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A New Dibenzocyclooctadiene Lignan from the Stems of *Schisandra neglecta* and Its Cytotoxicities

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A new dibenzocyclooctadiene lignan, neglignan H (**1**) was isolated from the stems of *Schisandra neglecta*. The structure of (**1**) was elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. Compound **1** was tested for its cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) and compound **1** exhibited modest cytotoxicity against NB4, A549 and MCF7 cell with IC₅₀ values of 8.1, 7.4 and 6.7 μ M, respectively.

Keywords: *Schisandra neglecta*, Lignan, Cytotoxicities.

INTRODUCTION

The stems and fruits of *Schisandra* plants are commonly used in traditional Chinese medicine for their diverse beneficial bioactivities^{1,2}. Previous studies showed that the plants of the *Schisandra* genus are rich in lignans and triperpenoids, especially dibenzocyclooctadiene lignans, which have been found to possess some beneficial pharmacological effects, including anti-HIV, antitumor, cytotoxic, antioxidant and antihepatotoxic effects³⁻⁵.

Schisandra neglecta A.C. Smith, one species of *Schisandra* genus, is a climbing plant mainly distributed in southwest China. In previous study, some new dibenzocyclooctadiene lignans were isolated from the fruits of *S. neglecta*⁶, and the stems of *S. neglecta*^{7,8}. In our continuing efforts to identify bioactive natural products from the medicinal plants of *Schisandra* ceae family, a chemical investigation on the stems of *S. neglecta* was carried out, which was collected from the Dali Prefecture, Yunnan Province of China. As a result, a new dibenzocyclooctadiene lignan (**1**) was separated from this plant. In addition, the cytotoxicities of compound **1** were also evaluated.

EXPERIMENTAL

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. CD spectra were measured on a JASCO J-810 spectropolarimeter.

1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C₁₈ (9.4 mm $\times$ 25 cm) column. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Zorbax SB-C₁₈ column (20 mm $\times$ 25 cm, 5 mm). Column chromatography was performed with Si gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40-63 μ m, Merck, Darmstadt, Germany) and MCI gel (75-150 μ m, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC and spots were visualized by heating Si gel plates sprayed with 5 % H₂SO₄ in EtOH.

The stems of *S. neglecta* were collected in Dali Prefecture of Yunnan Province, P.R. China, in July 2010. The plant material was verified by Prof. N. Yuan. A voucher specimen (YNNI10-7-12) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered stems of *S. neglecta* (4.5 kg) were extracted four times with 70 % aqueous Me₂CO (4 $\times$ 5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure and partitioned with EtOAc (3 $\times$ 4 L). The EtOAc partition (412 g) was applied to Si gel (200-300 mesh) column chromatography eluting with a CHCl₃-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to give five fractions A-F. The separation of fraction A (46.8 g) by Si gel column

chromatography eluted with petroleum ether-acetone (20:1-5:5) yielded mixtures A1-A6. Fraction A2 (11.6 g) was purified by preparative HPLC (60 % MeOH-H₂O, flow rate 25 mL/min) to give **1** (26.5 mg).

Marlignan A (1): C₂₈H₃₄O₁₀, white amorphous powder; $[\alpha]_D^{24.6}$ -42.5 (c 0.20, MeOH); CD (c 0.11, MeOH) $\lambda_{\max}$ nm ($\Delta\epsilon$) 250 (-55.2), 242 (-36.5), 210 (+42.8); UV (MeOH) $\lambda_{\max}$ (log ϵ) 300 (1.52), 254 (3.94), 210 (4.48) nm; IR (KBr): $\nu_{\max}$ 3480, 2969, 2931, 2882, 1728, 1635, 1605, 1583, 1492, 1462, 1413, 1358, 1196, 1128, 1053, 928, 876 cm⁻¹; ¹H and ¹³C NMR data (Table-1); ESIMS m/z 553; HRESIMS m/z 553.2058 [M+Na]⁺ (calcd. C₂₈H₃₄O₁₀Na for 553.2050).

TABLE-1
¹H AND ¹³C NMR DATA OF COMPOUND **1**
(δ ppm) MEASURED IN PYRIDINE-*d*₅

No.	δ_C (mult.)	δ_H (mult., J, Hz)
1	149.6 s	
2	139.2 s	
3	151.8 s	
4	112.9 d	7.28, s
5	133.8 s	
6	87.6 d	6.52, s
7	71.8 s	
8	44.5 d	2.18, m
9 α	36.8 t	3.10, dd, J = 13.6, 9.5
9 β		2.15, brd, J = 13.6
10	137.6 s	
11	104.1 d	6.85, s
12	148.4 s	
13	138.9 s	
14	142.8 s	
15	122.3 s	
16	123.9 s	
17	28.8 q	1.58, s
18	19.2 q	1.40, d, J = 7.2
OMe-2	60.4 q	3.88, s
OMe-3	55.8 q	3.85, s
OCH ₂ O	101.2 t	5.86, 5.98, s
1'	177.3 s	
2'	83.2 s	
3'	36.3 d	2.23, m
4'	72.8 t	4.18, m
5'	26.2 q	1.43, s
6'	12.9 q	1.40, d, J = 7.6
OMe-3'	55.9 q	3.18, s
Ar-OH-1		11.26, s

RESULTS AND DISCUSSION

A 70 % aqueous acetone extract prepared from the stems of *S. neglecta* was partitioned between EtOAc and H₂O. The EtOAc layer was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and semi-preparative HPLC to afford compound (**1**).

