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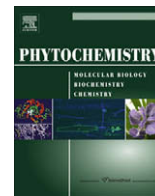


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Nortriterpenoids and lignans from *Schisandra sphenanthera*

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ABSTRACT

Nortriterpenoids, spenadilactone C (**1**) and spenasin A (**2**), together with four known lignans (**3–6**), were isolated from the leaves and stems of *Schisandra sphenanthera*. Their structures were elucidated by extensive analysis of 1D and 2D NMR spectroscopic data and compound **2** was further confirmed by single-crystal X-ray diffraction. Compound **1** features a partial enol moiety and an acetamide group in its structure. In addition, compounds **1**, **3–6** showed weak anti-HIV-1 activity with EC₅₀ values in the range of 15.5–29.5 µg/mL.

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1. Introduction

Genus *Schisandra* species are known to contain dibenzocyclooctadiene lignans, lanostane and cycloartane triterpenes, some of which possess antihepatitis, antitumor and anti-HIV activities (Chen et al., 1998, 1999, 2001; Hancke et al., 1999; Kuo et al., 1997; Sun et al., 1996). In 2003, our group discovered a highly oxidized nortriterpenoid, micrandilactone A, from the stems and leaves of *Schisandra micrantha* (Li et al., 2003a). Since then, our group have given considerable attention to *Schisandra* genus species and phytochemically investigated more than 10 species of them collected from 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 (Li et al., 2003a,b, 2004a,b, 2005a,b; Xiao et al., 2005a,b, 2006a,b,c,d, 2007; Huang et al., 2007a,b), some of which exhibited anti-HIV-1 activities (Li et al., 2005a; Xiao et al., 2006c). A phytochemical study of *Schisandra sphenanthera* Rehd. et Wils led to the isolation of six novel nortriterpenoids, spenadilactones A–B and spenalactones A–D (Xiao et al., 2006b, 2007). Reinvestigation of this plant resulted in the isolation of two new minor nortriterpenoids, spenadilactone C (**1**) and spenasin A (**2**), together with four known lignans, schizandrin (**3**) (Ikeya et al., 1988), gomisins J (**4**) (Ikeya et al., 1980), schisantherin A (**5**)

(Ikeya et al., 1990), and schisantherin D (**6**) (Ikeya et al., 1982) (Fig. 1). Compound **1**, characterized by the presence of a partial enol structure, is the first example of acetamide-bearing highly oxygenated nortriterpenoid from cycloartane in nature, compound **2**, by contrast, features a biosynthetically modified seven-membered lactone ring and the loss of C-25, C-26 and C-27. In addition, compounds **1**, **3–6** were tested for their anti-HIV-1 activity and compound **1** was further tested its cytotoxicity against Hela and HepG2 cell lines. However, all compounds showed no obvious bioactivities. Described in this paper are isolation, structure elucidation, and biological activity of these compounds.

2. Results and discussion

Spenadilactone C (**1**) was isolated as an amorphous white powder with $[\alpha]_D^{25} = +59.1$ (c 0.26, CH₃OH). Its molecular formula was determined as C₃₁H₃₉NO₁₂ by HR-ESIMS ([M+Na]⁺, found 640.2382, calcd. 640.2369), indicating 13 degrees of unsaturation. Its IR spectrum showed absorptions at 3441 and 1776 cm⁻¹, indicating the presence of hydroxyl and γ-lactone groups (Li et al., 2003a). The ¹H NMR spectrum displayed signals due to six tertiary methyls. The ¹³C NMR spectrum exhibited 31 carbon resonances, which were assigned by chemical shifts and HMQC spectrum as three ester or amide groups, one carbonyl group, nine quaternary carbons (two olefinic ones), seven methines (three oxygenated ones), five methylenes, and six methyls. The data suggested that **1** may be a highly oxygenated triterpenoid that contained eight rings.

