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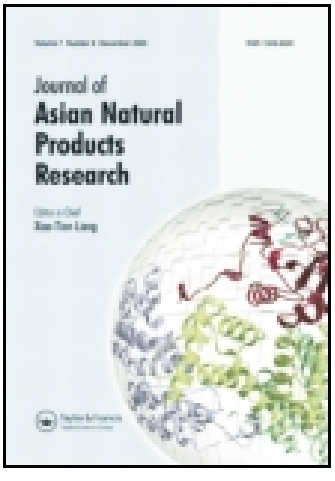

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7,8-Secolignans from the fruits of Schisandra neglecta

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7,8-Secolignans from the fruits of *Schisandra neglecta*

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Two new 7,8-secolignans, neglectahenols E and F (**1** and **2**), together with four known 7,8-secolignans (**3**–**6**), were isolated from the fruits of *Schisandra neglecta*. The structures were elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. Compounds **1**–**6** were tested for their anti-tobacco mosaic virus (anti-TMV) activities at the concentration of 20 μM. Compounds **1** and **6** showed high anti-TMV activities with inhibition rates of 38.2% and 32.7%, respectively. These rates are higher than that of a positive control. Compounds **2**–**5** also showed modest anti-TMV activities with inhibition rates in the range of 22.8–28.7%. These rates are close to that of a positive control.

Keywords: *Schisandra neglecta*; 7,8-secolignans; anti-tobacco mosaic virus (anti-TMV) activities

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1. Introduction

The stems and fruits of plants in the genus *Schisandra* are used commonly in Traditional Chinese Medicine for their diverse beneficial bioactivities [1,2]. Previous studies have shown that these species are rich in lignans and triterpenoids, especially dibenzocyclooctadiene lignans, which have been found to possess some potentially beneficial activities, including anti-hepatotoxic, anti-HIV, antioxidant, antitumor, and cytotoxic effects [3–5].

Schisandra neglecta A.C. Smith (Schisandraceae) is a climbing plant distributed mainly in southwest of mainland China. In previous studies, several new dibenzocyclooctadiene lignans were isolated from the stems of *S. neglecta* [6–8]. In our continuing efforts to identify bioactive natural products from the medicinal plants of the family Schisandraceae, a chemical investigation on the fruit of *S. neglecta* was carried out, which were collected from the Xichang Prefecture, Sichuan Province, China. As a result, two new (**1** and **2**) and four known (**3**–**6**) 7,8-secolignans were isolated from this species. Described in this paper are the structure elucidation of compounds **1**–**6** and their anti-tobacco mosaic virus (anti-TMV) activities.

2. Results and discussion

The fruits of *S. neglecta* were extracted with 70% acetone. The extract produced was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18, and RP-HPLC, to afford two 7,8-secolignans, neglectahenols E and F (**1** and **2**), together with four known 7,8-secolignans (**3**–**6**). The structures of compounds **1**–**2** are shown in Figure 1, and the ^{1}H and ^{13}C NMR data of compounds **1** and **2** are listed in Table 1.

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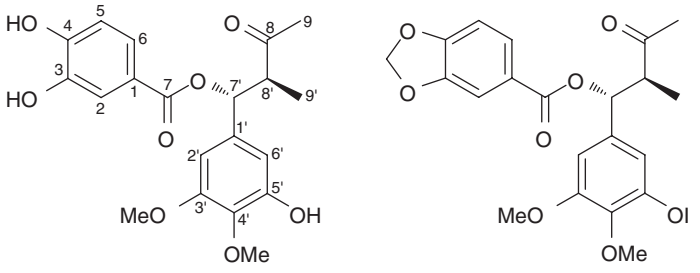

Figure 1. The structures of compounds **1**–**2**.

The known compounds were identified as marphenol A (**3**) [9], 7,8-secoholostylone B (**4**) [10], neglectahenol A (**5**) [11], and neglectahenol C (**6**) [11], by comparing their spectroscopic data with values reported in the literature.

