Asian Journal of Chemistry Vol. 21, No. 7 (2009), 5488-5492

A New Lignan from Fruit of *Schisandra wilsoniana* A.C.Smith and its Anti-HIV activity

YI-PING FAN†, LI-PING DUAN, XIU-HUA DONG, YUN DAI and GAN-PENG LI*
Department of Chemistry, Yunnan Nationalities University, Kunming 650031, P.R. China
E-mail: ganpeng_li@sina.com; huqiufena@yahoo.com.cn

A new lignan, willignan A, was isolated from the fruit of *Schisandra wilsoniana* A.C.Smith. It structures and stereochemistry was elucidated by analysis of spectroscopic data. This compound was evaluated for its inhibitory activity against HIV-1 and it shows an anti-HIV activity with EC_{50} (median effect concentration) value of 12.5 µg/mL and a Therapeutic Index (TI) above 16.00.

Key Words: *Schisandra wilsoniana* A.C.Smith, Lignan, Willignan A, Anti-HIV-1 activity.

INTRODUCTION

The family *Schisandraceae*, consisting of *Schisandra* and *Kadsura* genera, is medicinally important. Many plants of this family are commonly used in Traditional Chinese Medicine for their diverse beneficial bioactivities. Previous studies showed that the principal bioactive constituents of this family were lignans, especially the dibenzocyclooctadiene type[1], some of which possessed anti-HIV[2,3], antitumor[4], cytotoxic[5-9], antioxidant[10,11] and antihepatotoxic[12] effects.

Schisandra wilsoniana A.C.Smith is an evergreen liana, growing in the forests at elevations of 3080-3550 m in Yunnan Province, P.R. China[13]. Its fruit are used as a folk medicine to promote blood circulation and treat fractures and menstrual irregularities. However, the studies on the chemical constituents of this plant have not been reported. In this paper, a new lignan, willignan A was isolated from the fruit of *Schisandra wilsoniana* A.C.Smith. It structures was identified by means of MS and extensive NMR spectra and the absolute configurations of was determined by CD and ROESY experiments. The anti-HIV test show that the compound has *in vitro* anti-HIV Activity.

EXPERIMENTAL

Optical rotations were measured in Horiba SEPA-300 High Sensitive Polarimeter. The CD spectra were recorded on a JASCO J-810 spectropolarimeter. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS

†Department of Chemistry, Yuxi Teacher's College, Yuxi 653100, P.R. China.

were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on Bruker DRX-500 instruments with TMS as internal standard. On second separate used Agilent 1100 HPLC equipped with ZORBAX-C18 (9.4 × 250 nm, 5.0 mm) column and DAD detector. Column chromatography was performed on silica gel (200-300 mesh) or on silica gel H (10-40 mm, Qingdao Marine Chemical Inc., China). The fractions were monitored by HPLC.

The fruit of *Schisandra wilsoniana* A.C.Smith were collected in Dali Prefecture of Yunnan Province, PR China, in November 2007 and was identified by Prof. S.G. Wu. A voucher specimen (No. KIB 01-11-05) was deposited in our laboratory.

Extraction and isolation: The air-dried and powdered fruit of *S. wilsoniana* (0.8 kg) were extracted with 70 % aqueous Me_2CO (2.0 L × 3, 24 h each) at room temperature and the extract was partitioned successively with petroleum ether (1.0 L × 3) and EtOAc (1.0 L × 3), respectively. The EtOAc extract (29 g) was subjected to CC over silica gel eluting with a $CHCl_3$-Me_2CO (1:0-0:1, 180 L) gradient system to give fractions 1-5. Fraction 3 (0.8 g) was further purified by HPLC with mobile phase (MeOH-H_2O 7:3) to yield willignan A (12.8 mg).

Willignan A, white gum. $[\alpha]_D^{17.9}$ + 16.2. (c 0.43, $CHCl_3$); IR (KBr, ν_{max}, cm^{-1}): 3448(br), 2935, 1735, 1647, 1598, 1578, 1494, 1456, 1401, 1321, 1225, 1127, UV (MeOH) λ_{max} (log ε) 241 (4.24) nm. CD (c = 0.015, $CHCl_3$) $\Delta OD_{256.4}$ + 80.049, $\Delta OD_{232.6}$-16.136, $\Delta OD_{227.8}$ + 30.257, ΔOD_{210}-27.315 (mdeg). HRESI-MS (positive ion mode) m/z: found 537.2467 $[M+Na]^+$ calc. 523.22464). ^{1}H (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) data (Table-1).