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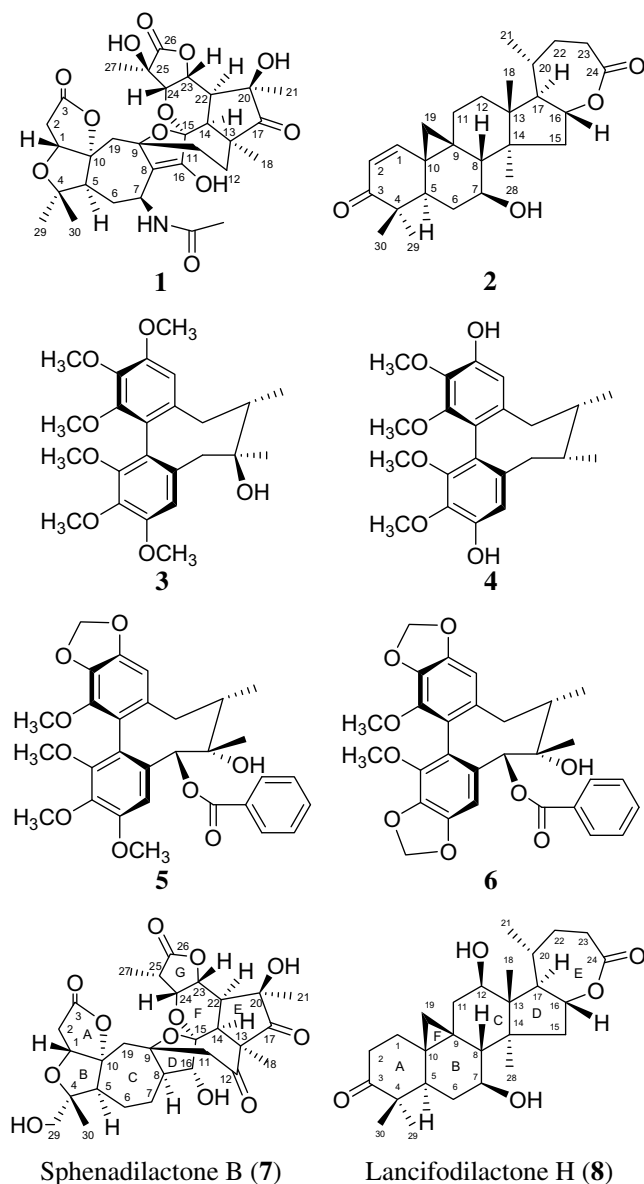


Fig. 1. The structures of compounds 1–8.

The three characteristic resonances appearing as an ABX spin system at δ_{H} 4.00 (*d*, $J = 4.8$ Hz), 2.55 (*d*, $J = 18.0$ Hz) and 2.65 (*dd*, $J = 4.8, 18.0$ Hz) were assigned to H-1, H-2 α and H-2 β , respectively (Li et al., 2004a,b). The HMBC spectrum displayed the following correlations: two geminal methyl groups, CH₃-29 and CH₃-30, with C-4 and C-5; a methine doublet at δ_{H} 4.00 (H-1) with C-2, C-3, C-4, C-5, C-10 and C-19; a methine proton at δ_{H} 4.92 (H-7) with C-5, C-6, C-8 and C-9; isolated methylene protons at δ_{H} 1.97 and 2.09 (H₂-19) with C-5, C-8 and C-9. The presence of an acetamide group at C-7 was evident from the characteristic ^1H and ^{13}C spectroscopic data (Table 1) and HMBC correlations of H-7 with a carbonyl carbon (δ_{C} 173.3), and NH (δ_{H} 9.79) with C-6 and C-7. The above evidence, along with two proton spin systems observed from ^1H - ^1H COSY spectrum, H-1/H-2 and H-5/H-6/H-7/NH, strongly suggested that compound 1 possessed similar rings A–C with those of sphenadilactone B (7) (Xiao et al., 2006b), which led to the establishment of partial structure 1a (Fig. 2).

Analysis of the HMBC spectrum of 1 also showed obvious correlations from CH₃-18 (δ_{H} 1.06) to C-12, C-13, C-14, and C-17, from CH₃-21 to C-17, C-20 and C-22, from CH₃-27 to C-24, C-25 and