Compound **1** was obtained as a white amorphous powder, and was assigned the molecular formula $C_{20}H_{22}O_8$ by HRESIMS at *m/z* 391.1399 $[M + H]^+$. Its ^1H and ^{13}C NMR (Table 1) spectra showed signals of 22 protons and 20 carbons, respectively, corresponding to a 1′,3′,4′,5′-tetra-substituted benzyl (δ_C 133.3 s, 102.9 d, 152.3 s, 139.8 s, 148.8 s, 109.1 d; δ_H 6.88 s, 7.26 s) [9], a 1,3,4-tri-substituted benzyl (δ_C 126.3 s, 108.1 d, 150.9 s, 147.2 s, 109.9 d, 125.3 d; δ_H 7.68 s, 6.83 d $J = 8.2$, 7.80 d $J = 8.2$) [9], two carbonyl carbons (δ_C 165.0 s, 209.0 s), two methyl

Table 1. ^1H and ^{13}C NMR spectral data of **1** and **2** (δ ppm, J Hz) measured in pyridine-d_5.

	1		**2**	
No.	δ_C(m)	δ_H(mult,*J*,Hz)	δ_C(m)	δ_H(mult,*J*,Hz)
1	126.3 s		125.2 s	
2	108.1 d	7.68, s	111.5 d	7.72, d, $J = 1.9$
3	150.9 s		148.5 s	
4	147.2 s		151.0 s	
5	109.9 d	6.83, d, $J = 8.2$	109.0 d	7.04, d, $J = 8.2$
6	125.3 d	7.80, d, $J = 8.2$	124.5 d	7.85, dd, $J = 8.2$, 1.9
7	165.0 s		165.3 s	
8	209.0 s		208.7 s	
9	29.7 q	2.23, s	29.3 q	2.25, s
1′	133.3 s		136.0 s	
2′	102.9 d	6.88, s	105.2 d	6.73, s
3′	152.3 s		154.9 s	
4′	139.8 s		139.3 s	
5′	148.8 s		154.9 s	
6′	109.1 d	7.26, s	105.2 d	6.73, s
7′	78.9 d	6.42, d, $J = 9.9$	79.1 d	6.40, d, $J = 9.9$
8′	51.3 d	3.44–3.46, m	53.0 d	3.44–3.46, m
9′	14.3 q	1.01, d, $J = 7.1$	13.6 q	1.06, d, $J = 7.1$
OMe-3′	56.0 q	3.73, s	55.8 q	3.76, s
OMe-4′	60.7 q	3.79, s	60.8 q	3.83, s
OMe-5′			55.8 q	3.76, s
Ar–OH-3		10.80, br s		
Ar–OH-4		10.68, br s		
Ar–OH-5′		11.10, br s		
$-OCH_2O-$			101.6 t	5.92, 5.97, s

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groups (δ_C 29.7 q, 14.3 q; δ_H 2.23 s, 1.01 d $J = 7.1$), one methine group (δ_C 51.3 d; δ_H 3.45 m), one oxidated methine group (δ_C 78.9 d; δ_H 6.42 d $J = 9.9$), two methoxyl groups (δ_C 56.0 q, 60.7 q; δ_H 3.73 s, 3.79 s), and three phenolic hydroxy groups (δ_H 10.80 br s, 10.68 br s, 11.10 br s), which were in accordance with the molecular formula $C_{20}H_{22}O_8$. Strong absorption bands accounting for hydroxy group (3445 cm^{-1}), carbonyl group (1745, 1709 cm^{-1}), and aromatic group (1638, 1582, 1518, 1485 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maximum at 272 nm which confirmed the existence of the aromatic functions. The results of HMBC experiment (Figure 2) supported the presence of ketone (δ_C 209.0), methyl (δ_C 29.7), and veratryl ester ($\delta_{C=O}$ 165.0) groups. The HMBC experiment also showed that the carbonyl carbon C-8 (δ_C 209.0) was correlated to both H_3-9 (δ_H 2.23, s) and H_3-9′ (δ_H 1.01, d, $J = 7.1$). Furthermore, the prominent absorption bands at 1709 cm^{-1} observed in the IR spectrum were in accordance with ketone and conjugated ester carbonyl groups in the molecule. A $^1H-^1H$ COSY experiment showed that H-7′ (δ_H 6.42, d, $J = 9.9$) was coupled to H-8′ (δ_H 3.45, m) which, in turn, was also coupled to H_3-9′. The ^1H and ^{13}C NMR data of **1** were very similar to those of known compound, marphenol B [9], except for the appearance of two phenolic hydroxy signal at (δ_H 10.80 and 10.68) and the disappearance of a methylenedioxyl signal at (δ_C 102.5 t; δ_H 5.99, 6.01, each s). These indicated the methylenedioxyl group in marphenol B was replaced by two phenolic hydroxy groups in **1**. These two phenolic hydroxy groups located at C-3 and C-4 were also supported by the HMBC correlations of one phenolic hydroxy proton signal (δ_H 10.80) with C-2 (δ_C 108.1), C-3 (δ_C 150.9), and C-4 (δ_C 147.2); and of another phenolic hydroxy proton signal (δ_H 10.68) with C-3 (δ_C 150.9), C-4 (δ_C 147.2), and C-5 (δ_C 109.9), respectively. Considering the magnitude of the coupling constant between H-7′ and H-8′ ($J = 9.9$) as well as the similar ^1H and ^{13}C NMR data and optical rotation with those of reported marphenol B, the structure of **1** could be established as (7′*R*, 8′*S*)-7,8-secolignans [9]. Thus, the structure of **1**, neglectahenol E, was established.

