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Schilancidilactones A and B: two novel tetranortriterpenoids with an unprecedented skeleton from *Schisandra lancifolia*

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ABSTRACT

Schilancidilactones A (**1**) and B (**2**), two novel tetranortriterpenoids possessing an unprecedented skeleton, have been isolated from the stems of *Schisandra lancifolia*. Their structures were elucidated on the basis of extensive spectroscopic analysis. The relative configurations of **1** were further determined by single-crystal X-ray crystallography. Compounds **1** and **2** were tested for their cytotoxicity against four tumor cell lines NB4, A549, SH-SY5Y, and PC-3, and compound **1** was further tested for its anti-HIV-1 activity.

The family Schisandraceae, a group of climbing plants with important economical and medicinal values, contains two genera *Schisandra* and *Kadsura*. There are about 50 species of this family worldwide, and 29 species of them distributed in China. Recent phytochemical research of our group found that the plants of genus *Schisandra* produced structurally interesting *Schisandra* nortriterpenoids, which were characterized with highly oxygenated and complex polycyclic skeletons.[1] *Schisandra* nortriterpenoids may be further grouped into six main skeleton types based on their structural characteristics, including schisanartane,[1,2] schiartane,[1,3] 18-norschiartane,[1,4,5] 18(13/14)-abeo-schiartane,[1,6] pre-schisanartane,[1,7] and wuweiziartane types.[1,8] Some of them showed considerable bioactivity.[1]

Schisandra lancifolia (Rehd.et.Wils.) A. C. Smith was distributed widely in the west of China and was used as a Chinese traditional medicine to staunch cut, treat fractures, and eliminate stasis.[9] In order to find structurally interesting and bioactive metabolites, we conducted the research on the chemical constituents of this species and led to the identification of a series of *Schisandra* nortriterpenoids, and some of which showed weak to moderate anti-HIV-1 activity.[10] Continued investigation on *S. lancifolia* from different source led to the isolation of two novel tetranortripenoids, schilancidilactones A (**1**) and B (**2**). Compounds **1** and **2** were characterized with an unprecedented backbone for the highly oxygenated cleavage, rearrangement, and loss of four carbons, which make them different from other types of *Schisandra* nortriterpenoids previously found from the genus *Schisandra*.[1] In addition, compounds **1** and **2** were tested for their cytotoxicity against four tumor cell lines (NB4, A549, SH-SY5Y, and PC-3) and **1** was further evaluated for its anti-HIV-1 activity. Herein, we report the isolation and structural elucidation of the two compounds and their bioactivities.

The plant material of *S. lancifolia* were collected from Nujiang prefecture of Yunnan province, mainland China, in October 2007, and identified by Prof. Hong Wang. The air-dried and powered stems (5.0 kg) were percolated with 70% aqueous Me_2CO at room temperature, and the crude extract was successively extracted with petroleum and EtOAc. The EtOAc extract (125 g) was chromatographed on a silica gel column eluted with gradient $CHCl_3$/Me_2CO (9:1 to 0:1) to yield six fractions A–F. Fraction C was chromatographed over open columns packed with reverse phase C_{18} and Sephadex LH-20 repeatedly. Further purification with

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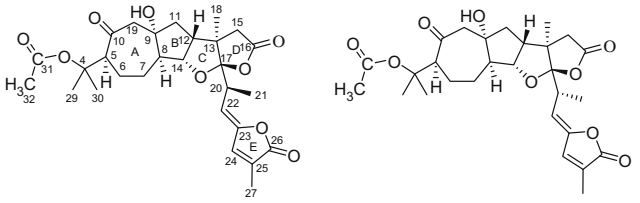
semipreparative HPLC (Agilent 1100 HPLC system, Zorbax, SB-C_{18}, Agilent, 9.4 × 25 mm, MeOH–H_2O 1:1) led to the isolation of compounds **1** (20 mg, retention time 15.1 min) and **2** (2 mg, retention time 17.2 min).