Compound **1**, obtained as white amorphous powder, was assigned the m.f. C₂₈H₃₄O₁₀ by HRESIMS { m/z 553.2058 [M+Na]⁺ (calcd 553.2050)}. The ¹H and ¹³C NMR spectra of **1** indicated the presence of 12 aromatic carbons, two aromatic protons, one methylenedioxy group and three methoxyl groups, suggesting the presence of a biphenyl moiety⁹. HMBC correlations of H-11 (δ_H 6.85, s) with C-9 (δ_C 36.8, t), C-10 (δ_C

137.6, s) and C-15 (δ_C 122.3, s) and of H-4 (δ_H 7.28, s) with C-5 (δ_C 133.8, s), C-6 (δ_C 87.6, s) and C-16 (δ_C 123.9, s), together with ¹H-¹H COSY correlations of H-9/H-8/H-17 (Fig. 2) and UV absorption bands at 210 and 254 nm, implied that **1** was a dibenzocyclooctadiene lignan¹⁰. The ¹H and ¹³C NMR spectra of compound **1** were similar to those of gomisin D¹¹ (Table-1). In addition to the dibenzocyclooctadiene structure, the molecule still contained a structural unit consisting of a methoxy group and a six carbon chain [δ_C 177.3 (s), 83.2 (s), 36.3 (d), 72.8 (t), 26.2 (q) and 12.9 (q)]. The ¹H-¹H COSY spectrum indicated the existence of a -OCH₂CH(CH₃)-fragment. HMBC correlations from -OMe (δ_C 3.18, s) and H-4' (δ_H 4.18, m) to C-2' (δ_C 83.2, s), H-3' (δ_H 2.23, m) to C-1' (δ_C 177.3, s), Me-5' (δ_H 1.43, s) to C-1' (δ_C 177.5, s) and Me-6' (δ_H 1.40, d, J = 7.6 Hz) to C-2' (δ_C 83.2, s), C-3' (δ_C 36.3, d) and C-4' (δ_C 72.8, t) suggested the above structural unit could be -OCH₂CH(CH₃)C(OCH₃)(CH₃)COO-. Furthermore, the HMBC correlations from H-4' (δ_H 4.18, m) to C-14 (δ_C 142.8, s) and H-6 (δ_H 6.52, s) to C-1' (δ_C 177.3, s) indicated that the above unit was connected to the dibenzocyclooctadiene skeleton (Fig. 1). The planar structure of **1** was initially deduced by comparison of the 1D NMR spectrum of **1** with those of gomisin D, the structure of which was determined by X-ray crystallographic analysis of its 4,11-dibromo derivative¹¹. The

Fig.1. The structure of compound **1**

Fig. 2. Selected HMBC (↷) and ¹H-¹H COSY (→) correlations of compound **1**

only difference between **1** and gomisins D was that a hydroxy group at C-2' was substituted by a methoxy group and a methoxy group at C-1 was substituted by a hydroxy group. The HMBC correlations of -OMe (δ_{H} 3.18, s) with C-2' (δ_{C} 83.2, s) indicated a methoxy group was attached to C-2'. The HMBC correlations of the phenolic hydroxyl proton (δ_{H} 11.26) with C-1 (δ_{C} 149.6), C-2 (δ_{C} 139.2) and C-16 (δ_{C} 123.9) indicated that the phenolic hydroxy was at C-1. Thus, the planar structure of **1** was established.

Since the CD spectra of dibenzocyclooctadiene lignans are dominated by the axial chirality of the biphenyl chromophore, the absolute configuration of the biphenyl axis of compound **1** could be determined from its CD curve, which showed a negative Cotton effect around 250 nm and a positive one around 210 nm. This suggested that **1** possessed an S-biphenyl configuration⁹. With the axial chirality defined, a ROESY experiment was used to establish the relative configuration of the remaining stereocenters. The ROESY correlations (Fig. 3) of H-9 (δ_{H} 3.10, dd, $J = 13.6, 9.5$ Hz; δ_{H} 2.15, brd, $J = 13.6$ Hz) with H-11 (δ_{H} 6.85, s), H-4 (δ_{H} 7.28, s) with H-6 (δ_{H} 6.52, s), H₃-18 (δ_{H} 1.40, d, $J = 7.2$ Hz) with H-9 (δ_{H} 3.10, dd, $J = 13.6, 9.5$ Hz; δ_{H} 2.15, brd, $J = 13.6$ Hz) and H-8 (δ_{H} 2.18, m) with H-11 (δ_{H} 6.85, s) suggested that **1** possessed a twisted boat-chair conformation of the cyclooctadiene ring and C-6 (S), C-8 (S) and C-9 (R) relative configurations. However, the absence of correlation between H₃-17 (δ_{H} 1.58, s) and H-4 (δ_{H} 7.28, s) indicated a quasi-axial 7-OH and thus C-7 (S) relative configuration^{11,12}. In addition, the ROESY correlations of H-3' (δ_{H} 2.23, m) with H₃-5' (δ_{H} 1.41, s) confirmed the relative configurations of C-2' (S), C-3' (R) relative configuration. As a result, the structure of **1** was determined as shown and given the trivial name of neglignan H.

Fig. 3. Key ROESY correlations (↔) of compound **1**

Some of dibenzocyclooctadiene lignans from *Schisandra* genus species exhibited cytotoxicities. The compound **1** was tested for its cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) using the MTT method as reported previously¹³. Taxol was used as the positive control. The results shown that the compound **1** exhibited moderate cytotoxicity against NB4, A549 and MCF7 cell with IC₅₀ values of 8.1, 7.4 and 6.7 μM , respectively.

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