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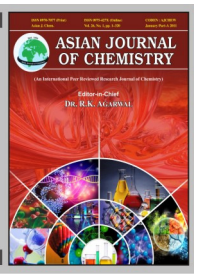

A New Dibenzocyclooctadiene Lignan from Stems of *Schisandra lancifolia* and its Bioactivities

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A new dibenzocyclooctadiene lignan, lanciphenol A (**1**) was isolated from the stems of *Schisandra lancifolia*. The structure of **1** was elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques. Compound **1** was evaluated for its anti HIV activity and cytotoxicity. It showed anti HIV-1 activity with therapeutic index value of 64.8 and cytotoxicity against NB4, A549 and MCF7 cell with IC_{50} values of 6.8, 7.5 and 8.4 μM, respectively.

Keywords: Dibenzocyclooctadiene lignan, *Schisandra lancifolia*, Anti HIV activity, Cytotoxicity.

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 triperpenoids, especially dibenzocyclooctadiene lignans, which have been found to possess some beneficial activities, including anti HIV, antitumor, cytotoxic, antioxidant and antihepatotoxic effects[3-5].

Schisandra lancifolia belongs to the genus *Schisandra* of the family *Schisandraceae*. It is a climbing plant mainly distributed in Mainland of China[6]. In previous studies, some new dibenzocyclooctadiene lignans were isolated from the fruits of *S. lancifolia* from of Erlang Mountain area of Sichuan Province[7]. In our continuing efforts to identify bioactive natural products from the medicinal plants of the Schisandraceae, a chemical investigation on the stems of *S. lancifolia* from Lijiang Country, Yunnan Province was carried out. As a result, a new dibenzocyclooctadiene lignan *i.e.*, lanciphenol A (**1**) was separated from this plant. In addition, the anti HIV-1 and cytotoxicity of **1** was evaluated. In this paper its structure elucidation and biological activities are described.

EXPERIMENTAL

Optical rotations were measured in a Horiba SEPA-300 polarimeter. UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer and CD spectra were measured on a JASCO J-810 spectropolarimeter. A Tenor 27 spectrophotometer was used for scanning IR spectra (KBr pellets). 1D and 2D NMR spectra were recorded on a DRX-500 spectrometer with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to TMS. HRESIMS was performed on an API QSTAR spectrometer or a VG Autospec-3000 spectrometer. Preparative HPLC was performed on a Shimadzu LC-8A liquid chromatograph equipped with Zorbax PrepHT GF (21.2 mm × 25 cm, 7 μm) column or Venusil MP C_{18} (20 mm × 25 cm, 5 μm) column. Column chromatography was performed using Si gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich Corp. -St Louis, USA), or MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Column fractions were monitored by TLC and the spots were visualized by heating the plates after spraying with 5 % H_2SO_4 in EtOH.

The stems of *S. lancifolia* were collected in Lijiang Country of Yunnan Province, People's Republic of 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 11-9-52) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation: The air-dried and powdered stems of *S. lancifolia* (2.6 kg) were extracted four times with 70 % acetone (4 × 3 L) at room temperature and filtered, with the filtrate evaporated under reduced pressure and partitioned with EtOAc (3 × 1 L). The EtOAc partition (186 g) was applied

to silica gel (200-300 mesh) column chromatography, eluting with a $CHCl_3$-Me_2CO gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give five fractions, A-F. The further separation of fraction B (42.7 g) by silica gel column chromatography, eluted with petroleum ether-acetone (20:1-1:2), yielded mixtures B1-B6. Fraction B2 (4.6 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (68 % MeOH-H_2O, flow rate 12 mL/min) to give **1** (16.5 mg).