1 **2**

Schilancidilactone A (**1**),[11] $[\alpha]_D^{27.9} = +72.06$ (*c*, 0.303, $CHCl_3$) was crystallized as a colorless prism, giving a molecular formula of $C_{28}H_{36}O_9$ as determined by HRESIMS at *m/z* 539.2252 $[M+Na]^+$ (calcd 539.2257). The IR spectrum of 1 revealed the presence of hydroxyl groups (3508 cm^{-1}). The 1D NMR data (Table 1) and HSQC spectrum showed the existence of four tertiary methyls at δ_H 1.22, 1.47, 1.50, and 2.00 (each 3H, s), a secondary methyl at δ_H 1.20 (3H, d, *J* = 7.4 Hz), and an acetyl group (δ_H 1.98 (3H); δ_C 22.4 and 169.9). The ^{13}C and DEPT NMR spectra of **1** displayed 28 carbons, including one carbonyl group, two ester groups, one acetyl group, two trisubstituted double bonds, four quaternary carbons (three oxygenated), five methines (one oxygenated), five methylenes, and six methyls. Considering that the NMR data of **1** have a distinct difference from those of the known triterpene skeleton, we first tried to establish the possible structure of **1** by an analysis of two-dimensional NMR spectroscopic data. HMBC spectrum showed obvious correlations from Me-27 (δ_H 2.00) to C-24, C-25, and C-26, H-22 to C-23 and C-24, and Me-21 (δ_H 0.82, d, *J* = 7.4 Hz) to C-20 and C-22. This evidence, along with a proton spin system deduced from ^1H–^1H COSY correlations, H-21/H-20/H-22, led to the establishment of partial structure **1a** (Fig. 1). Further study of the COSY spectrum revealed the presence of another proton spin system, H-5/H_2-6/H_2-7/H-8/H-14/H-12/H_2-11. This information coupled with the observed HMBC correlations: Me-29 and Me-30 with C-4 and C-5; H-19 with C-5, C-8, C-9, and C-10; H-5 with C-10; H-14 with C-7, C-9, and C-11; Me-18 with C-12, C-13, C-15, and C-17; H_2-15 with C-16, and H-12 with C-17, showed the presence of the structural fragment **1b** (Fig. 1). In addition, the HMBC correlations from Me-21 and H-22 to C-17 permitted partial structures **1a** and **1b** to be joined to get the partial structure **1c** (Fig. 1). However, we cannot determine the correct connections

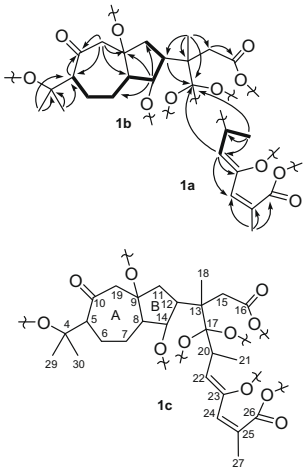


Figure 1. Fragments and key COSY (▬), and HMBC (→) correlations of **1**.

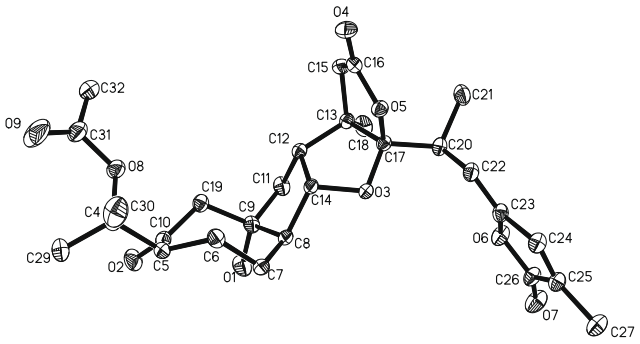


Figure 2. X-ray structures of **1** showing relative configuration.

between C-4, C-9, C-14, C-16, C-17, C-23, and C-26, and where the acetyl group attached due to the absence of enough evidence from 2D NMR data. Therefore, a single crystal of **1** was obtained from methanol after repeated recrystallization and an X-ray diffraction experiment was conducted (Fig. 2), which indicated the acetyl group to be attached at C-4, and other three rings C, D, and E are connected through the linkages of C-14–O–C-17, C-16–O–C-17, and C-23–O–C-26.

The stereochemistry of **1** was also determined by X-ray analysis[12] and ROESY experiment. The *Z* geometry of the double bond between C-22 and C-23 was deduced from ROESY correlation of H-22 with H-24. Biogenetically, H-5 was α-orientated. According to the IUPAC sequence rule,[13] the chiral center with the lowest lo-

Table 1
^1H and ^{13}C NMR data of **1** and **2**[a]

No.	1		2	
	δ_H (mult., *J*, Hz)	δ_C	δ_H (mult., *J*, Hz)	δ_C
4		82.2		82.3
5	2.76 (m)	62.4	2.78 (m)	62.5
6α	1.75–2.00 (m)	19.6	1.80–1.91 (m)	19.6
6β	1.75–2.00 (m)		1.80–1.91 (m)	
7α	1.46–1.51 (m)	23.3	1.48–1.53 (m)	23.3
7β	1.96–2.02 (m)		1.99–2.02 (m)	
8	2.20 (m)	53.2	2.23 (m)	53.4
9		80.8		80.7
10		211.2		211.1
11α	2.03 (overlapped)	41.7	2.08 (overlapped)	41.8
11β	1.76 (overlapped)		1.78 (overlapped)	
12	2.39 (m)	50.8	2.38 (m)	50.8
13		50.1		50.2
14	4.54 (dd, 6.0, 7.5)	84.0	4.57 (dd, 6.4, 8.0)	83.6
15α	2.71 (d, 18.0)	46.3	2.67 (d, 18.0)	46.4
15β	2.64 (d, 18.0)		2.47 (d, 18.0)	
16		173.0		173.6
17		121.4		121.4
18	1.22 (s)	19.1	1.19 (s)	18.8
19α	2.26 (ABd, 11.5)	50.5	2.27 (ABd, 11.6)	50.2
19β	3.13 (ABd, 11.5)		3.13 (ABd, 11.6)	
20	3.34 (m)	35.9	3.37 (m)	36.3
21	1.20 (d, 7.4)	15.9	1.26 (d, 6.8)	16.0
22	5.28 (d, 9.9)	113.4	4.99 (d, 10.4)	111.3
23		147.7		148.4
24	7.01 (d, 1.2)	137.7	7.00 (d, 1.6)	137.4
25		130.0		130.8
26		170.6		170.2
27	2.00 (s)	10.6	2.02 (s)	10.6
29	1.47 (s)	24.8	1.49 (s)	24.9
30	1.50 (s)	24.5	1.53 (s)	24.6
OAc	1.98 (s)	22.4	1.99 (s)	22.4
		169.9		169.9