TABLE-1
^{1}H AND ^{13}C NMR SPECTRAL DATA FOR WILLIGNAN A IN CD_3OD[a]

Position	δ_H (mult, *J*, Hz)	δ_C	Position	δ_H (mult, *J*, Hz)	δ_C
1		151.7s	15		122.9s
2		141.8s	16		122.8 s
3		151.9s	17	0.98(d,6.7)	18.9q
4	6.71(s)	110.8d	18	0.77(d,6.6)	14.1q
5		132.2s	1'		166.9s
6	5.81(d,7.6)	80.7d	2'		128.2s
7	1.95 (m)	37.4d	3'	5.82 (m)	137.5d
8	1.95 (m)	36.4d	4'	1.61(d,6.9)	11.7q
9α	2.26	36.8t	5'	1.57(s)	14.1q
9β	2.19		OMe-1	3.90(s)	60.8q
10		136.6s	OMe-2	3.89(s)	60.7q
11	6.52(s)	106.7d	OMe-3	3.87(s)	55.8q
12		152.5s	OMe-12	3.56(s)	55.9q
13		139.9s	OMe-13	3.55(s)	60.4q
14		151.6s	OMe-14	3.53(s)	60.1q

[a]Data were recorded on Bruker AM-400 MHz spectrometer; assignments were confirmed by HMBC and NOESY.

HIV-1 Inhibition assays cytotoxicity assay: The cellular toxicity of compounds on MT4 cells was assessed by MTT colorimetric assay as described previously. Briefly, 50 µL of MT4 cells was seeded onto a microtiter plate, 100 µL of various concentrations of compounds was added and incubated at 37 ºC in a humidified atmosphere of 5 % CO_2 for 72 h. Discard 100 µL supernatant, MTT reagent was added and incubated for 4 h, 100 µL 50 % DMF-10 % SDS was added. After the formazum was dissolved completely. The plates were read on a Bio-Tek ELx 800 enzyme-linked immunosorbent assay (ELISA) reader at 595 nm/630 nm. The results were shown by absorbance values.

Inhibition assay for the cytopathic effects of HIV-1. Antimicrobial peptides serially diluted the RPMI-1640 medium were added to triplicate wells of a 96 % well flat bottomed microtiter plate. Then 8×10^5 MT4 cells and 200 $TCID_{50}$ (50 % tissue culture infectious dose) of HIV-1_{IIIB} stock solution were added immediately to each well. After incubation at 37 ºC for 72 h without changing medium, syncytial cells from five different fields of each well were examined and counted under an inverted microscope (100×). The inhibition percentage of syncytial cell formation was calculated by percentage of syncytial cell number in the sample treated culture to that in infected control culture. The concentration of the antiviral sample reducing HIV-1 replication by 50 % (EC_{50}) was determined from the dose response curve. The Therapeutic index (TI) was calculated from the ratio of CC_{50}/EC_{50}. Anti-HIV-1 activity date see Table-2.

TABLE-2
ANTI-HIV-1 ACTIVITY, CYTOTOXICITY, THERAPEUTIC INDEX FOR WILLIGNAN A[a]

Compound	Cytotoxity (CC_{50}) (µg/mL)[b]	Syncytium	
		(EC_{50}) (µg/mL)[c]	TI[d]
Willignan A	>200	12.5	>16.00

[a]Data are expressed as means of three dependent measurements.
[b]Concentration required to reduce MT_4 cells viability by 50 %.
[c]Concentration required to reduce HIV-1_{IIIB} induced syncytiurn formation by 50 % on MT_4 cells. [d]Therapeutic index (TI): ratio CC_{50}/EC_{50}.

