

Full Paper

Neglschisandrins A-B: Two New Dibenzocyclooctene Lignans from *Schisandra neglecta*

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Abstract: Two new dibenzocyclooctene lignans, neglschisandrins A-B (**1-2**), were isolated from the stems of *Schisandra neglecta*. Their structures and stereochemistries were elucidated by spectroscopic methods, including 1D- and 2D-NMR and HR-ESI-MS techniques.

Keywords: *Schisandra neglecta*, dibenzocyclooctene lignans, neglschisandrins A-B.

Introduction

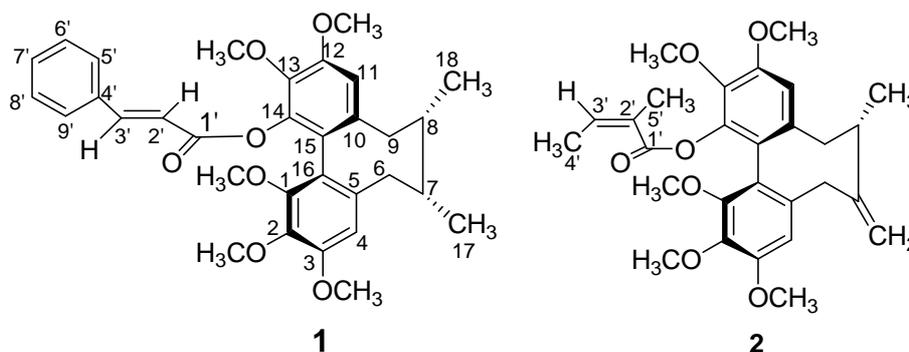
The stems or fruits of plants in the Schisandraceae family are widely used in China as tonic and astringent drugs for the treatment of rheumatic arthritis, traumatic injuries and related diseases [1]. Plants of the Schisandraceae are rich in lignans, especially dibenzocyclooctene lignans, which have

been found to possess some beneficial effects such as anti-HIV effect, antitumor-promoting effect, calcium antagonism effect, and anti-lipid peroxidation effect, etc. [2-6]. In our previous studies, some new dibenzocyclooctene lignans from the Schisandraceae family were reported [7-9]. To search for new biological active natural products, another Schisandraceae plant, *Schisandra neglecta*, was investigated. Our examination of fractions extracted from the stems of *S. neglecta* with Et₂O led to the isolation and identification of two new lignans, neglschisandrins A-B (**1-2**). This paper deals with the isolation and characterization of these new compounds.

Results and Discussion

Repeated column chromatography of the Et₂O extract of the stem of *S. neglecta* yielded two new lignans, neglschisandrins A-B (**1-2**, Figure 1).

Figure 1. Structures of Compounds **1-2**.



Neglschisandrin A (**1**), obtained as colorless powder, has the molecular formula C₃₂H₃₆O₇, based on HR-ESI-MS data [*m/z* 555.2330 ([*M*+Na]⁺)]. The UV spectrum, with maximum absorptions at 220, 251 and 280 nm, along with corresponding ¹H- and ¹³C-NMR spectra (Table 1) indicated that **1** is a dibenzocyclooctene lignan [10].

The ¹H-NMR spectrum of **1** showed signals due to two Me groups at δ_H 1.00 and 0.80 (each 3H, *d*, *J*=7.1 Hz), assignable to the *cis*-oriented 7-Me and 8-Me, respectively [11]. The presence of four methylene signals (δ_H 2.33, 1H, *dd*, *J*=13.4, 9.6 Hz; 2.06, 1H, *d*, *J*=12.8 Hz; 2.56, 1H, *dd*, *J*=13.7, 1.9 Hz and 2.64, 1H, *dd*, *J*=13.7, 7.4 Hz) indicated that, like the known schisanhenol [12], **1** has no substitution at C-6 and C-9. Based on the HMQC spectrum, the protons at δ_H 2.33 and 2.06 were attached to the same carbon (δ_C 35.5). Similarly, the protons at δ_H 2.56 and 2.64 were attached to the same carbon at δ_C 39.2. Furthermore, HMBC correlations of δ_H 2.06 with δ_C 21.6 (C-17) and δ_C 40.5 (C-7) and of δ_H 2.56 with δ_C 12.9 (C-18) and δ_C 33.8 (C-8) indicated that δ_H 2.33 and 2.06 were H₂-6 and that δ_H 2.56 and 2.64 were H₂-9 (Figure 2).

