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Article

Neglschisandrins C-D: Two New Dibenzocyclooctadiene Lignans from *Schisandra neglecta*

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Abstract: Two new dibenzocyclooctadiene lignans, neglschisandrins C-D (**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*, dibenzocyclooctadiene lignans, neglschisandrins C-D.

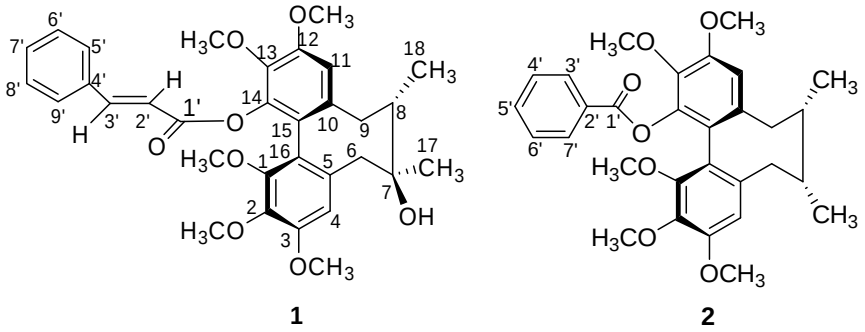
Introduction

The stems or fruits of plants in the Schisandraceae family are widely used in China as tonics and astringent drugs for the treatment of rheumatic arthritis, traumatic injuries and related diseases [1]. Plants of the Schisandraceae are rich in lignans, especially dibenzocyclooctadiene ones, which have been found to possess some beneficial effects such as anti-HIV, antitumor, calcium antagonism and anti-lipid peroxidation properties, etc. [2-6]. In a previous study, two new dibenzocyclooctadiene lignans from the *Schisandra neglecta* were reported [7]. In our continuing efforts to identify bioactive natural products from the stems of *Schisandra* medicinal plants, a chemical investigation on the stems of *Schisandra neglecta* (Schisandraceae), indigenous to the Tibet Autonomous Region of China, was tested for inhibition of tumor cells growth and showed cytotoxic activity. Bioactivity-directed fractionation of this extract led to the isolation and identification of two new dibenzocyclooctadiene lignans, named neglschisandrins C-D (**1-2**). This paper deals with the isolation and characterization of these new compounds.

Results and Discussion

Repeated column chromatography of the Et_2O-soluble fraction of the ethanol extract of the stems of *S. neglecta* yielded two new lignans **1** and **2** (Figure 1), which have been named neglschisandrins C-D, respectively.

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



Neglschisandrin C (compounds **1**) was obtained as a colorless powder. A molecular formula of $C_{32}H_{36}O_8$ was established by HR-ESI-MS (*m/z* 571.2302 $[M+Na]^+$). The UV spectrum, with absorption maxima at 217, 252 and 280 nm, along with the ^{1}H-NMR and ^{13}C-NMR data mentioned below indicated that compound **1** was a dibenzocyclooctadiene lignan [8].

The ^{1}H-NMR spectrum of **1** (Table 1) showed a singlet methyl signal (δ_H 1.28, 3H) and a doublet methyl signal (δ_H 0.88, *J*=7.2 Hz, 3H), indicating the presence of a tertiary methyl group attached to a carbon carrying a hydroxyl (δ_H 2.13, 1H, br s) and a secondary methyl group, which could be assigned to CH_3-17 and CH_3-18, respectively [9]. The presence of four methylene signals (δ_H 2.74, 1H, *d*, *J*=13.7 Hz; 2.37, 1H, *d*, *J*=13.7 Hz; 2.73, 1H, *dd*, *J*=14.3, 1.3 Hz and 2.44, 1H, *dd*, *J*=14.3, 7.7 Hz) indicated that, like the known heteroclitin H [10], compound **1** had no substitution at C-6 and C-9.

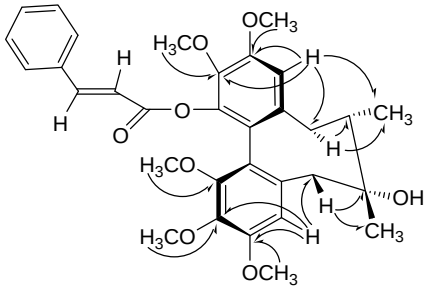
Based on the HMQC spectrum, the protons at δ_H 2.74 and 2.37 were attached to the same carbon (δ_C 40.7), as were the protons at δ_H 2.73 and 2.44 (δ_C 34.4). Furthermore, HMBC correlations of δ_H 2.37 with δ_C 29.8 (C-17) and δ_C 71.9 (C-7) and of δ_H 2.73 with δ_C 15.9 (C-18) and δ_C 41.9 (C-8) indicated that δ_H 2.74 and 2.37 were H_2-6 and that δ_H 2.73 and 2.44 were H_2-9 (see Figure 2).