RESULTS AND DISCUSSION

Willignan A, was obtained as a white gum and had the molecular formula $C_{29}H_{38}O_8$ as revealed by its HR-ESIMS ($[M + Na]^+$, m/z 537.2467, calcd. for 537.2464). The UV spectrum, with maximum absorption at 241 (4.24) nm and the IR spectrum, with bands at 3448 (-OH), 1712 (ester) and 1638, 1598, 1485 cm^{-1} (aromatic), suggested that willignan A was a dibenzocyclooctadiene lignan possessing an ester linkage. The ^1H and ^{13}C NMR spectra revealed that willignan A possessed six methoxyls on the aromatic rings, an angeloyl group and also three secondary methyl and a methylenes on the cyclooctadiene ring. The chemical shift of the angeloyl group (1.57, 3H, s; 1.61, 3H, d, 6.9 Hz; 5.82, lH, m) in the ^1H NMR

spectrum indicated that it was shielded by the aromatic ring, suggesting it being attached to C-6 or C-9 of the cyclooctadiene ring. In HMBC spectrum (Fig. 1), H-6 (d H 5.81, d, J = 7.6 Hz) correlated to the tertiary methyl (Me-17, δc 18.9), C4 (δc 110.8), C-16 (δc 122.8) and C-1' (δc 166.9); H-9 (δH 2.26, 2.19) correlated to the secondary methyl (Me-18, δc 14.1), C-11 (δc 106.7) and C-15 (δc 122.9); H-7 (δH 1.95, m) correlated to C-5 (δc 132.2), which established the angeloyl group being located at C-6.

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 willignan A could be determined by CD. The CD curve showed a negative Cotton effect around 250 nm and a positive one around 220 nm, suggesting that willignan A possessed an S-biphenyl configuration[14]. With the axial chirality defined, a NOESY experiment was used to establish the relative configuration of the remaining stereocenters. In NOESY spectrum, the NOE correlation between H-4 and H-6 further supported the above assignment. The stereochemical assignments in the cyclooctadiene ring were strengthened by the NOESY spectrum. The H-11 had NOE correlations with H-9β, 12-OCH_3 and 18-CH_3 [which was also correlated with H-9α]; the H-4 with H-6 and 3-OCH_3, the H-8β with Me-17 indicating a twist-boat-chair conformation (Fig. 2).

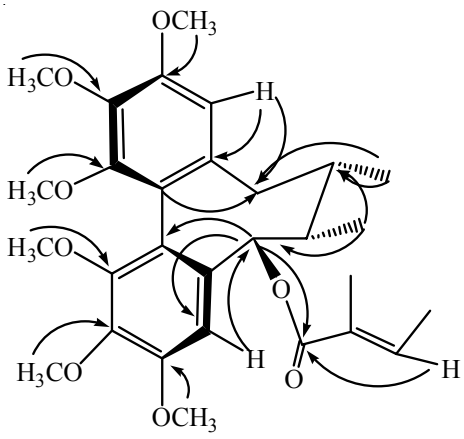

Fig. 1. Key HMBC correlations for willignan A

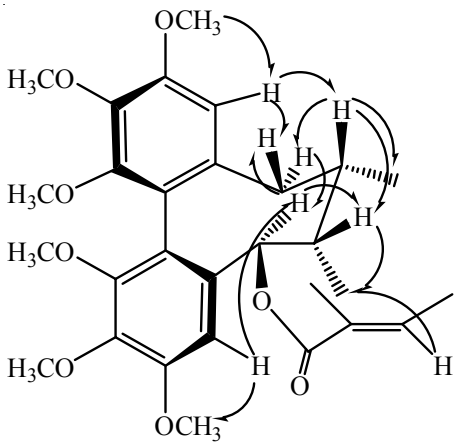

Fig. 2. Key NOESY correlations for willignan A

The potencies of willignan A in preventing the cytopathic efects of HIV-1 in MT_4 cells,as well as compound-induced cytotoxicity in MT_4 cells in parallel with the antiviral activity were evaluated. The results from the cell-based assays demonstrated potent anti-HIV-1 activity with EC_{50} (median effect concentration) value of 12.5 μg/mL and a TI (Therapeutic index) of greater than 16.00 12.5 μg/mL. Willignan A shows a strong anti-HIV activity.

ACKNOWLEDGEMENTS

Financial support was provided by grants from the Young Academic and Technical Leader Raising Foundation of Yunnan Province (NO. 2007PY01-27) and the Natural Science Foundation of Yunnan Province (NO.2005B0027Q).

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(*Received*: 13 October 2008; *Accepted*: 30 April 2009) AJC-7496