Lanciphenol A (1): Yellow gum; $[\alpha]_D^{24.6}$ + 36.8 (c 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.64), 242 (3.85), 320 (1.27), nm; CD (c 0.05, MeOH), nm (Δε) 250 (-43.8), 235 (-25.6), 220 (+23.4), 210 (+4.6); IR (KBr, ν_{max}, cm^{-1}) 3415, 3082, 2951, 2867, 1618, 1569, 1492, 1450, 1408, 1325, 1272, 1018, 968, 862; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz), see Table-1; ESIMS (positive ion mode) *m/z* 497 $[M + Na]^+$; positive ESIMS *m/z* 483 $[M + Na]^+$; HRESIMS *m/z* 497.2522 $[M + Na]^+$. Calcd. (%) for $C_{27}H_{38}NaO_7$, 497.2515.

TABLE-1
^1H AND ^{13}C NMR DATA OF COMPOUND **1** (δ IN ppm, OBTAINED IN C_5D_5N)

No.	δ_C	δ_H (*J* in Hz)	No.	δ_C	δ_H (*J* in Hz)
1	152.6 s	–	14	151.2 s	–
2	141.8 s	–	15	122.5 s	–
3	153.3 s	–	16	123.8 s	–
4	112.4 d	6.61 s	17	17.5 q	0.89 overlap
5	132.8 s	–	18	17.5 q	0.89 overlap
6	89.2 d	4.08d(8.2)	1'	72.2 t	3.68 d (6.8)
7	38.5 d	1.68 m	2'	26.7 d	2.13 (m)
8	37.0 d	1.80 m	3', 4'	19.8 q	0.98 d (6.8)
9α	39.4 t	2.08 m	OMe-1	60.2 q	3.79 s
9β	–	2.27 m	OMe-2	60.4 q	3.85 s
10	135.7 s	–	OMe-3	55.8 q	3.86 s
11	108.4 d	6.84 s	OMe-13	60.5 q	3.88 s
12	148.2 s	–	OMe-14	60.6 q	3.81 s
13	139.8 s	–	Ar-OH	–	11.45 brs

Anti HIV-1 assay: The cytotoxicity assay against C8166 cells (CC_{50}) was assessed using the MTT method and anti HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) using azidothymidine (AZT) as a positive control[8]. All experiments were performed in triplicate.

Cytotoxicity assay: The cytotoxicity tests for these compounds were performed by against NB4 (human acute promyelocytic leukemia cells), A549 (human lung adenocarcinoma epithelial cells), SHSY5Y (human neuroblastoma cells), PC3 (human prostate cancer cell) and MCF7 (human breast adenocarcinoma cells) tumor cell lines by MTT-assay with paclitaxel as the positive control[9]. All experiments were performed in triplicate. The IC_{50} was defined as the concentration of the test compound resulting in a 50 % reduction of absorbance compared with untreated cells.

RESULTS AND DISCUSSION

The stems of *S. lancifolia* 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 the new dibenzocyclooctadiene lignan, lanciphenol A (**1**). Its structure was shown in Fig. 1 and its ^1H and ^{13}C NMR spectroscopic data were listed in Table-1.

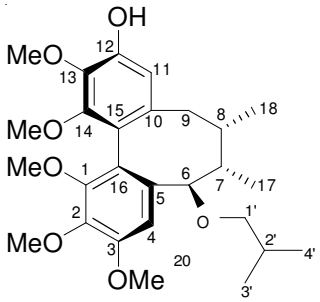

Fig. 1. Structure of lanciphenol A (**1**)

