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Sphenadilactones A and B, Two Novel Nortriterpenoids from *Schisandra sphenanthera*

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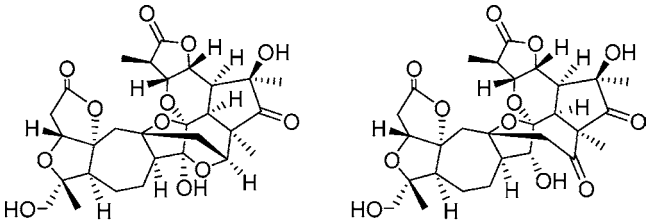
ABSTRACT



Sphenadilactone A (1) Sphenadilactone B (2)

Two novel nortriterpenoid compounds, sphenadilactones A (1) and B (2), have been isolated from the leaves and stems of *Schisandra sphenanthera*. The structural elucidation of 1 and 2 was accomplished by extensive NMR analysis. The relative stereochemistry of 1 was established by single-crystal X-ray crystallography. Both compounds were tested for their cytotoxicities against K562, A549, and HT-29, and compound 1 was further tested for its anti-HIV-1 activity.

Since the discovery of a highly oxidized, rearranged cycloartane nortriterpenoid, micrandilactone A,[1] from *Schisandra micrantha* in 2003, our group has phytochemically studied several species of *Schisandra* genus collected in the Yunnan Province of China, and this led to the isolation of a series of nortriterpenoids with a diversity of highly oxygenated structures,[2−9] some of which showed anti-HIV-1 activities.[6−9]

With the aim of identifying more novel natural compounds with biological activities from this genus, we investigated the leaves and stems of *Schisandra sphenanthera* Rehd et Wils collected from the Sichuan Province of China. The fruits

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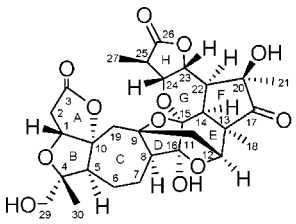
of which were previously reported to be used as anti-tussive and tonic agents in traditional Chinese medicine,[10] and lignans are considered to possess liver-protective, anti-inflammatory, anti-oxidant, anti-tumor, and anti-HIV activities.[11,12] Our present studies have led to the isolation of two novel highly oxygenated nortriterpenoids, sphenadilactones A (**1**) and B (**2**). Both compounds feature an unprecedented rearranged backbone derived from cycloartane, and the structure of **1** was confirmed by X-ray diffraction analysis. In addition, compounds **1** and **2** were tested for their cytotoxicities against human tumor cell lines of K562, A549, and HT-29, and compound **1** was further tested for its anti-HIV-1 activity. Described herein are the isolation, structure elucidation, and biological activities of the two compounds.



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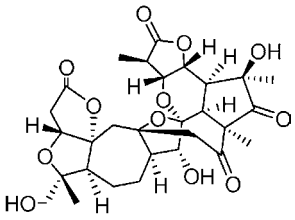

2

The leaves and stems of *S. sphenanthera* were collected in Maoxian county of Sichuan Province, People's Republic of China, in August 2004, and identified by Prof. Xi-Wen Li. The air-dried and powdered stems and leaves (2.5 kg) were extracted with 70% aqueous Me_2CO (4 × 5 L) at room temperature and concentrated in vacuo to give a crude extract (102 g), which was partitioned between H_2O and EtOAc. The EtOAc part (57.0 g) was chromatographed on silica gel column, RP-18, and sephadex LH-20 (MeOH) repeatedly. Further purification with semipreparative HPLC (Agilent 1100 HPLC system, Germany; Zorbax SB-C-18, Agilent, 9.4 mm × 25 cm, U.S.A., MeCN−H_2O 35:65) yielded sphenadilactones A (**1**, 33 mg) and B (**2**, 4 mg).