Table 1. NMR data of compounds **1-2** (400 MHz for ^1H , 100 MHz for ^{13}C) in CDCl_3 (δ in ppm, J in Hz).

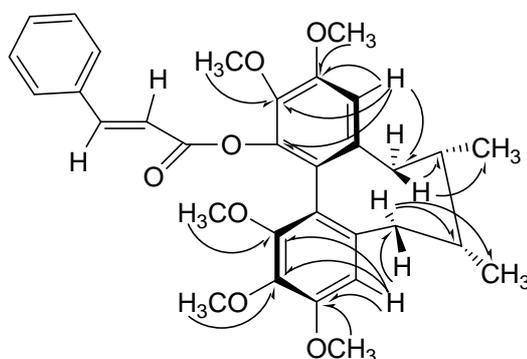
No	1		2	
	δ_{C}	δ_{H} (Mult., J)	δ_{C}	δ_{H} (Mult., J)
1	151.2		151.1	
2	139.4		139.8	
3	153.0		153.2	
4	107.5	6.48 (<i>s</i>)	107.2	6.60 (<i>s</i>)
5	140.1		137.7	
6	35.5	6 α : 2.33 (<i>dd</i> ,13.4/9.6) 6 β : 2.06 (<i>d</i> ,12.8)	36.9	2.98 (<i>d</i> ,12.6) 2.98 (<i>d</i> ,12.6)
7	40.5	1.80 (<i>m</i>)	154.0	
8	33.8	1.94 (<i>m</i>)	38.7	2.70 (<i>m</i>)
9	39.2	9 α : 2.64 (<i>dd</i> ,13.7/7.4) 9 β : 2.56 (<i>dd</i> , 13.7/1.9)	37.8	9 α : 2.58 (<i>m</i>) 9 β : 2.58 (<i>m</i>)
10	133.9		133.1	
11	113.1	6.72 (<i>s</i>)	112.8	6.73 (<i>s</i>)
12	151.5		151.8	
13	139.6		139.9	
14	142.3		142.4	
15	123.6		124.1	
16	120.9		121.2	
17	21.6	1.00 (<i>d</i> , 7.1)	111.0	4.72 (<i>d</i> ,1.5) 4.83 (<i>d</i> ,1.5)
18	12.9	0.80 (<i>d</i> ,7.1)	20.5	1.04 (<i>d</i> ,7.2)
1-OMe	60.7	3.61 (<i>s</i>)	60.5	3.56 (<i>s</i>)
2-OMe	60.9	3.75 (<i>s</i>)	60.8	3.81 (<i>s</i>)
3-OMe	55.9	3.82 (<i>s</i>)	55.9	3.86 (<i>s</i>)
12-OMe	56.1	3.92 (<i>s</i>)	56.1	3.92 (<i>s</i>)
13-OMe	60.9	3.86 (<i>s</i>)	60.9	3.85 (<i>s</i>)
Cin: 1'	164.4			
2'	117.3	6.37 (<i>d</i> ,16.0)		
3'	145.6	7.62 (<i>d</i> ,16.0)		
4'	134.3			
5'/9'	128.1	7.44 (<i>m</i>)		
6'/8'	128.8	7.34 (<i>m</i>)		
7'	130.3	7.34 (<i>m</i>)		
Ang: 1'			165.7	
2'			127.8	
3'			137.0	5.88 (<i>m</i>)
4'			15.3	1.78 (<i>d</i> ,6.1)
5'			20.3	1.77 (<i>s</i>)

The ^1H -NMR spectrum of **1** also showed signals due to two aromatic protons (δ_{H} 6.48, 6.72, each 1H, *s*) and five methoxy groups (δ_{H} 3.92, 3.86, 3.82, 3.75 and 3.61, each 3H, *s*) on two aromatic rings. The ^1H -NMR spectrum also showed the presence of a trans-cinnamic acid ester, with proton signals at

δ_H 6.37 and 7.62 (each 1H, *d*, $J=16.0$ Hz) and aromatic proton signals at δ_H 7.44 (2H, *m*) and 7.34 (3H, *m*). Carbon signals at δ_C 117.3, 145.6, 134.3, 128.1(x2), 128.8(x2) and 130.3, as well as carbonyl carbon at δ_C 164.4, supported this deduction [13].

