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New Triterpenoids from the Fruits of *Schisandra wilsoniana* and Their Biological Activities

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Investigation of an organic extract of the fruits *Schisandra wilsoniana* led to the isolation of two new highly oxygenated nortriterpenoids, named schilancidilactones V-W (**1-2**). Their structures were elucidated by spectroscopic evidence. Compounds **1-2** feature a double bond between C-7 and C-8 compared with related known nortriterpenoids isolated from the genus *Schisandra*. Compounds **1** and **2** were tested for their *anti*-HIV-1 activities and cytotoxicity. The results revealed that compounds **1** and **2** showed moderate *anti*-HIV-1 activities with EC_{50} 3.05 and 2.87 μg/mL, respectively, and compound **1** showed high cytotoxicity against KB and MDA-MB-231 cell with IC_{50} values of 3.18 and 5.22 μM, respectively.

Key Words : *Schisandra wilsoniana*, Nortriterpenoid, *Anti*-HIV-1 activity, Cytotoxicity

Introduction

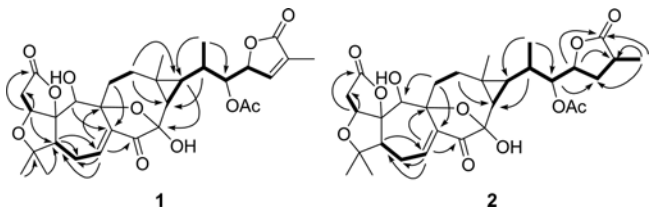
The family Schisandraceae comprises two genera *Kadsura* and *Schisandra*. Previously, a series of novel highly oxygenated triterpenoids with unusual norcycloartane skeletons were isolated from plants of the genus *Schisandra*.[1-15] Phytochemical studies on species of the genus *Schisandra* have revealed that they are rich sources of lignans, highly oxygenated nortriterpene derivatives possessing various beneficial pharmacological effects, and over 19 species are widely used as sedative and tonic agents in traditional Chinese medicine.[1-15] As a part of our ongoing phytochemical investigation on the species of the genus, *Schisandra wilsoniana* A.C Smith (Schisandraceae), a species native to Yunnan province,[16] was chosen for chemical constituent studies. Previously, a series of lignans, nortriterpene and sesquiterpenes are reported from this species.[6-14] Our continuing study of the fruits this plant has led to the isolation of two new nortriterpenoids, schilancidilactones V-W (**1-2**), along with six related known compounds identified as pre-schisanartanin (**3**),[1] pre-schisanartanin I (**4**),[2] wuweizidilactone B (**5**),[3] lancifodilactone K (**6**),[4] lancifodilactone N (**7**),[4] micarndilactone G (**8**),[5] Compounds **1-2** were tested for their *anti*-HIV-1 activities and cytotoxicity. This paper describes the isolation, structure elucidation, and biological activities of the new compounds.

Results and Discussion

Powdered dried fruits of *S. wilsoniana* were extracted in 70% aqueous acetone. The filtrate was concentrated and partitioned with EtOAc. The EtOAc fraction was submitted to successive chromatographic fractionation and purification to yield compounds **1-8**.

The positive ion ESIMS of **1** exhibited a pseudo-molecular ion at *m/z* 609 for $[M+Na]^+$. The molecular formula was established as $C_{31}H_{38}O_{11}$ by HRESI-MS (*m/z* 609.2299 $[M+Na]^+$), suggesting thirteen degrees of unsaturation. Examination of the ^1H and ^{13}C NMR data of **1** showed the presence of six methyls, four methylenes, eight methines, seven quaternary carbons (four oxygenated), three ester groups, and one carbonyl group. In addition, the presence of two olefinic moieties required the presence of six rings to satisfy the degrees of unsaturation.

Considering that a series of oxygenated nortriterpenoids had been isolated from the genus *Schisandra*,[15] it was reasonable to presume that **1** was also a nortriterpenoid derivative. Comparison of ^1H and ^{13}C NMR data of **1** with those of pre-schisanartane A (**3**),[1] along with a detailed analysis of HSQC, ^1H-^1H COSY, and HMBC spectra revealed that **1** possessed the same rings as **3**. However, different carbon and proton chemical shifts for C-7 and C-8 indicated that the ring C in



1 **2**

Figure 1. Selected HMBC (→) and ^1H-^1H COSY (—) correlations of **1** and **2**.