[a] Data were recorded in $CDCl_3$, δ in ppm, ^1H NMR at 400 MHz, ^{13}C NMR at 100 MHz, and assignments were based on HSQC, COSY, HMBC, and ROESY experiments.

cant, the configuration of the eight chiral centers, C-5, C-8, C-9, C-12, C-13, C-14, C-17, and C-20, was deduced as S^*, R^*, S^*, R^*, R^*, S^*, R^*, and S^*, respectively.

Schilancidilactone B (**2**),[14] $[\alpha]_D^{26.3} = -76.72$ (*c*, 0.063, $CHCl_3$), has the molecular formula $C_{28}H_{36}O_9$ as established by analysis, ^1H and ^{13}C NMR spectral data (Table 1), together with HRESIMS at *m/z* 539.2252 $[M+Na]^+$ (calcd 539.2257). Initial observation of ^1H and ^{13}C NMR spectral data showed that the structure of **2** was very close to that of **1**. Side-by-side comparison of their 1D NMR data and an analysis of 2D NMR data indicate that **1** and **2** possess the same planar structure. The almost identical 1D NMR data of **2** through C-4 to C-17 with those of **1**, and the evidences from NOESY spectrum of **2,** especially the observation of correlations between H-12 and H-14, and between Me-18 and H-20, indicated that **2** has the same rings A-D as those of **1**. The double bond between C-22 and C-23 in **2** was deduced to be *Z* geometry from ROESY correlation of H-22 with H-24, which is the same as that of **1**. Therefore, the reason responsible for the minor difference of chemical shifts between **2** and **1** can be rationalized to the different configuration of C-20. Thus compound **2** was deduced to be the C-20 epimer of **1**. It is interesting that another case of C-20 epimer of nortriterpenoids, schintrilactones A and B, was found from *S. chinensis*[15].

Compounds **1** and **2** represented a new class of *Schisandra* nortriterpenoids from the plants of Schisandraceae family and featured with a unique C_{26} skeleton. Both were screened for their cytotoxicity against NB4 (acute promyelocytic leukemia), A549 (lung cancer), SHSY5Y (neuroblastoma), PC-3 (prostate cancer), and MCF-7 (breast cancer) cell lines, using taxol as positive control.[16] Compounds **1** and **2** showed no obvious inhibitory activities with IC_{50} values more than 50 μg/ml. In addition, compound **1** was further tested for cytotoxicity assay against C8166 cells (CC_{50}), and anti-HIV-1 activity evaluated by the inhibition assay for the cytopathic effects of HIV-1_{IIIB} (EC_{50}), using AZT as a positive control (EC_{50} 0.0034 μg/ml and CC_{50} >200 μg/ml).[17] It exerted minimal cytotoxicity against C8166 cells (CC_{50} >200 μg/ml) and showed considerable anti-HIV-1 activity with EC_{50} 8.51 μg/ml and selectivity index more than 23.5. Compound **2** was not tested for further bioactivities due to its limited mass.

Acknowledgments

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Supplementary data

Supplementary data (detailed description of the experimental procedures, crystallographic data of 1, and 1D and 2D NMR spectra of schilancidilactones A (**1**) and B (**2**)) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.08.051.

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11. Schilancidilactone A (**1**): colorless prism, mp 192.6–193.8 °C; $[\alpha]_D^{27.9} = +72.06$ (*c* 0.303, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 276 (4.01) nm; IR (KBr) υ_{max} 3508, 2986, 2951,1765, 1732, 1692, 1620, 1461, 1369, 1245, 1063, 922 cm^{-1}; NMR can be found in Table 1; positive ESIMS ($[M+Na]^+$): *m/z* 539; HR-ESIMS ($[M+Na]^+$) found 539.2252, calcd for $C_{28}H_{36}O_9Na$ 539.2257.
12. Crystallographic data for **1** can be seen in Supplementary data. CCDC 696627 contains the supplementary crystallographic data for this Letter. This data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
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14. Schilancidilactone B (**2**): colorless crystals, mp 192.3–193.7 °C; $[\alpha]_D^{26.3} = -76.72$ (*c* 0.063, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 276(4.03) nm; IR (KBr) υ_{max} 3441, 2926, 1768, 1702, 1628, 1461, 1370, 1253, 1060, 914 cm^{-1}; NMR can be found in Table 1; positive ESIMS: *m/z* 539, $[M+Na]^+$; HR-ESIMS ($[M+Na]^+$) found 539.2252, calcd for $C_{28}H_{36}O_9Na$ 539.2257.
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