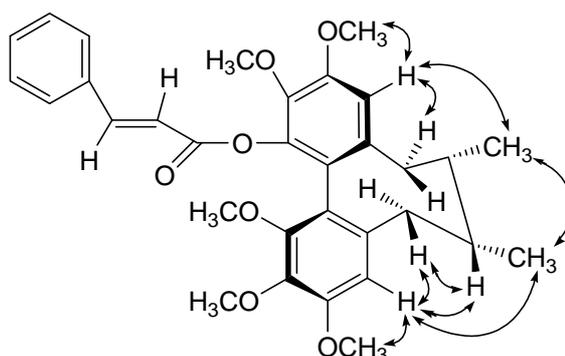
HMBC correlations of δ_H 6.48 with δ_C 35.5 (C-6) and δ_H 6.72 with δ_C 39.2 (C-9) suggested that these two protons were H-4 and H-11, respectively. Their corresponding carbon signals were assigned as δ_C 107.5 and 113.1, respectively, by HMQC techniques. Based on HMBC correlations of H-4 with the aromatic carbons at δ_C 139.4, 151.2 and 153.0 and of H-11 with δ_C 139.6, 142.3 and 151.5, these six carbons were assigned to C-2, -1, -3, -13, -14 and -12, respectively. The positions of the five methoxy substituents were elucidated from the HMBC cross peaks of δ_H 3.75, 3.61, 3.82, 3.86 and 3.92 with δ_C 139.4 (C-2), 151.2 (C-1), 153.0 (C-3), 139.6 (C-13) and 151.5 (C-12), respectively. Thus, the cinnamoyl group should be located at the C-14 position (Figure 2).

Figure 2. Key HMBC Correlations of **1**.



The circular dichroism (CD) spectrum showed a negative *Cotton* effect at 216 nm and a positive *Cotton* effect at 249 nm, indicating that **1** has a *R*-biphenyl configuration [14]. The NOESY cross peaks (Figure 3) for H-4 with CH₃-17, H-11 with H-9 and H-11 with CH₃-18 in **1** suggested a twist-boat-chair (TBC) conformation for the cyclooctadiene ring [15]. The stereochemical assignments in the cyclooctadiene ring of **1** were supported by other NOESY correlations of H-4 with H-6 β , CH₃-17 with CH₃-18, H-4 with 3-OMe, H-6 β with H-7, H-11 with 12-OMe, and H-4 with H-7 (Figure 3). From the above data, the structure of **1** was elucidated as (6*R*, 7*S*, *R*-biar)- 3-phenyl-acrylic acid-2,3,10,11,12-pentamethoxy-6,7-dimethyl-5,6,7,8-tetrahydro -dibenzo[a,c]cycloocten-1-yl ester.

Figure 3. Key NOESY Correlations of **1**.



Neglschisandrin B (**2**), obtained as colorless powder, has the molecular formula $C_{28}H_{34}O_7$ according to HR-ESI-MS [m/z 505.2181 ($[M+Na]^+$)]. The UV absorptions (216, 248 and 280 nm) and NMR spectra (Table 1) indicated that **2** was also a dibenzocyclooctene-type lignan. Its IR, UV, CD and NMR spectra were similar to those of **1**. The differences between **2** and **1** were the substituent at C-14 and the formation of an exocyclic double bond between C-7 and C-17.

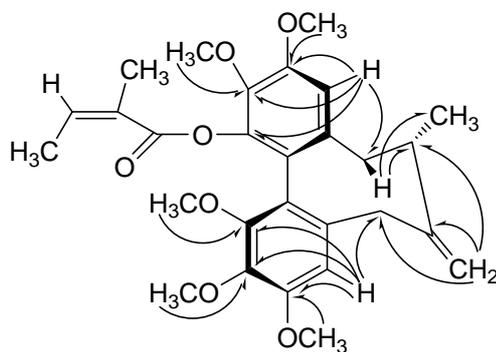
The 1H -NMR spectrum of **2** showed signals due to one Me group at δ_H 1.04 (3H, *d*, $J=7.2$ Hz), assignable to 8-Me. The presence of four methylene signals (δ_H 2.98, 2H, *d*, $J=12.6$ Hz; 2.58, 2H, *m*) indicated that **1** has no substitution at C-6 and C-9. Based on the HMQC spectrum, the protons at δ_H 2.58 (2H) were attached to the same carbon (δ_C 37.8) as well as the protons at δ_H 2.98 (2H) attached to the carbon at δ_C 36.9. Furthermore, HMBC correlations of δ_H 2.58 with δ_C 20.5 (C-18) and δ_C 38.7 (C-8) indicated that δ_H 2.58 (2H, *m*) were H₂-9 (Figure 4). Then, the δ_H 2.98 (2H) were located at H₂-6.