Table 1. NMR data of compounds **1-2** in $CDCl_3$ (δ in ppm, J in Hz).

No	Compound **1**		Compound **2**	
	δ_C	δ_H (*mult.*, *J*)	δ_C	δ_H (*mult.*, *J*)
1	151.8		151.2	
2	140.4		139.4	
3	152.5		152.9	
4	110.3	6.72(*s*)	107.4	6.44 (*s*)
5	132.9		140.1	
6	40.7	6α: 2.74 (*d*, *J*=14.7) 6β: 2.37 (*d*, *J*=13.7)	35.4	2.36 (*dd*, *J*=13.4/9.7) 2.04 (*d*, *J*=13.3)
7	71.9		40.6	1.80 (*m*)
8	41.9	1.90 (*m*)	33.8	1.94 (*m*)
9	34.4	9α: 2.73 (*dd*, *J*=14.3/1.3) 9β: 2.44 (*dd*, *J*=14.3/7.7)	39.1	9α: 2.56 (*dd*, *J*=13.6/1.8) 9β: 2.66 (*dd*, *J*=13.6/7.4)
10	133.8		133.9	
11	113.0	6.56 (*s*)	113.1	6.74 (*s*)
12	151.7		151.5	
13	139.8		139.7	
14	142.2		142.4	
15	123.1		123.5	
16	122.6		120.9	
17	29.8	1.28 (*s*)	21.6	1.00 (*d*, *J*=7.1)
18	15.9	0.88 (*d*, *J*=7.2)	12.9	0.81 (*d*, *J*=7.1)
7-OH	-	2.13 (*br s*)		
1-OMe	60.7	3.61 (*s*)	60.5	3.53 (*s*)
2-OMe	60.9	3.78 (*s*)	60.6	3.63 (*s*)
3-OMe	55.9	3.83 (*s*)	55.8	3.78 (*s*)
12-OMe	56.1	3.93 (*s*)	56.1	3.93 (*s*)
13-OMe	61.0	3.86 (*s*)	60.9	3.85 (*s*)
Cin: 1'	164.4	-		
2'	117.0	6.36 (*d*, *J*=16.0)		
3'	145.8	7.62 (*d*, *J*=16.0)		
4'	134.2	-		
5'/9'	128.1	7.44 (*m*)		
6'/8'	128.9	7.35 (*m*)		
7'	130.5	7.35 (*m*)		
Ben: 1'			164.1	
2'			129.8	
3'/7'			129.9	7.97 (*d*, *J*=7.3)
4'/6'			128.1	7.34 (*t*, *J*=7.8)
5'			132.9	7.49 (*t*, *J*=7.3)

The ^{1}H-NMR spectrum of compound **1** also showed signals due to two aromatic protons (δ_H 6.72, 6.56, each *s*, 1H) and five methoxy group singlets (δ_H 3.61, 3.78, 3.83, 3.86 and 3.93, each 3H) on two aromatic rings. The ^{1}H-NMR spectrum also showed the presence of a trans-cinnamic acid ester with proton signals at δ_H 6.36 and 7.62 (each 1H, *d*, *J*=16.0 Hz) and aromatic proton signals at δ_H 7.44 (2H, *m*) and 7.35 (3H, *m*). Carbon signals at δ_C 117.0, 145.8, 134.2, 128.1 (×2), 128.9 (×2) and 130.5, as well as carbonyl carbon at δ_C 164.4, supported this deduction [7].