Sphenadilactones A (**1**)[13] were obtained as colorless and optically active crystals. Its molecular composition of $C_{29}H_{36}O_{12}$ was established from HRESIMS (found $[M + Na]^+$ 599.2118, calcd 599.2123) and ^{13}C NMR spectroscopic data, indicating 12 degrees of unsaturation. The IR spectrum showed absorptions at 3463 and 1776 cm^{-1}, revealing the presence of hydroxyl and γ-lactone groups.[14] The ^1H NMR of **1** displayed the presence of four methyl groups. Detailed analysis of the ^1H and ^{13}C NMR (Table 1) and HSQC spectra revealed that **1** contained two ester groups, one carbonyl group, four methyls, six methylenes (including oxygenated one), nine methines (including four oxygenated ones), and seven quaternary carbons (including six oxygenated ones). These suggested that compound **1** was a highly oxygenated nortriterpenoid. Importantly, the lack of any olefinic moieties required the presence of nine rings to satisfy the degrees of unsaturation.

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

	1		**2**	
no.	δ_H (mult., *J*, Hz)	δ_C	δ_H (mult., *J*, Hz)	δ_C
1	4.19 (d, 5.5)	80.4 d	4.15 (d, 5.2)	80.5 d
2α	2.65 (d, 22.2)	35.6 t	2.62 (d, 22.7)	35.7 t
2β	2.85 (dd, 5.5, 22.2)		2.73 (dd, 5.2, 22.7)	
3		174.8 s		174.6 s
4		87.7 s		87.7 s
5	2.72 (br d, 14.2)	51.9 d	2.59 (br d, 15.1)	51.7 d
6α	1.55 (m)	22.7 t	1.48 (m)	23.1 t
6β	1.31 (m)		1.24 (m)	
7α	1.86 (m)	18.9 t	1.63 (m)	22.8 t
7β	1.99 (overlapped)		1.91 (m)	
8	3.34 (dd, 7.9, 15.7)	43.7 d	3.30 (m)	46.9 d
9		83.8 s		80.9 s
10		96.8 s		97.1 s
11α	2.22 (br d, 19.0)	33.2 t	3.27 (AB d, 14.4)	51.4 t
11β	2.00 (overlapped)		2.93 (AB d, 14.4)	
12	4.35 (br d, 5.5)	77.3 d		206.6 s
13		53.3 s		64.4 s
14	2.46 (d, 8.2)	39.3 d	2.57 (d, 8.1)	46.9 d
15		100.6 s		100.8 s
16		102.9 s	4.00 (d, 6.3)	80.2 d
17		221.3 s		219.1 s
18	1.24 (3H, s)	23.8 q	1.38 (3H, s)	20.3 q
19α	1.92 (d, 16.2)	41.6 t	1.88 (d, 16.2)	44.0 t
19β	2.19 (d, 16.2)		2.41 (d, 16.2)	
20		76.5 s		75.3 s
21	1.48 (3H, s)	25.8 q	1.58 (3H, s)	26.4 q
22	2.98 (br d, 8.2)	41.4 d	3.22 (br d, 8.1)	42.9 d
23	4.79 (br s)	73.8 d	4.91 (br s)	73.8 d
24	4.95 (br d, 3.9)	72.3 d	5.02 (br d 5.1)	71.8 d
25	3.15 (m)	42.1 d	3.09 (m)	42.4 d
26		178.2 s		178.1 s
27	1.62 (3H, d, 7.2)	8.6 q	1.56 (3H, d, 7.1)	8.6 q
29	3.63 (d, 11.7)	67.8 t	3.57 (d, 14.5)	67.7 t
	3.75 (d, 11.7)		3.71 (d, 14.5)	
30	1.12 (3H, s)	16.7 q	1.12 (3H, s)	16.8 q

[a] Data were recorded in C_5D_5N on Bruker AM-400 MHz (^1H, ^{13}C) and Bruker DRX-500 MHz spectrometers (HSQC, COSY, HMBC, and ROESY); chemical shifts (δ) were expressed in parts per million with reference to the most downfield signal of C_5D_5N (δ 8.71 ppm) for ^1H, and to the center peak of the most downfield signal of C_5D_5N (δ 149.9 ppm) for ^{13}C, respectively.

