Asian Journal of Chemistry; Vol. 26, No. 7 (2014), 1948-1950

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.15583

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

YAN-QING YE[1], CONG-FANG XIA[1], YIN-KE LI[1,2], XIAN-XUE WU[2], GANG DU[1], QIU-FEN HU[1] and XUE-MEI GAO[1,*]

[1]Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission and Ministry of Education, Yunnan University of Nationalities, Kunmig 650031, P.R. China
[2]Collge of Resource and Environment, Yuxi Normal University, Yuxi 653100, P.R. China

*Corresponding author: Fax: +86 871 5910017; Tel: +86 871 5910013; E-mail: gao_xuemei@hotmail.com

Received: 4 April 2013; *Accepted*: 1 August 2013; *Published online*: 22 March 2014; AJC-14933

A new dibenzocyclooctadiene lignan, neglignan H (**1**) was isolated from the stems of *Schisandra neglecta*. The structures 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 IC_{50} values of 8.1, 7.4 and 6.7 μM, 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 effects[3-5].

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-C_{18} (9.4 mm × 25 cm) column. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Zorbax SB-C_{18} column (20 mm × 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 μm, Merck, Darmstadt, Germany) and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC and spots were visualized by heating Si gel plates sprayed with 5 % H_2SO_4 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 Me_2CO (4 × 5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure and partitioned with EtOAc (3 × 4 L). The EtOAc partition (412 g) was applied to Si 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 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-H_2O, flow rate 25 mL/min) to give 1 (26.5 mg).

Marlignan A (1): $C_{28}H_{34}O_{10}$, white amorphous powder; $[\alpha]_D^{24.6}$ -42.5 (*c* 0.20, MeOH); CD (*c* 0.11, MeOH) λ_{max} nm ($\Delta\varepsilon$) 250 (-55.2), 242 (-36.5), 210 (+42.8); UV (MeOH) λ_{max} (log ε) 300 (1.52), 254 (3.94), 210 (4.48) nm; IR (KBr): ν_{max} 3480, 2969, 2931, 2882, 1728, 1635, 1605, 1583, 1492, 1462, 1413, 1358, 1196, 1128, 1053, 928, 876 cm^{-1}; ^1H and ^{13}C NMR data (Table-1); ESIMS *m/z* 553; HRESIMS *m/z* 553.2058 $[M+Na]^+$ (calcd. $C_{28}H_{34}O_{10}Na$ for 553.2050).

TABLE-1
^1H AND ^{13}C 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
9α	36.8 t	3.10, dd, *J* = 13.6, 9.5
9β		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_2O	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 H_2O. 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. $C_{28}H_{34}O_{10}$ by HRESIMS {*m/z* 553.2058 $[M+Na]^+$ (calcd 553.2050)}. The ^1H and ^{13}C 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 moiety[9]. 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 lignan[10]. The ^1H and ^{13}C NMR spectra of compound **1** were similar to those of gomisin D[11] (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 -$OCH_2CH(CH_3)$- 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 -$OCH_2CH(CH_3)C(OCH_3)(CH_3)COO$-. 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 these of gomisin D, the structure of which was determined by X-ray crystallographic analysis of its 4,11-dibromo derivative[11]. The

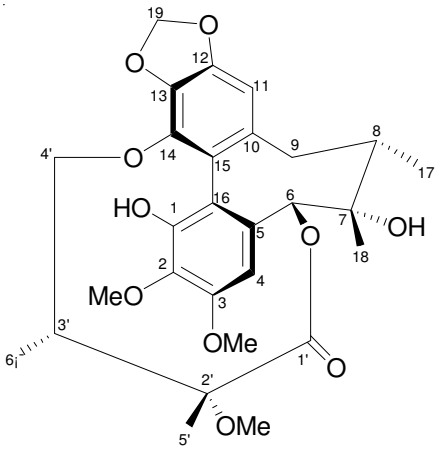

Fig.1. The structure of compound **1**

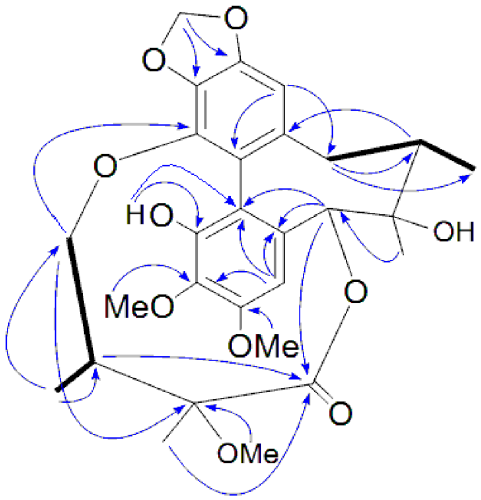

Fig. 2. Selected HMBC (⌒) and 1H-1H COSY (–) correlations of compound **1**

only difference between **1** and gomisin 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 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 configuration[9]. 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), H_3-18 (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 H_3-17 (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 H_3-5′ (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.