Compound **1** was obtained as yellow gum. It was assigned the molecular formula of $C_{27}H_{38}NaO_7$, from its HRESIMS at *m/z* 497.2522 $[M + Na]^+$ (calcd *m/z* 497.2515). Its ^1H, ^{13}C and DEPT NMR spectra showed signals for 27 carbons and 38 hydrogens. The NMR data (Table-1) and CD spectrum indicated that **1** is an S-biphenyl configured dibenzocyclooctadiene lignan possessing five methoxy groups and one phenolic hydroxyl group on the aromatic rings. In addition, an O-isobutyl group [δ_C 72.2 t, 26.7 d, 19.8 q (2C); δ_H 3.68 d, *J* = 6.8 Hz, (2H), 2.13 m, (1H), 0.98 d, *J* = 6.8 Hz (6H)] was also observed in **1**. The UV absorption bands at 210 and 242 nm, the ^1H ^1H COSY correlations of H-6/H-7/H-8/H-9, H-7/H-17 and H-8/H-18, together with HMBC correlations (Fig. 2) of H-11 with C-9, C-10 and C-15, of H-9 with C-10, C-11 and C-15 and of H-6 with C-4, C-5 and C-16, implied the structure of dibenzocyclooctadiene lignan[10,11]. In dibenzocyclooctadiene lignans, the chemical shifts of methoxy groups at C-3 and C-12 occur at δ_C 55-56, whereas the methoxy groups at C-1, C-2, C-13, C-14 are found to be δ_C 60-61[10,12]. Base on this, four methoxy groups (δ_C 60.2, 60.4, 55.8, 60.5 and 60.6) should be located C-1, C-2, C-13 and C-14. These were also confirmed by the analysis of its HMBC spectrum in **1**. The remainder methoxy group (δ_C 55.8) located at C-3 was confirmed by the HMBC correlation of the methoxyl proton signal (δ_H 3.93) with C-3 (δ_C 151.9). The HMBC correlations of the phenolic hydroxyl proton (δ_H 11.24) with C-11, C-12 and C-13 supported the phenolic hydroxyl group located at C-12. Finally, the HMBC correlations of H-1′ (δ_H 3.68) with C-6 (δ_C 89.2) and of H-6 (δ_H 4.08) with C-1′ (δ_C 72.2) indicated the O-isobutyl group should be located at C-6.

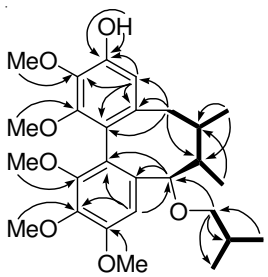

Fig. 2. Selected HMBC ( ) and ^1H ^1H COSY (–) correlations of **1**

The CD spectrum of **1** (negative Cotton effect at 250 nm and a positive Cotton effect at 220 nm) indicated that **1** has a

S-biphenyl configuration[10,11]. The ROESY correlations between H-4/CH_3-17 and H-11/H-9β in **1** suggested a twist-boat-chair (TBC) conformation for the cyclooctadiene ring[13]. The configuration of the O-isobutyl group attached to C-6 was deduced as being β-oriented by the chemical shift (δ_C 89.2), which was similar to β-oriented derivatives of the dibenzocyclooctadiene lignan[14] and was distinct from that of 6-α-oriented components in dibenzocyclooctadiene lignan family[15]. This was also confirmed by the ROESY correlations (Fig. 3) between H-4/H-6α and H-4/CH_3-17. Thus, the structure of **1** was established, as shown and given the trivial name of lanciphenol A.

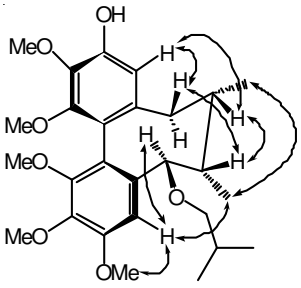

Fig. 3. Key ROESY correlations of **1**

Since some dibenzocyclooctadiene lignans from *Schisandra* species are reported to possess anti HIV-1 activity and cytotoxicity for cancer cell lines[7,8], These activities were tested for compound **1**.

In anti HIV-1 assay, the cytotoxicity assay against C8166 cells (CC_{50}) and anti HIV-1 activity were evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using azidothymidine (AZT) as a positive control (EC_{50} = 0.034 μM and CC_{50} > 200 μM)[8]. The results revealed that compound **1** showed anti HIV-1 activities with EC_{50} of 1.26 μM, CC_{50} of 81.6 μM and therapeutic index value of 64.8.

The cytotoxicity tests for compounds were performed against NB4, A549, SHSY5Y, PC3 and MCF7 tumor cell lines by MTT-assay with paclitaxel as the positive control[9]. The results shown that the compound **1** exhibited moderate cytotoxicity against NB4, A549 and MCF7 cell with IC_{50} values of 6.8, 7.5 and 8.4 μM, respectively.

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