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(13) Sphenadilactone A (**1**): colorless crystals, mp 210−211 °; $[\alpha]_D^{14.7}$ = +118.6 (*c* 0.116, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ): 211 (3.32) nm; IR (KBr) ν_{max} 3463, 2949, 2917, 1776, 1750, 1643, 1381, 1257, 1188, 1057, 598 cm^{-1}; NMR can be found in Table 1; positive ESIMS: *m/z* (rel int.) 599 (100, $[M + Na]^+$); HR-ESIMS, found 599.2118; calcd for $C_{29}H_{36}O_{12}$-Na 599.2123.

(14) Chen, G. F.; Li, Z. L.; Pan, D. J.; Tang, C. M.; He, X.; Xu, G. Y.; Chen. K.; Lee, K. H. *J. Nat. Prod.* **1993**, *56*, 1114−1122.

The three characteristic resonances appearing as an ABX spin system at δ 4.19 (d, J = 5.5 Hz), 2.65 (d, J = 22.2 Hz), and 2.85 (dd, J = 5.5, 22.2 Hz) were assigned to H-1, H-2α, and H-2β, respectively.[4,5] In addition, HMBC spectrum displayed the following correlations: the methyl signal at δ 1.12 (H-30) with C-4, C-5, and C-29; the methine doublet at δ 4.19 (H-1) with C-2, C-3, C-5, C-10, and C-19; the methine proton at δ 2.72 (H-5) with C-6 and C-7; the proton signal at δ 3.34 (H-8) with C-9 and C-19. The above evidence, along with two proton spin systems observed from the ^{1}H−^{1}H COSY spectrum, H-1/H-2, and H-5/H-6/H-7/H-8, strongly suggested that compound **1** possessed A−C rings similar to those of lancifodilactone G,[8] which led to the establishment of partial structure **1a** (Figure 1).

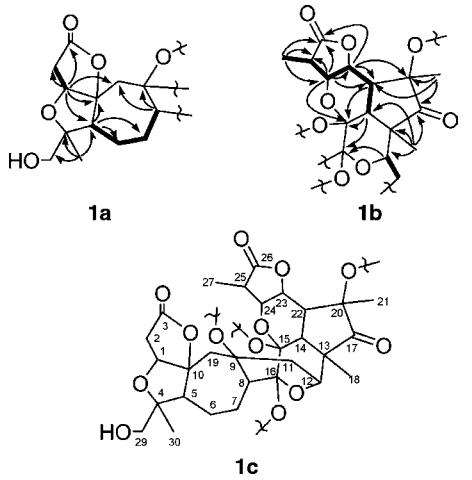

Figure 1. Fragments and key COSY (—) and HMBC (→) correlations of **1**.

Analysis of the HMBC spectrum of **1** also showed obvious correlations from CH_3-18 (δ 1.24) to C-12, C-13, C-14, and C-17, from CH_3-21 to C-17, C-20, and C-22, from CH_3-27 to C-24, C-25, and C-26, from H-24 to C-15, C-22, C-23, C-25, and C-26, from H-23 to C-26, from H-22 to C-15, from H-14 to C-15 and C-16, and from H-12 to C-16. The above observed HMBC correlations, coupled with two proton spin systems, H-11/H-12 and H-14/H-22/H-23/H-24/H-25/H-27, established by ^{1}H−^{1}H COSY correlations, gave rise to another partial structure **1b** (Figure 1).

Further HMBC correlations of H-19 to C-11 and H-8 to C-15 and C-16 established the direct linkages of C-9 with C-11 and C-8 with C-16, which permitted fragments **1a** and **1b** to be joined to get the third partial structure, **1c**.

Since C-9, C-15, C-16, and C-20 were fully substituted carbons and the 2D NMR spectra did not provide sufficient information to elucidate the pattern of connection of these carbons, further solid evidence, such as X-ray diffraction, was necessary. Fortunately, after many attempts with different solvents, a single crystal of compound **1** was finally obtained from Me_2CO−MeOH (1:2) solvent, and an X-ray crystallographic analysis was realized (Figure 2),[15] which clarified the still uncertain structural details.