The 1H -NMR spectrum of **2** also showed signals due to two aromatic protons (δ_H 6.60, 6.73, each 1H, *s*) on two aromatic rings. Their corresponding carbon signals were assigned as δ_C 107.2 and 112.8, respectively, by HMQC techniques. The chemical shifts of the protons and carbons indicated that they are located in H-4/11 and C-4/11 similar to the authentic sample [2]. HMBC correlations of δ_H 6.73 with δ_C 37.8 (C-9) suggested this proton was H-11. Therefore the another proton (δ_H 6.60) should be H-4, which was confirmed by the HMBC correlations of δ_H 6.60 with 36.9 (C-6).

Five methoxy groups (δ_H 3.56, 3.81, 3.86, 3.92 and 3.85, each 3H, *s*) on two aromatic rings were also observed from the 1H -NMR spectrum. An angeloyl group was observed in 1H -NMR (δ 5.88 (1H, *m*), 1.78 (3H, *d*, $J=6.1$ Hz), 1.77 (3H, *s*) and ^{13}C -NMR data (δ 165.7, 127.8, 137.0, 15.3 and 20.3) [16]

Based on HMBC correlations of H-4 with the aromatic carbons at δ_C 139.8, 151.1 and 153.2 and of H-11 with δ_C 139.9, 142.4 and 151.8, these six carbons were assigned to C-2, C-1, C-3, C-13, C-14 and C-12, respectively (Figure 4). The positions of the five methoxy substituents were elucidated from the HMBC cross peaks of δ_H 3.81, 3.56, 3.86, 3.92 and 3.85 with δ_C 139.8 (C-2), 151.1 (C-1), 153.2 (C-3), 139.9 (C-11) and 151.8 (C-12), respectively. Thus, the angeloyl group was located at C-12 position.

Figure 4. Key HMBC Correlations of **2**.

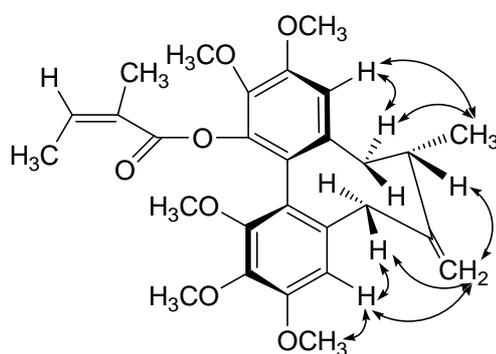


The ^{13}C NMR spectrum of **2** showed the presence of a quaternary olefinic carbon at δ 154.0 and an olefinic methylene carbon at 111.0, indicating the existence of the double bond. HMQC spectrum suggested the corresponding proton signal of δ_C 111.0 were assigned to δ_H 4.72 and 4.83 (each 1H, *d*, $J=1.5$ Hz). The correlations of δ_H 4.72, 4.83 with δ_C 38.7 (C-8), 36.9 (C-6), and 154.0 based on HMBC suggested δ_C 111.0 and 154.0 were located at C-17 and at C-7, respectively (Figure 4), revealed the

formation of an exocyclic double bond between C-7 and C-17, similar to that of the authentic sample of schisandrene reported in the literature [17].

The CD spectrum of **2** had a negative *Cotton* effect at 211 nm and a positive *Cotton* effect at 247 nm indicating that **2** has a *R*-biphenyl configuration. The NOESY correlations of H-4 with H-6 β , H-4 with CH₂-17, H-9 α with CH₃-18, and H-9 α with H-11 in **2** suggested a twist-boat-chair (TBC) conformation for the cyclooctadiene ring (Figure 5). The substituent positions and stereochemical assignments in the cyclooctadiene ring of **2** were supported by other NOESY correlations of H-4/MeO-3, H-8/CH₂-17, H-6 β /CH₂-17, and H-11/CH₃-18. Thus, the structure of **2** was determined as that shown in Figure 1.

Figure 5. Key NOESY Correlations of **2**.



