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Sphenalactones A–D, a new class of highly oxygenated trinortriterpenoids from *Schisandra sphenanthera*

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Abstract—Four novel highly oxygenated trinortriterpenoids, sphenalactones A–D (**1**–**4**), were isolated from the leaves and stems of *Schisandra sphenanthera* and their structures were elucidated by extensive analysis of 1D and 2D NMR data. Compounds **1**–**4** featured a C_{27} backbone and showed anti-HIV-1 activity with EC_{50} values in the range of 35.5–89.1 μg/mL with low cytotoxicity against C8166 cells ($CC_{50} > 200$ μg/mL).
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Since 2003, in the continuous search for bioactive constituents from the genus *Schisandra* plants of the Schisandraceae family, our group has phytochemically investigated more than 10 species of this genus distributed in south-west region of China. The most distinguishing result is the discovery of a series of novel nortriterpenoids with a diversity of highly oxygenated structures biogenetically related to cycloartane,[1–10] and some of them showed remarkable anti-HIV-1 activities with a low toxicity.[4,5] As a result, these discoveries brought great interests in the chemists devoted to the phytochemical investigation on plants of the Schisandraceae family,[11–16] and total synthesis of these kinds of nortriterpenoids.[17,18] For us, current interest is aimed at finding the structure interesting and bioactive triterpenoids from the genus *Schisandra*.

Schisandra sphenanthera Rehd. et Wils has long been used in traditional Chinese medicine as an important sedative and tonic agent and widely distributed in the south-west region of China. As part of the continuing investigation for more bioactive substances from the *Schisandra* species, this plant was phytochemically studied which led to the isolation of two novel nortriterpenoids, sphenadilactones A and B.[3] Reinvestigation of this plant has resulted in the isolation of four unique trinortriterpenoids, sphenalactones A–D (**1**–**4**). These compounds feature an unusual C_{27} backbone derived from cycloartane due to the loss of C-2, C-3, and CH_3-28, which is apparently the first class of naturally occurring highly oxygenated and unsaturated trinortriterpenoids from *Schisandra* species.[1–11] To our knowledge, even in the cycloartane triterpenoid family, they are the first cases, of which C-2 and C-3 degraded in their molecules. In addition, all compounds were tested for their anti-HIV activities and compounds **1** and **2** were further tested for their cytotoxicities against Hela and HepG2 cell lines. Described in this paper are isolation, structure elucidation, and biological activities of these new compounds.

The leaves and stems of *S. sphenanthera* were collected from the Maoxian county of Sichuan province, PR China, in August 2004. The specimen was identified by Professor Xi-Wen Li and a voucher specimen (no. KIB 2004-07-12) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy

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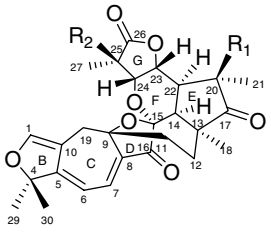
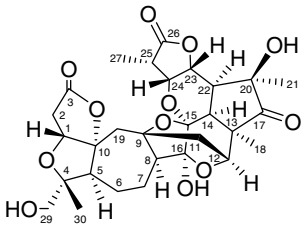
of Sciences. The air-dried and powdered stems and leaves (2.5 kg) were extracted with 70% aqueous Me_2CO (4 × 5 L) at room temperature. The crude extract (102 g) was partitioned with EtOAc and water. The EtOAc fraction (57.0 g) was subjected to repeated column chromatography to give sphenalactones A (**1**, 10.2 mg), B (**2**, 11.6 mg), C (**3**, 4.3 mg), and D (**4**, 4.1 mg).



1 R_1 = OH R_2 = H
2 R_1 = H R_2 = OH
3 R_1 = OH R_2 = OH
4 R_1 = H R_2 = H



Sphenadilactone A (**5**)

Sphenalactone A (**1**) was obtained as a light yellow powder and showed the molecular formula of $C_{27}H_{30}O_8$ as determined by analysis of ^1H, ^{13}C, and DEPT NMR spectral data, which was verified by HR-FABMS ($[M+H]^+$, found 483.2009, calcd 483.2020), requiring 13° of unsaturation. The ^1H NMR spectrum displayed signals due to four tertiary methyls and a secondary methyl. The ^{13}C NMR spectrum exhibited 27 carbon signals, which were classified by chemical shifts and HMQC spectrum as 1 ester group, 2 carbonyl groups, 8 quaternary carbons (three olefinic ones), 8 methines (two oxygenated ones and three olefinic carbons), 3 methylenes, and 5 methyls. The data suggested that **1** was a highly oxygenated trinortriterpenoid and contained seven rings.