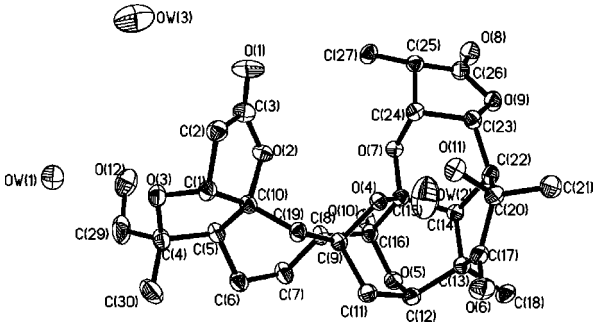

Figure 2. X-ray structure of **1** showing relative configuration.

The relative stereochemistry of **1** was also determined by X-ray analysis, together with ROESY correlations. Biogenetically, C-29 was α-oriented, while CH_3-30 was in β-orientation. The cross-peaks observed between CH_3-30/H-1, H-29/H-5, and H-5/H-8 in the ROESY spectrum demonstrated that H-1 was β-oriented, while H-5 and H-8 possessed α-orientation, respectively. In addition, according to the IUPAC sequence rule,[16] based on the chiral center with the lowest locant, the relative stereochemistry of the four quaternary carbons C-9, C-10, C-15, and C-16 was deduced as *S**, *R**, *S**, and *S** configuration, respectively.

Sphenadilactone B (**2**)[17] was isolated as colorless crystals, and the molecular formula of $C_{29}H_{36}O_{12}$ was established by HRESIMS (found $[M + Na]^+$ 599.2105, calcd 599.2104) and ^{13}C NMR spectroscopy, which was the same as that of **1**. The IR spectrum showed that hydroxyl (3453 cm^{-1}), carbonyl (1747 cm^{-1}), and γ-lactone (1778 cm^{-1}) functionalities were present in **2**. The ^{1}H NMR spectrum of **2** (Table 1) exhibited signals due to three tertiary methyls and a secondary methyl. The ^{13}C NMR spectrum showed signals for 29 carbons, and the DEPT NMR spectrum indicated the presence of two ester groups, two carbonyl groups, four methyls, six methylenes (including oxygenated one), nine

(15) Crystallographic data for **1**: $C_{29}H_{36}O_{12}$, M = 576.60, monoclinic, space group $P2_1$, a = 8.255 (1) Å, b = 18.920 (1) Å, c = 9.657 (1) Å, V = 1508.3 (5) Å^3, Z = 2, d = 1.389 g/cm^3, crystal dimensions 0.15 × 0.20 × 0.30 mm was used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator (ω-2θ scans, $2\theta_{max}$ = 50.0°), Mo Kα radiation. The total number of independent reflections measured was 2942, of which 2941 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: R_1 = 0.0582, wR_2 = 0.1591, S = 1.215, $(\Delta/\sigma)_{max}$ = 0.009, $(\Delta\rho)_{min}$ = − 0.274 e/Å^3, $(\Delta\rho)_{max}$= 0.353 e/Å^3. The crystal structure of **1** was solved by direct method SHELXS-97 (Sheldrich, G. M. University of Gottingen: Gottingen, Germany, 1997) and expanded using difference Fourier techniques, refined by the program SHELXL-97 (Sheldrich, G. M. University of Gottingen: Gottingen, Germany, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 294053). Copies of these 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).

(16) *IUPAC Nomenclature of Organic Chemistry*; Pergamon: New York, 1979, Sections A−H. Recommendation for section A, Spiro hydrocarbons.

(17) Sphenadilactone B (**2**): colorless crystals, mp 199−200 °C; $[\alpha]_D^{15.3}$ = +106.7 (c 0.146, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ): 214 (3.57) nm; IR (KBr) ν_{max} 3453, 2951, 2947, 1778, 1747 1653, 1389, 1244, 1177, 1056, 578 cm^{-1}; NMR can be found in Table 1; positive ESIMS: m/z (rel int.) 599 (100, $[M + Na]^+$); HR-ESIMS, found 599.2105; calcd for $C_{29}H_{36}O_{12}$-Na 599.2104.