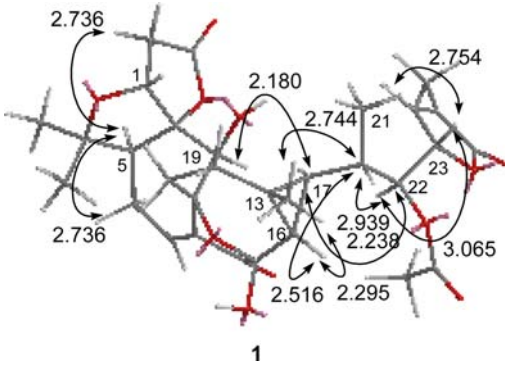


Figure 2. Computer generated molecular model showing key ROESY correlations and corresponding interatomic distance (Å) of compound **1**.

structure of **1** differed from **3**. A detailed analysis of HSQC, HMBC and ^{1}H-^{1}H COSY spectra revealed that compound **1** featured a double bond between C-7 and C-8. This double bond was elucidated by the downfielded signals of C-7 and C-8 (δ = 135.6 and 137.6 ppm), and upfielded signal of C-14 (δ = 200.4 ppm). Further analysis of the HMBC spectrum of **1** (Figure 1) showed the following correlations: H-5 and H-6 with C-7; H-6, H-11 and H-19 with C-8; H-7 with C-5, C-6, C-8, C-9 and C-14. This evidence, together with obvious spin system deduced from ^{1}H-^{1}H COSY spectra of **1**, H-5/H6/H-7, established the planar structure of **1** as shown.

The relative stereochemistry of **1** was determined from analysis of molecular models, energy minimized using the MM2 force field in CS Chem 3D V 8.0 overlaid with key correlations observed in the ROESY NMR spectrum (Figure 2). The relative configurations of C-19, C-15, C-18, C-20, C-22, and C-23 were determined to be same as those of **3** by ROESY correlations. Biogenetically, the relative orientation of H-5 was α, and CH_3-30 was β. The relative configurations of the stereocenters in **1** were the same as those of **3**. Compared with related nortriterpenoid **3**, compound **1** features a double bond between C-7 and C-8.

Schilancidilacone W (**2**) was obtained as colorless crystals. Its molecular formula was established to be $C_{31}H_{40}O_{11}$ by the HRESI-MS data (*m/z* 611.2466 $[M + Na]^+$, calcd. 611.2442). The ^{13}C NMR spectrum showed 30 carbon signals including six methyls, six methylenes, eight methines, six quaternary carbons, three ester groups, and one carbonyl group. This suggested that **2** also was a nortriterpenoid derivative. Comparison of ^{1}H and ^{13}C NMR data of **2** with those of **1** suggested that **2** was structurally related to **1**. Particularly noteworthy in the ^{13}C NMR data for **2** were the downfield shifted signal for C-26 (δ = 182.0 ppm), and the upfield shifted signals for C-24 (δ = 33.0 ppm) and C-25 (δ = 34.5 ppm), suggesting reduction of Δ.[24,25] The differences between **1** and **2** can be explained by reduction of double bond between C-24 and C-25. The ROESY correlations of H_3-27 with H-24β, and of H-25 with H-23α and H-24α placed Me-27 at β-orientation in **2**. According to the observed ROESY correlations and comparison of ^{1}H and ^{13}C data with those of **1**, all of the chiral centers of **2** were established to be identical with those of **1** (Table 1 and Figure 2).

Table 1. ^{1}H and ^{13}C NMR data for Schilancidilactones V-W (**1-2**)[a]

No.	Compound **1**		Compound **2**	
	δ_H (mult, *J*, Hz)	δ_c	δ_H (mult, *J*, Hz)	δ_c
1	4.79 (d, 6.5)	77.7 d	4.77 (d, 6.5)	77.3 s
2α	2.62 (d, 18.8)	35.7 t	2.59 (d, 18.1)	35.6 t
2β	2.85 (dd, 18.8, 6.5)		2.81 (dd, 18.1, 5.2)	
3		175.8 s		175.8 s
4		83.6 s		83.5 s
5	2.12-2.15 (m)	56.9 d	2.17 (ov)	57.0 d
6α	2.59-2.62 (ov)	23.1 t	2.59-2.62 (ov)	23.1 t
6β	2.15-2.17 (m)		2.17 (ov)	
7	7.06 (t, 8.0)	135.6 d	7.07 (t, 8.0)	135.5 d
8		137.6 s		137.5 s
9		83.0 s		83.0 s
10		97.4 s		97.3 s
11α	1.98-2.00 (m)	33.0 t	1.98-2.00 (m)	32.2 t
11β	2.37-2.39 (m)		2.21-2.23 (m)	
12α	1.82-1.85 (m)	29.5 t	1.82-1.85 (m)	29.5 t
12β	1.90-1.93 (ov)		1.93-1.96 (m)	
13		21.2 s		21.1 s
14		200.4 s		200.5 s
15		100.0 s		100.0 s
16	1.13 (d, 8.9)	30.2 d	1.11 (d, 8.9)	30.0 d
17	0.68 (t like, 7.3)	34.6 d	0.75 (t like, 7.3)	34.5 d
18	0.87 (s)	30.8 q	0.86 (s)	30.7 q
19	3.83 (d, 6.2)	73.6 d	3.78 (d, 6.2)	73.9 d
20	3.70-3.75 (m)	30.2 d	3.67-3.70 (m)	29.5 d
21	1.31 (d, 10.5)	18.8 q	1.30 (ov)	18.8 q
22	4.85-4.88 (m)	75.7 d	4.88-4.91 (m)	79.7 d
23	4.93-4.95 (m)	81.0 d	4.91-4.94 (m)	77.7 d
24α	7.01 (br s)	146.6 d	2.22-2.26 (m)	33.0 t
24β			2.11-2.14 (m)	
25		130.6 s	2.74-2.77 (m)	34.5 d
26		175.2 s		182.0 s
27	1.92 (br s)	10.4 q	1.25 (ov)	16.8 q
29	1.25 (s)	21.1 q	1.31 (s)	21.8 q
30	1.29 (s)	28.6 q	1.26 (s)	28.5 q
OAc		170.1 s		170.1 s
	2.00 (s)	21.4 q	2.17 (br s)	21.1 q

[a]Data were recorded in $CDCl_3$ on Bruker AM-125 (^{13}C-NMR) and AM-500 (^{1}H-NMR) MHz; chemical shifts (δ) are expressed in ppm.