The co-occurrence of **1** with sphenadilactone A (**5**),[3] whose relative stereochemistry was determined by X-ray crystallography, triggers us to establish the possible structure of **1** by comparing its NMR spectral data with those of **5,** and together with detailed analysis of its two-dimensional NMR spectral data.

Interpretation of HMBC data showed the following correlations: CH_3-18 with C-12, C-13, C-14, and C-17; CH_3-21 with C-17, C-20, and C-22; CH_3-27 with C-24, C-25, and C-26; H-14 with C-15 and C-16; H-22 with C-14 and C-15; and H-24 with C-15. This, along with comparison of ^1H and ^{13}C NMR data with those of **5** and two proton spin systems deduced from ^1H–^1H COSY correlations, H-14/H-22/H-23/H-24/H-25/H-27 and H-11/H-12, led to the establishment of partial structure **1a** (Fig. 1). HMBC correlations observed from two geminal methyl groups, CH_3-29 and CH_3-30, to C-4 and C-5, H-1 to C-4 and C-5, H-6 to C-4, C-8 and C-10, H-7 to C-5, C-8 and C-9, and from H_2-19 to C-1, C-5, C-8, C-9, and C-10, along with the proton spin system deduced from ^1H–^1H COSY correlations, H-6/H-7, established the partial structure **1b** (Fig. 1). Furthermore, HMBC correlations from H-7 to C-16, and from H_2-19 to C-11 required direct connections of C-8 with

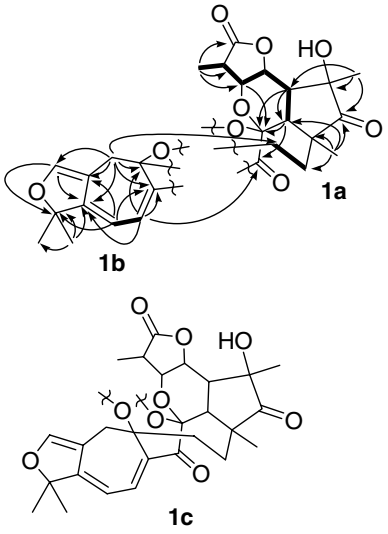


Figure 1. Fragments and selected 2D NMR correlations of **1**. The bond in bold indicates ^1H–^1H COSY, the arrow indicates HMBC.

C-16 and C-9 with C-11 and permitted fragments **1a** and **1b** to be joined to get **1c** (Fig. 1). At last, an oxygen-bridge between C-9 and C-15 was established by the molecular formula.

The relative stereochemistry of **1** was construed from the ROESY spectrum, together with the 1D NMR data compared with those of sphenadilactone A (**5**). The ROESY correlations of H-1/H-19α, H-19α/H_2-11, CH_3-18/H-14, CH_3-21/H-14, CH_3-21/H-22, and CH_3-27/H-22, H-23/H-24 suggested that all chiral centers of **1** the same as those of **5**.

Furthermore, a computer-generated 3D structure was obtained by CHEM 3D ULTRA V 8.0, with MM_2 force-field calculations for energy minimization (Fig. 2). The calculated interatomic distances between H-1/H-19α (2.616 Å), H-19α/H_2-11 (2.617 Å), CH_3-18/H-14 (2.472 Å), CH_3-21/H-14 (3.168 Å), CH_3-21/H-22 (2.506 Å), CH_3-27/H-22 (2.378 Å), and H-23/H-24 (2.500 Å) are all less than 4.00 Å; this further supported the observed ROESY correlations.