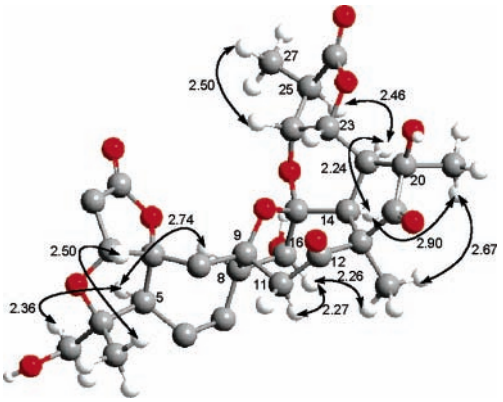

Figure 3. Key ROESY correlations of **2** and corresponding interatomic distance (Å), and some protons hidden for concision of the picture.

methines (including four oxygenated ones), and six quaternary carbons (including five oxygenated ones). The above analysis suggested that compound **2** was also a highly oxygenated nortriterpene, and gross comparison of the NMR spectra of **1** and **2** suggested that they are structurally similar (Table 1). The structure elucidation of **2** was mainly restricted to the differences with respect to **1** and analysis of 2D NMR spectral data. Detailed analysis of extensive 2D NMR spectra, including HSQC, ^{1}H−^{1}H COSY, HMBC, and ROESY spectra, showed the presence of rings A, B, C, F, G, and H. The major differences were the oxygenated methine at C-12 and the ketal carbon at C-16 in **1** replaced by a carbonyl group and an oxygenated methine, respectively. This was confirmed by HMBC correlations of CH_3-18 (δ 1.38) with the signal at δ 206.6 (C-12) and H-14 (δ 2.57) with the signal at δ 80.2 (C-16), in conjunction with the ^{1}H−^{1}H COSY spin system of H-8/H-16. These differences led to the disappearance of the oxygen bridge between C-12 and C-16. Accordingly, the planar structure was established as shown.

The relative stereochemistry of **2** was construed from the ROESY spectrum, together with 1D NMR data comparison with those of **1** (Table 1). The configuration of the hydroxyl group at C-16 was deduced to be α-oriented from the ROESY correlation of H-16/H-11α. Further ROESY correlations of H-1/CH_3-30, H-5/H-8, H-16/ CH_3-18, CH_3-18/ CH_3-21, H-14/ CH_3-21, H-22/H-25, and H-24/ CH_3-27 suggested that other chiral centers of **2** were the same as those of **1** (Figure 3).

In addition, a computer-generated 3D structure was obtained by CHEM 3D ULTRA V 8.0, with MM2 force-field calculations for energy minimization (Figure 3). The calculated interatomic distances between H-16/H-11α (2.27 Å), H-1/CH_3-30 (2.50 Å), H-5/H-8 (2.74 Å), H-16/CH_3-18 (2.26 Å), CH_3-18/CH_3-21 (2.67 Å), H-14/ CH_3-21 (2.90 Å), H-22/H-25 (2.46 Å), and H-24/ CH_3-27 (2.50 Å) are all less than 4.00 Å; this further supported the well-defined ROESY correlations observed for each of these proton pairs.

Compounds **1** and **2** were evaluated for their cytotoxicities toward three human tumor cell lines, viz. K562, A549, and HT-29, using the same bioassay method as previously described,[18] and both compounds showed no obvious inhibitory activities with IC_{50} values greater than 100 μg/mL. In addition, compound **1** was 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).[19] It showed anti-HIV-1 activity with EC_{50} of 137.0 μg/mL and exerted minimal cytotoxicity against C8166 cells (CC_{50} > 200 μg/mL). Compound **2** was not tested for further bioactivities due to its limited mass.

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Supporting Information Available: One- and two-dimensional NMR spectra and crystallographic data of sphenadilactones A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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