Since some nortriterpenoids isolated from *Schisandra* genus are reported to have modest or strong *anti*-HIV activities,[6,13,14] the new compounds were tested for their ability to prevent the cytopathic effects of HIV-1 in C8166, and their cytotoxicity was measured in parallel with the determination of antiviral activity using AZT as a positive control (EC_{50} = 0.0043 μg/mL and CC_{50} > 200 μg/mL).[17] Compounds **1-2** showed *anti*-HIV-1 activities with EC_{50} values of 3.05 and 2.87 μg/mL respectively. Compounds **1** and **2** showed cytotoxicity against C8166 cells with CC_{50} values of 92.3 and 126.5 μg/mL, respectively.

Since some nortriterpenoids isolated from *Schisandra* genus are reported to have cytotoxiciy,[2,15] the new compounds

Table 2. Cytotoxicity of Compound **1** and **2** (IC_{50} values in μmol/L)

Compounds	Cell lines			
	HL-60	HepG2	KB	MDA-MB-231
1	11.5	16.2	3.18	5.22
2	4.15	12.2	8.21	12.4
Camptothecin	1.87	0.94	1.62	2.18

were evaluated for their cytotoxicity against four human tumor cell lines. The cytotoxicity tests for the isolates were performed using a previously reported procedure.[18] All treatments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic abilities against HL-60, Hep-G2, KB and MDA-MB-231 tumor cell lines by MTT-assay (with camptothecin as the positive control) were shown in Table 2.

Experimental Section

General Procedures. Melting points were obtained on a XRC-1 micro melting point apparatus and are uncorrected. 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. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Mass spectra were performed on a VG Autospec-3000 spectrometer under 70 eV. Column chromatography was performed with silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). Semi-preparative 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 Shimadzu PRC-ODS (K) column. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The fruits of *S. wilsoniana* were collected in August 2009 from Dali Prefecture of Yunnan Province, China. The specimen was identified by Prof. Xi-Wen Li. A voucher specimen, No. KIB-2009-09, has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Air-dried and powdered fruits (5.0 kg) were extracted with 70% aqueous Me_2CO (4 × 50 L) at room temperature and concentrated *in vacuo* to give a crude extract, which was partitioned between H_2O and EtOAc. The EtOAc part (180 g) was decolorized by MCI. The decolorized part (185 g) was chromatographed on a silica gel column eluting with $CHCl_3$-acetone (1:0, 1:1, and 0:1) to afford fractions I-III. Fraction II was repeatedly chromatographed on silica gel and Sephadex LH-20, and then by semi-preparative HPLC (CH_3OH: H_2O, 45:55 and CH_3OH:CH_3CN:H_2O, 10:40:50) to yield compounds **1** (10 mg), **2** (7.3 mg), **3** (6.9 mg), **4** (2.0 mg), **5** (10.6 mg), **6** (2.2 mg), **7** (10.3 mg), and **8** (8.8 mg).

***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}).[17]

Cytotoxicity Assay. The cytotoxicity tests for these compounds were performed by against HL-60, Hep-G2, KB, and MDA-MB-231 tumor cell lines by MTT-assay (with camptothecin as the positive control). [18]

Schilancidilactone V (1): $C_{31}H_{38}O_{11}$; pink crystal; mp 178-179 °C; $[\alpha]_D^{19}$ +37.8 (c 0.05, CH_3OH); UV (CH_3OH) λ_{max}(log ε) 210 (0.67) nm; IR (KBr) ν_{max}: 3439, 2972, 2871, 1746, 1657, 1461, 1373, 1238, 1072, 1034, 943 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive ESIMS: *m/z* 609 $[M+Na]^+$; HRESIMS: *m/z* 609.2299 $[M+Na]^+$, calcd for $C_{31}H_{38}O_{11}Na$: *m/z* 609.2284.

Schilancidilactone W (2): $C_{31}H_{40}O_{11}$; colorless crystal; mp 182-183 °C; $[\alpha]_D^{19}$ +66.9 (c 0.07, CH_3OH); UV (CH_3OH) λ_{max}(log ε) 210 (0.30), 240 (0.40), 362 (0.01) nm; IR (KBr) ν_{max}: 3443, 2939, 2881, 1763, 1741, 1660, 1461, 1373, 1236, 1071, 1033, 945 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive ESIMS: *m/z* 611 $[M+Na]^+$; HRESIMS: *m/z* 611.2466 $[M+Na]^+$, calcd for $C_{31}H_{40}O_{11}Na$: *m/z* 611.2442.

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