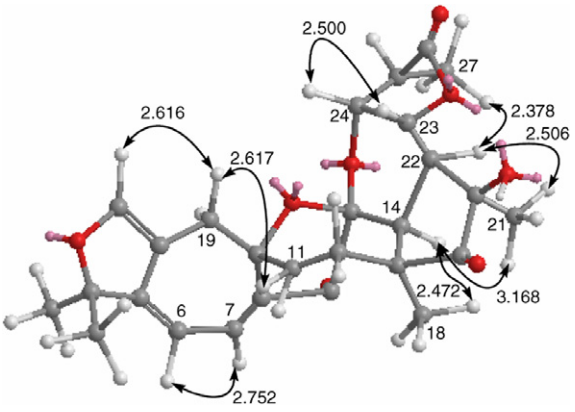


Figure 2. Key ROESY correlations of **1** and corresponding interatomic distance (Å).

Table 1. ^{1}H and ^{13}C NMR assignments of **1**–**4**[a]

No.	**1**		**2**		**3**		**4**	
	δ_H (mult., *J*, Hz)	δ_C	δ_H (mult., *J*, Hz)	δ_C	δ_H (mult., *J*, Hz)	δ_C	δ_H (mult., *J*, Hz)	δ_C
1	7.07 (s)	158.9 d	7.24 (s)	158.9 d	7.07 (s)	159.0 d	7.23 (s)	158.9 d
4		92.2 s		92.2 s		92.2 s		92.2 s
5		165.6 s		165.7 s		165.7 s		165.6 s
6	5.53 (d, 7.7)	106.2 d	5.55 (d, 7.7)	106.2 d	5.54 (d, 7.8)	106.2 d	5.53 (d, 7.7)	106.3 d
7	7.27 (d, 7.7)	129.9 d	7.29 (d, 7.7)	129.8 d	7.31 (d, 7.8)	130.0 d	7.28 (d, 7.7)	129.7 d
8		114.2 s		114.4 s		114.3 s		114.4 s
9		84.7 s		84.3 s		84.8 s		84.2 s
10		130.7 s		131.1 s		130.7 s		131.2 s
11α	1.79 (m)	34.8 t	1.78 (m)	34.9 t	1.79 (m)	34.7 t	1.80 (m)	34.9 t
11β	1.35 (overlapped)		1.43 (m)		1.43 (m)		1.34 (overlapped)	
12α	1.85 (m)	31.4 t	1.75 (m)	32.2 t	1.89 (m)	31.0 t	1.90 (m)	31.3 t
12β	1.48 (m)		1.47 (m)		1.48 (m)		1.45 (m)	
13		50.4 s		51.2 s		50.4 s		51.2 s
14	2.85 (d, 8.3)	45.4 d	2.80 (d, 8.2)	45.3 d	2.92 (d, 8.4)	45.4 d	2.75 (d, 7.0)	44.9 d
15		99.8 s		100.6 s		100.7 s		100.3 s
16		196.8 s		197.0 s		196.8 s		197.2 s
17		219.9 s		220.3 s		220.0 s		220.4 s
18	1.00 (3H, s)	27.4 q	0.96 (3H, s)	26.8 q	1.04 (3H, s)	26.7 q	0.94 (3H, s)	26.8 q
19α	2.44 (AB d, 14.1)	35.2 t	2.51 (AB d, 14.0)	35.3 t	2.48 (AB d, 14.1)	35.3 t	2.48 (AB d, 13.9)	35.3 t
19β	2.73 (AB d, 14.1)		2.86 (AB d, 14.0)		2.73 (AB d, 14.1)		2.86 (AB d, 13.9)	
20		74.6 s	2.81 (m)	44.8 d		76.7 s	2.88 (m)	45.5 d
21	1.60 (3H, s)	24.6 q	1.27 (3H, d, 6.9)	14.3 q	1.62 (3H, s)	23.0 q	1.29 (3H, d, 6.9)	14.6 q
22	3.18 (d, 8.3)	42.0 d	2.95 (dd, 8.2, 11.0)	40.3 d	3.26 (br d, 8.4)	45.4 d	2.89 (overlapped)	40.3 d
23	5.00 (br s)	73.7 d	5.32 (br s)	74.8 d	4.93 (br s)	75.8 d	4.67 (br s)	75.2 d
24	4.87 (br s)	71.6 d	4.74 (br s)	72.5 d	5.72 (br s)	73.4 d	4.70 (br s)	68.3 d
25	3.16 (m)	42.4 d		76.9 s		74.7 s	3.10 (m)	42.5 d
26		177.8 s		177.3 s		177.3 s		177.9 s
27	1.32 (3H,d, 7.2)	8.4 q	1.80 (3H, s)	18.2 q	1.81 (3H, s)	18.2 q	1.31 (3H,d, 7.2)	8.4 q
29[b]	1.30 (3H, s)	26.7 q	1.33 (3H, s)	26.8 q	1.30 (3H, s)	27.4 q	1.30 (3H, s)	26.8 q
30[b]	1.28 (3H, s)	27.8 q	1.30 (3H, s)	27.4 q	1.28 (3H, s)	27.8 q	1.27 (3H, s)	27.4 q