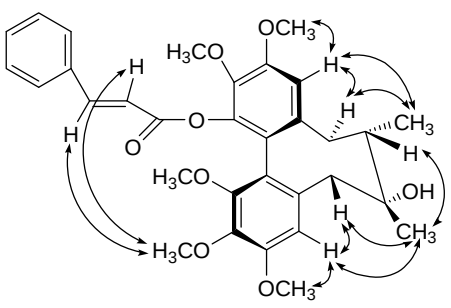
Figure 2. Key HMBC Correlations of compound **1.**



HMBC correlations (Figure 2) of δ_H 6.56 with δ_C 40.7 (C-6) and δ_H 6.72 with δ_C 34.4 (C-9) suggested that these two protons were H-4 and H-11, respectively. Their corresponding carbon signals were assigned as δ_C 110.3 and 113.0, respectively, by HMQC techniques. Based on HMBC correlations of H-4 with the aromatic carbons at δ_C 140.4 and 152.5 and of H-11 with δ_C 139.8, 142.2 and 151.7, these five carbons were assigned to C-2, -3, -13, -14 and -12, respectively. The positions of the five methoxy substituents were elucidated from the HMBC cross peaks of δ_H 3.78, 3.61, 3.83, 3.86 and 3.93 with δ_C 140.4 (C-2), 151.8 (C-1), 152.5 (C-3), 139.8 (C-13) and 151.7 (C-12), respectively. Thus, the cinnamoxyl group should be located at C-14 position.

The circular dichroism (CD) spectrum showed a negative *Cotton* effect at 215 nm and a positive *Cotton* effect at 249 nm, indicating that compound **1** has a *R*-biphenyl configuration [11]. The NOESY cross peaks (see Figure 3) for H-11/CH_3-18, H-11/H-9α, H-4/H-6β and H-9α/CH_3-18 in compound **1** suggested a twist-boat-chair (TBC) conformation for the cyclooctadiene ring [12]. The stereochemical assignments in the cyclooctadiene ring of compound **1** were supported by other NOESY correlations of H-4/3-OMe, H-11/12-OMe, 2-OMe/H-2', 2-OMe/H-3', H-6β/CH_3-17, H-4/CH_3-17 and H-8/CH_3-17. From the above data, the structure of compound **1** was elucidated as *(6R,7S,R-biar)-3-phenylacrylic acid-7-hydroxy-2,3,10,11,12-pentamethoxy-6,7-dimethyl-5,6,7,8-tetrahydrodibenzo[a,c]cycloocten-1-yl ester.*

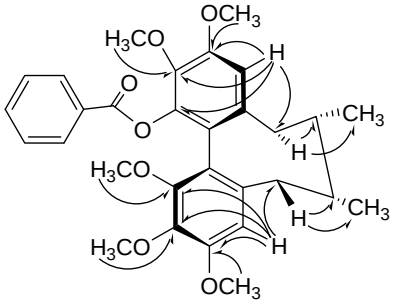
Figure 3. Key NOESY Correlations of compound **1.**



Neglschisandrin D (**2**), obtained as a colorless powder, had the molecular formula $C_{30}H_{34}O_7$ according to HR-ESI-MS [*m/z* 529.2180 ([*M*+Na]$^+$)]. The UV absorptions (226, 250 and 279 nm) and NMR spectra (Table 1) indicated that compound **2** was also a dibenzocyclooctadiene-type lignan. Its IR, UV, CD and NMR spectra were similar to those of compound **1**, the differences between both compounds being the substituents at C-14 and the C-7.

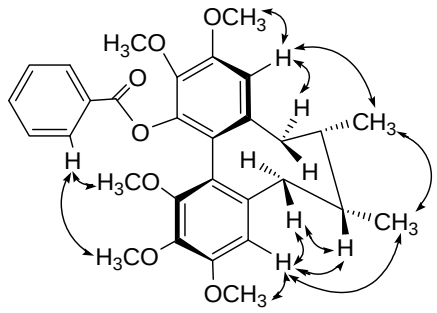
In the cyclooctadiene ring, two *doublet* methyl signals (δ1.00, 0.81, each 3H, *J*=7.1Hz) were assigned to 7-Me and 8-Me, respectively. This suggested that there was no substitution at C-7 and C-8, and these two methyl groups were in *cis*-orientation [13]. Comparing the NMR spectrum of compound **2** with that of compound **1** (Table 1), the aromatic proton signals at δ_H 7.97 (2H, *d, J*=7.3Hz), 7.34 (2H, *t, J*=7.8 Hz) and 7.49 (1H, *t, J*=7.3 Hz) and carbon signals at δ_C 129.8, 129.9(x2), 128.1(x2) and 132.9, as well as carbonyl carbon at δ_C 164.1, showed that the cinnamoyl group in compound **1** was replaced by an benzoyl group in compound **2** (see Figure 4).