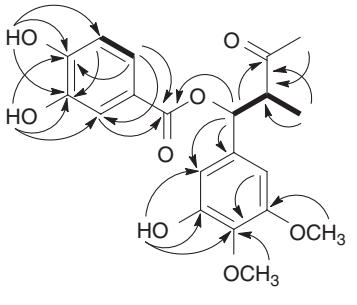

Figure 2. Key $^1H-^1H$ COSY ((—)) and HMBC ((↷)) correlations of **1.**

Neglectahenol F **(2)**, obtained as a white amorphous powder solid, had the molecular formula $C_{22}H_{24}O_8$ as revealed by its HRESIMS at *m/z* 439.1363 $[M + Na]^+$. The ^1H and ^{13}C NMR spectra were very similar to those of marphenol A (**3**) [9]. Analysis of HSQC, $^1H-^1H$ COSY, and HMBC spectra of **2** showed that obvious chemical-shift differences were resulted from the substituents in aromatic ring, and a phenolic hydroxy group at C-5′ in marphenol A was replaced by a methoxyl group in **2**. The appearance of typical aromatic proton signals (δ_H 6.73, s, 2H) and the two equivalent methoxyl proton signals (δ_H 3.76 s) also supported the 3',4',5'-trimethoxyl substituted aromatic for ring B. Compound **2** is therefore the 5'-methoxyl derivative of marphenol A.

Because certain 7,8-secolignans exhibit potential anti-virus activities [9,11], compounds **1–6** were tested for their anti-TMV activities. The anti-TMV activities were tested using the half-leaf method [12,13]. Ningnanmycin (a commercial product for plant disease in China), was

Table 2. TMV infection inhibition activities of compounds **1–6**.

Compounds	Inhibition rate (%)	Compounds	Inhibition rate (%)
1	38.2 ± 3.5	**5**	25.5 ± 3.0
2	25.6 ± 3.4	**6**	32.7 ± 2.5
3	22.8 ± 2.5	Ningnanmycin	31.8 ± 3.0
4	28.7 ± 2.7		

All results are expressed as mean ± SD; $n = 3$ for all groups.

used as a positive control. Their antiviral inhibition rates at the concentration of 20 μM were listed in Table 2. Compounds **1** and **6** showed high anti-TMV activities with inhibition rates of 38.2% and 32.7%, respectively. These rates are higher than that of a positive control. Compounds **2–5** also showed modest anti-TMV activities with inhibition rates in the range of 22.8–28.7%. These rates are close to that of a positive control.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). A Tensor 27 spectrophotometer (Bruker Optics, Ettlingen, Germany) was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers (Bruker Optics, Ettlingen, Germany) with TMS as an internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS was performed on an API QSTAR time-of-flight spectrometer (Applied Biosystems, Foster City, American) and a VG Autospec-3000 spectrometer (VG, Manchester, England), respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph (Shimadzu, Kyoto, Japan) with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 μm) column or a Venusil MP C18 (20 mm × 25 cm, 5 μm) column. Column chromatography was performed with Si gel (200–300 mesh; Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40–63 μm; Merck, Darmstadt, Germany), and MCI gel (75–150 μm; Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 5% H_2SO_4 in EtOH.