[a] Data were recorded in C_5D_5N on Bruker AM-125 MHz (^{13}C) and Bruker DRX-500 MHz spectrometers (^{1}H); chemical shifts (δ) are in ppm.
[b] ^{1}H and ^{13}C NMR data of C-29 and C-30 are exchangeable.

Sphenalactone B (**2**) was obtained as a yellow powder and the molecular formula of $C_{27}H_{30}O_8$ was established by HR-FABMS ($[M+H]^+$, found 483.2011, calcd 483.2019) and ^{13}C NMR spectroscopy, which was the same as that of **1**. The ^{1}H NMR spectrum of **2** (Table 1) exhibited signals due to four tertiary methyls and a secondary methyl. The ^{13}C NMR spectrum showed signals for 27 carbons. The above analysis hints that compound **2** was an analog of **1**. Gross comparison of the NMR spectra of **1** and **2** suggested that they are structurally similar and the major difference was a hydroxy group located at C-25 in **2** rather than located at C-20 in **1**. This was confirmed by HMBC correlations of CH_3-21 (δ_H 1.27) with C-17 (δ_C 220.3), C-20 (δ_C 44.8), and C-22 (δ_C 40.3) and CH_3-27 (δ_H 1.80) with C-24 (δ_C 72.5), C-25 (δ_C 76.9), and C-26 (δ_C 177.3). The ROESY correlations of CH_3-21 with H-14, and CH_3-27 with H-22 determined that both CH_3-21 and CH_3-27 are α-orientated. Thus, the structure of **2** was established as shown.

Sphenalactone C (**3**) was assigned the molecular formula, $C_{27}H_{30}O_9$, as deduced from HR-FABMS data ($[M+H]^+$, found 499.2005, calcd 499.2012). Comparisons of the spectroscopic data of **3** with those of **1** revealed that the difference was a methine at C-25 in **1** was replaced by an oxygenated quaternary carbon for an additional hydroxy group located at C-25 in **3**. This was confirmed by HMBC correlations from CH_3-27 (δ_H 1.81) to C-24 (δ_C 73.4), C-25 (δ_C 74.7), and C-26 (δ_C 177.3). In addition, the hydroxy group (OH-25) was deduced to be β-orientated by the ROESY correlation of CH_3-27 with H-22.

Sphenalactone D (**4**) was assigned the molecular formula, $C_{27}H_{30}O_7$, on the basis of its HR-FABMS ($[M+H]^+$, found 467.1887, calcd 467.1892) and NMR spectral data. Comparison of the spectroscopic data of **4** with those of **1** (Table 1) showed similarities except that an oxygenated quaternary carbon at C-20 in **1** was replaced by a methine group (δ_C 45.5) in **4**. HMBC correlation of CH_3-21 (δ_H1.29) with C-17 (δ_C 220.4), C-20 (δ_C 45.5) and C-22 (δ_C 40.3) further confirmed this deduction. In addition, the ROESY correlation of CH_3-21 with H-14 determined CH_3-21 to be α-orientated. A further examination of its extensive 2D NMR data leads to the full assignment of **4**.

Compounds **1**–**4** were tested for cytotoxicity assay against C8166 cells (CC_{50}), and the 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] All exerted minimal cytotoxicity against C8166 cells ($CC_{50} > 200$ μg/mL) and showed anti-HIV-1 activity with EC_{50} of 89.1, 74.1, 52.5, 35.5 μg/mL, respectively.

In addition, compounds **1** and **2** were further evaluated for their cytotoxicities toward Hela and HepG2 cell lines, using the same bioassay method as previously described,[20] and both compounds showed no obvious inhibitory activities with IC_{50} values more than 100 μg/mL. Compounds **3** and **4** did not evaluate for cytotoxicities due to their limited mass.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.05.152.

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