer was neutralized with $NaHCO_3$ and then concentrated to dryness under reduced pressure and purified by Sephadex LH-20 chromatography to give a sugar fraction. The sugar fraction was analyzed by HPLC under the following conditions: column, Hypersil NH_2 (250 mm × 4.6 mm, 5 μm); column temperature, 30°C; mobile phase, acetonitrile–water (85/15, v/v); flow rate, 0.8 ml min^{-1}. Identification of D-glucose and D-apiose was carried out by comparison of these retention times and optical rotations with those of authentic samples. D-Glucose: t_R 5.8 min, positive optical rotation; D-apiose: t_R 3.2 min, positive optical rotation.

3.4 *Antimicrobial assay* in vitro

The antimicrobial activities of compounds **1**–**8** were evaluated by disk diffusion method [23,24] using *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Bacillus subtitles*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. All screening concentrations of the samples were 10 mg ml^{-1} and the positive reference standards, gentamicin, were 0.5 mg ml^{-1}; both of test volumes were 40 μl per disc. Comparing with gentamicin, compounds **3**, **4**, **7**, and **8** had moderate inhibition activities against *M. luteus*, and their inhibition zone diameters were 24.37 ± 1.73, 9.28 ± 0.62, 10.13 ± 1.09, 10.02 ± 0.54, and 9.80 ± 0.45 mm, respectively.

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Note

1. The description of Chinese *Miao Minzu* medicine: the Chinese *Miao Minzu*, also known as *Miao* minority or Hmong, has a population of approximately 8 million in China. Majority of them can be found in the provinces of Guizhou, part of Sichuan, Yunnan, Hunan, and Guangxi province. They have a long history of using herbs to protect health, treat illness, and have gradually established their own theoretical system of medicinal uses. In Chinese *Miao Minzu* medicine theory, medicines can be divided into three categories: hot medicines, cold medicines, and the He Xing medicine that is between the hot and the cold ones. The hot medicines can be used to treat 'cold' diseases, the cold medicines can be used to treat 'hot' diseases, whereas the He Xing medicines can be used to vitalize, strengthen, and rejuvenate the entire body. The Chinese *Miao Minzu* medicine theory could be described as 'offset' method, which is very similar to the 'Yin and Yang' theory of Chinese traditional medicines, in order to mobilize body's own defense system to treat illness.

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