Figure 4. Key HMBC and NOESY Correlations of compound **2.**



The circular dichroism (CD) spectrum showed a negative *Cotton* effect at 210 nm and a positive one at 247 nm, indicating that compound **1** has a *R*-biphenyl configuration. The NOESY cross peaks (Figure 5) for H-4 with CH_3-17, H-4/H-6β, H-11 with H-9α and H-11 with CH_3-18 in compound **2** suggested a twist-boat-chair (TBC) conformation for the cyclooctadiene ring. The stereochemical assignments in the cyclooctadiene ring of **1** were supported by other NOESY correlations of H-4/3-OMe, H-11/12-OMe, H-6β/H-7, H-4/H-7, CH_3-17/CH_3-18, H-7'/1-OMe and H-7'/2-OMe (see Figure 4). From the above data, the structure of compound **2** was elucidated as *(6S,7S,R-biar)benzoic acid 2,3,10,11,12-pentamethoxy-6,7-methyl-5,6,7,8-tetrahydrodibenzo[a,c]cycloocten-1- yl ester*.

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



Experimental

General

TLC: Silica-gel plates GF_{254} (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 [in anh. MeOH; λ_{max} in nm (log ε)]: Hitachi U-3010 spectrophotometer. CD Spectra [λ in nm (Δε in mdeg)]: Jasco-810 spectropolarimeter. Optical rotation (ORD): JASCO P-1020 spectropolarimeter. IR Spectra (KBr pellets; in cm^{-1}): Avatar 360-ESP spectrophotometer (Thermo Nicolet). ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra in $CDCl_3$ soln.; δ in ppm rel. to Me_4Si, J in Hz): Bruker DRX400 Spectrometer. ESI-MS (*m/z*): Bio TOF Q spectrometer; HR-ESI-MS (*m/z*): Bruker Dalonics-BioToF Q spectrometer.

Plant Material

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

Air-dried stems of *Schisandra neglecta* (5 kg) were ground and extracted exhaustively with 95% ethanol at room temperature. The EtOH extract was evaporated *in vacuo* to yield a semisolid (430 g), which was suspended in H_2O (1 L) and extracted with Et_2O (5×1L). This ether solution was concentrated to yield 112 g of residue, which was subjected to CC [SiO_2, 1.5 kg, petroleum ether (PE)/acetone gradient]. Fraction 4 (eluted with PE/acetone 9:1) was subjected to repeated CC (eluted with PE/EtOAc 15:1) and prep. RP-HPLC (MeOH/H_2O 70:30) to yield compound **2** (2 mg). Fraction 5 (eluted with PE/acetone 8:2) was subjected to repeated CC (eluted with PE/$CHCl_3$ 1:1) and prep. RP-HPLC (MeOH/H_2O 80:20) to give compound **1** (29 mg).

(6R,7S,R-biar)-3-phenylacrylic acid-7-hydroxy-2,3,10,11,12-pentamethoxy-6,7-dimethyl-5,6,7,8-tetrahydrodibenzo[a,c]cycloocten-1-yl ester (*neglschisandrin C*, **1**). Colorless powder; UV: 217 (4.44), 252 (4.05), 280 (3.23); CD (*c*=0.08, MeOH): nm (Δε) 249 (+39.76), 234 (+55.71), 215 (-76.79); $[\alpha]_D^{22}$ =+72.1°(*c*=0.48, MeOH); IR: 3415, 1726, 1636, 1594, 1494; ^{1}H-NMR and ^{13}C-NMR: see Table 1; ESI-MS *m/z*: 571.1 ([*M*+Na]$^+$); HR-ESI-MS: *found* 571.2290 ([*M*+Na]$^+$, $C_{32}H_{36}O_8Na$, *calc.* 571.2302).

(6S,7S,R-biar)-benzoic acid-2,3,10,11,12-pentamethoxy-6,7-methyl-5,6,7,8-tetrahydrodibenzo[a,c]-cycloocten-1-yl ester (neglschisandrin D, **2**). Colorless powder; UV: 226 (4.42), 250 (4.12), 279 (3.71); CD (*c*=0.4, MeOH): nm (Δε) 247 (+312.52), 233 (+389.88), 211 (-278.28); $[\alpha]_D^{22}$

=+79.6°(*c*=1.30, MeOH); IR: 1738, 1597, 1493, 707; ^{1}H-NMR and ^{13}C-NMR: see Table 1; ESI-MS *m/z*: 529.5 ([*M*+Na]$^{+}$); HR-ESI-MS: *found* 529.2180 ([*M*+Na]$^{+}$, $C_{30}H_{34}O_7Na$, *calc.* 529.2197).

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