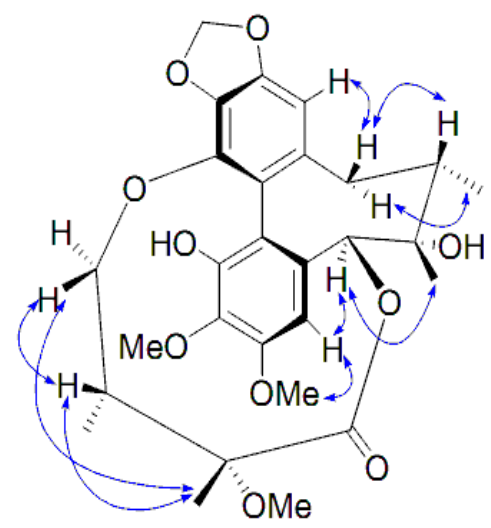

Fig. 3. Key ROESY correlations ( ) of compound **1**

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 previously[13]. Taxol was used as the positive control. The results shown that the compound 1 exhibited moderate cytotoxicity against NB4, A549 and MCF7 cell with IC_{50} values of 8.1, 7.4 and 6.7 μM, respectively.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 21002085), the Excellent Scientific and Technological Team of Yunnan High School (2010CI08), the Yunnan University of Nationalities Green Chemistry and Functional Materials Research for Provincial Innovation Team (2011HC008) and Open Research Fund Program of Key Laboratory of Ethnic Medicine Resource Chemistry (Yunnan University of Nationalities) (No. MZY100105).

REFERENCES

1. Committee of Pharmacopoeia of China, Pharmacopoeia of the People's Republic of China (Part 1), Pharmacopoeia Commission of the Ministry of Public Health of PRC, Beijing, China, pp. 454-455 (1990).
2. L.J. Xu, H.T. Liu, Y. Peng and P.G. Xiao, *J. System. Evol.*, **46**, 692 (2008).
3. J.B. Chang, J. Reiner and J.X. Xie, *Chem. Rev.*, **105**, 4581 (2005).
4. W.L. Xiao, R.T. Li, S.X. Huang, J.X. Pu and H.D. Sun, *Nat. Prod. Rep.*, **25**, 871 (2008).
5. X.G. Li, Q. Gao, W. Wen, P.F. Zhang, F. Xiao and H.M. Luo, *J. Chin. Med. Mat.*, **28**, 156 (2005).
6. Y.X. Duan, J.L. Cao, R.R. Wen, G.Y. Yang, J.X. Pu, H.D. Sun, W.L. Xiao and G.P. Li, *J. Asian Nat. Prod. Res.*, **13**, 592 (2011).
7. M. Chen, X.M. Xu, Z.H. Liao, L. Dong, L. Li and C.Z. Huang, *Molecules*, **13**, 548 (2008).
8. M. Chen, Z.Z. Liao, X.M. Xu, Y. Wen, M. Sun, H.X. Zhang and W.X. Ma, *Molecules*, **13**, 1148 (2008).
9. G.Y. Yang, Y.K. Li, R.R. Wang, X.N. Li, W.L. Xiao, L.M. Yang, J.X. Pu, Y.T. Zheng and H.D. Sun, *J. Nat. Prod.*, **73**, 915 (2010).
10. G.Y. Yang, R.R. Wang, H.X. Mu, Y.K. Li, X.N. Li, L.M. Yang, Y.T. Zheng, W.L. Xiao and H.D. Sun, *J. Nat. Prod.*, **76**, 250 (2013).
11. Y. Ikeya, H. Taguchi, I. Yosioka, Y. Iitaka and H. Kobayashi, *Chem. Pharm. Bull. (Tokyo)*, **27**, 1395 (1979).
12. Y. Ikeya, H. Taguchi, I. Yosioka and H. Kobayashi, *Chem. Pharm. Bull. (Tokyo)*, **27**, 1383 (1979).
13. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).