3.2 Plant material

The fruits of *Schisandra neglecta* were collected in Xichang Prefecture, Sichuan Province, China, in September 2011. The identification of the plant material was verified by Prof. Xi-Wen Li of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KIB 11-9-38) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The air-dried and powdered fruits of *S. neglecta* (5.0 kg) were extracted four times with 70% aqueous Me_2CO (4 × 8 L) at room temperature and filtered, with the filtrate evaporated under reduced pressure and partitioned with EtOAc (4 × 5 L). The EtOAc partition (357 g) was applied to silica gel (200—300 mesh) column chromatography, eluting with a $CHCl_3$–MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. The further

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separation of fraction B (9:1, 52.5 g) by silica gel column chromatography, eluted with $CHCl_3$–acetone (20:1–2:1), yielded mixtures B1–B7. Fraction B2 (9:1, 38.4 g) was subjected to silica gel column chromatography using petroleum ether–acetone and preparative HPLC (65% MeOH–H_2O, flow rate 12 ml/min) to give **2** (16.4 mg, Rt 12 min) and **6** (14.8 mg, Rt 17 min). Fraction B3 (8:2, 26.7 g) was subjected to silica gel column chromatography using petroleum ether–acetone and preparative HPLC (60% MeOH–H_2O, flow rate 12 ml/min) to give **3** (12.8 mg, Rt 13 min), **4** (15.2 mg, Rt 19 min), and **5** (18.6 mg, Rt 25 min). Fraction B4 (7:3, 22.8 g) was subjected to silica gel column chromatography using petroleum ether–acetone (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) and preparative HPLC (55% MeOH–H_2O, flow rate 12 ml/min) to give **1** (14.8 mg, Rt 12 min).

3.3.1 *Neglectahenol E (**1**)*

$C_{20}H_{22}O_8$, obtained as a white amorphous powder; $[\alpha]_D^{24.8} + 28.6$ (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 272 (3.48), 356 (1.24) nm; IR (KBr) ν_{max}: 3445, 3078, 2936, 2288, 1745, 1709, 1638, 1582, 1518, 1485, 1423, 1367, 1321, 1236, 1122, 1052, 1008, 874, 768 cm^{-1}; for ^1H and ^{13}C NMR spectral data (C_5ND_5, 500 and 125 MHz), see Table 1; ESIMS (positive ion mode): *m/z* 391 $[M + H]^+$; HRESIMS (positive ion mode): *m/z* 391.1399 $[M + H]^+$ (calcd for $C_{20}H_{23}O_8$, 391.1393).

3.3.2 *Neglectahenol F (**2**)*

$C_{22}H_{24}O_8$, obtained as a white amorphous powder; $[\alpha]_D^{24.8} + 25.6$ (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 273 (3.42), 355 (1.08) nm; IR (KBr) ν_{max}: 3457, 3072, 2918, 2856, 1743, 1712, 1623, 1571, 1518, 1468, 1432, 1368, 1330, 1249, 1133, 1049, 1021, 923, 875 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; ESIMS (positive ion mode): *m/z* 439 $[M + Na]^+$; HRESIMS (positive ion mode): *m/z* 439.1363 $[M + Na]^+$ (calcd for $C_{22}H_{24}O_8Na$, 439.1369).

3.4 *Anti-TMV assays*

The anti-TMV activity was tested using the half-leaf method [14]. The inhibitory activity of the compounds against TMV replication was tested using two approaches. First, the half-leaf method was used to test the antiviral activity in the local lesion host *Nicotiana glutinosa in vivo*. Then, the leaf-disk method was used to evaluate the antiviral activity of the compounds in the systemic infection host *Nicotiana tabacum* cv. K326. Ningnanmycin (20 μM), a commercial product for plant disease in China, was used as a positive control.

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