Experimental

General

TLC: Silica-gel plates GF₂₅₄ (Yan-tai Institute of Chemical Technology). Column chromatography (CC): Silica gel (200-300 mesh or 300-400 mesh: Qingdao Marine Chemical Factory). Prep HPLC: Amersham UV-900, with RP-C18 column (250×10 mm). UV: Hitachi U-3010 spectrophotometer, in anh. MeOH; λ_{\max} in nm (log ϵ). CD Spectra: Jasco-810 spectropolarimeter; λ in nm ($\Delta\epsilon$ in mdeg). Optical rotation (ORD): JASCO P-1020 spectropolarimeter. IR Spectra: Avatar 360-ESP spectrophotometer (*Thermo Nicolet*), as KBr pellets; in cm^{-1} . ¹H-NMR and ¹³C-NMR spectra: Bruker DRX400 Spectrometer, in CDCl₃ soln; δ in ppm rel. to Me₄Si, *J* in Hz. HR-ESI-MS: Bruker Dalonics-BioToF Q spectrometer; in *m/z*.

Plant Material

The stems of *Schisandra neglecta* were collected in Lin-zhi County, Xi-zang Autonomous Region, People's Republic of China in September of 2004, and identified by Associate Professor Hong-ping Deng of the School of Life Sciences, SouthWest University. A voucher specimen (MC-LZ-040901) is deposited in the Herbarium of Medicinal Plant, School of Life Sciences, SouthWest University, Chongqing, People's Republic of China.

Extraction and Isolation

The air-dried stems (5 kg) of *Schisandra neglecta* were ground and extracted exhaustively with 95% ethanol at r. t. The EtOH extract was evaporated *in vacuo* to yield a semisolid (430 g), which was suspended in H₂O (1 L) and extracted with Et₂O (5×1L). This ether solution was concentrated to yield 112 g of residue, which was subjected to CC (SiO₂, 1.5 kg, petroleum ether (PE)/acetone gradient.) Fr. 4 (eluted with PE/acetone 9:1) was subjected to repeated CC (eluted with PE/EtOAc 10:1) and prep. RP-HPLC (MeOH/H₂O 75:25) to yield **1** (30 mg). Fr. 5 (eluted with PE/acetone 8:2) was subjected to repeated CC (eluted with PE/EtOAc 5:1) and prep. RP-HPLC (MeOH/H₂O 75:25) to give **2** (46 mg).

Spectroscopic data for (6R,7S,R-biar)-3-phenylacrylic acid-2,3,10,11,12-pentamethoxy-6,7-dimethyl-5,6,7,8-tetrahydrodibenzo[a,c]cycloocten-1-yl ester (neglschisandrin A, 1). Colorless powder; UV (MeOH): 220 (4.80), 251 (4.48), 280 (4.47); CD (*c*=0.022, MeOH): nm ($\Delta\epsilon$) 249 (+13.76), 216 (-23.86); $[\alpha]_D^{22}$ = +40.2° (*c*=0.53, MeOH); IR (KBr): 3417, 2927, 1729, 1596, 1493, 1456, 765, 705; ¹H-NMR and ¹³C-NMR: see Table 1; ESI-MS *m/z*: 555.4 ([M+Na]⁺); HR-ESI-MS: 555.2330 ([M+Na]⁺, C₃₂H₃₆O₇Na; *calc* 555.2353).

Spectroscopic data for (7S,R-biar)-2-methylbut-2-enoic acid 2,3,10,11,12-pentamethoxy-6-methyl-7-methylene-5,6,7,8-tetrahydrodibenzo[a,c]cycloocten-1-yl ester (neglschisandrin B, 2). Colorless powder; UV (MeOH): 216 (4.21), 248 (3.77), 280 (3.26); CD (*c*=0.0088, MeOH): nm ($\Delta\epsilon$) 247 (+1.43), 236 (+1.68), 211 (-2.03); $[\alpha]_D^{22}$ = +17.9° (*c*=0.11, MeOH); IR (KBr): 2933, 1734, 1597, 1491, 735; ¹H-NMR and ¹³C-NMR: see Table 1; ESI-MS *m/z*: 505.3 ([M+Na]⁺); HR-ESI-MS: 505.2181 ([M+Na]⁺, C₂₈H₃₄O₇Na, *calc* 505.2197).

Acknowledgements

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Sample Availability: Samples of compounds **1** and **2** are available from the authors.