Table 1

 ^1H and ^{13}C NMR spectroscopic assignments of 1 and 2^a

Position	1		2	
	δ_{H} (mult., <i>J</i> , Hz)	δ_{C} (mult.)	δ_{H} (mult., <i>J</i> , Hz)	δ_{C} (mult.)
1	4.00 (<i>d</i> , 4.8)	80.4 <i>d</i>	6.79 (<i>d</i> , 10.1)	153.5 <i>d</i>
2 α	2.55 (<i>d</i> , 18.0)	35.4 <i>t</i>	6.16 (<i>d</i> , 10.1)	127.2 <i>d</i>
2 β	2.65 (<i>dd</i> , 4.8, 18.0)			
3		174.8 <i>s</i>		203.9 <i>s</i>
4		84.1 <i>s</i>		45.9 <i>s</i>
5	2.10 (overlapped)	55.7 <i>d</i>	2.27 (<i>dd</i> , 3.5, 13.2)	43.2 <i>d</i>
6 α	1.76 (<i>m</i>)	28.2 <i>t</i>	1.44 (overlapped)	30.9 <i>t</i>
6 β	1.76 (<i>m</i>)		2.00 (<i>m</i>)	
7	4.92 (<i>m</i>)	45.2 <i>d</i>	3.87 (<i>m</i>)	68.3 <i>d</i>
8		108.8 <i>s</i>	2.17 (<i>d</i> , 6.4)	52.5 <i>d</i>
9		88.2 <i>s</i>		24.9 <i>s</i>
10		95.7 <i>s</i>		30.3 <i>s</i>
11 α	2.08 (overlapped)	39.7 <i>t</i>	1.77 (<i>m</i>)	27.5 <i>t</i>
11 β	1.74 (overlapped)		1.58 (overlapped)	
12 α	2.08 (overlapped)	31.5 <i>t</i>	1.44 (overlapped)	31.7 <i>t</i>
12 β	1.95 (<i>m</i>)		1.57 (overlapped)	
13		50.5 <i>s</i>		47.9 <i>s</i>
14	2.91 (<i>d</i> , 8.4)	44.9 <i>d</i>		47.4 <i>s</i>
15 α		103.2 <i>s</i>	2.56 (<i>dd</i> , 7.7, 12.7)	44.0 <i>t</i>
15 β			2.14 (<i>br d</i> , 12.7)	
16		153.9 <i>s</i>	4.72 (<i>m</i>)	83.7 <i>d</i>
17		220.6 <i>s</i>	1.66 (<i>br d</i> , 6.4)	59.6 <i>d</i>
18	1.06 (3H, <i>s</i>)	28.1 <i>q</i>	0.99 (<i>s</i>)	17.4 <i>q</i>
19 α	1.97 (AB <i>d</i> , 12.9)	41.8 <i>t</i>	0.67 (<i>d</i> , 4.7)	28.4 <i>t</i>
19 β	2.09 (AB <i>d</i> , 12.9)		1.44 (<i>d</i> , 4.7)	
20		74.8 <i>s</i>	1.60 (overlapped)	35.9 <i>d</i>
21	1.55 (<i>s</i>)	24.2 <i>q</i>	0.84 (<i>d</i> , 6.3)	21.9 <i>q</i>
22 α	3.19 (<i>d</i> , 8.4)	42.5 <i>d</i>	1.34 (<i>m</i>)	33.8 <i>t</i>
22 β			1.68 (<i>m</i>)	
23 α	5.79 (<i>br s</i>)	73.6 <i>d</i>	2.60 (<i>m</i>)	34.4 <i>t</i>
23 β			2.79 (<i>m</i>)	
24	4.80 (<i>br s</i>)	75.6 <i>d</i>		175.2 <i>s</i>
25		77.3 <i>s</i>		
26		177.9 <i>s</i>		
27	2.25 (3H, <i>s</i>)	18.4 <i>q</i>		
28			1.14 (<i>s</i>)	19.5 <i>q</i>
29	0.91 (3H, <i>s</i>)	20.7 <i>q</i>	1.21 (<i>s</i>)	21.9 <i>q</i>
30	1.20 (3H, <i>s</i>)	27.9 <i>q</i>	0.99 (<i>s</i>)	19.3 <i>q</i>
Acetamide		173.3 <i>s</i>	7-OH: 6.02 (<i>d</i> , 4.8)	
NH	9.79 (<i>d</i> , 5.7)	22.1 <i>q</i>		

^a Data were recorded in C₅D₅N on Bruker AM-500 MHz; chemical shifts (δ) are expressed in ppm with reference to the signal at the highest frequency of C₅D₅N (δ 8.71 ppm) for ^1H and to the center peak of the most downfield signal of C₅D₅N (δ 149.9 ppm) for ^{13}C , respectively.

C-26, from H-24 to C-15, from H-23 to C-26, from H-22 to C-14 and C-15, from H-14 to C-15 and C-16. From the above observed HMBC correlations, coupled with the comparison of ^1H and ^{13}C NMR spectroscopic data of 1 with those of sphenadilactone B (7) and ^1H - ^1H COSY correlations, H-11/H-12 and H-14/H-22/H-23/H-24, another partial structure 1b was deduced (Fig. 2). Further HMBC correlations of H₂-19 to C-11 and H-7 to C-16 established the presence of direct linkages of C-9 with C-11 and C-8 with C-16, which permitted fragments 1a and 1b join to get the third partial structure 1c.

According to the molecular formula deduced by HR-ESIMS, another ring should exist in its structure. As shown in structure 1c, this ring can only be formed through three ways, i.e., by the oxygenated-bridge connection of C-15 with C-16, or C-9 with C-15 or C-16. Aided by a Dreiding molecular model analysis, we found that this ring could only form through the oxygenated-bridge connection of C-9 with C-15 for the rigid ring system. Accordingly, an enol moiety was established at C-8 and C-16, which was similar with that of lancifodilactone G (Xiao et al., 2005b). The presence of an enol moiety was consistent with the chemical shift of C-8 (δ_{C} 108.8) and C-16 (δ_{C} 153.9). Thus, compound 1 was established as shown in Fig. 1.

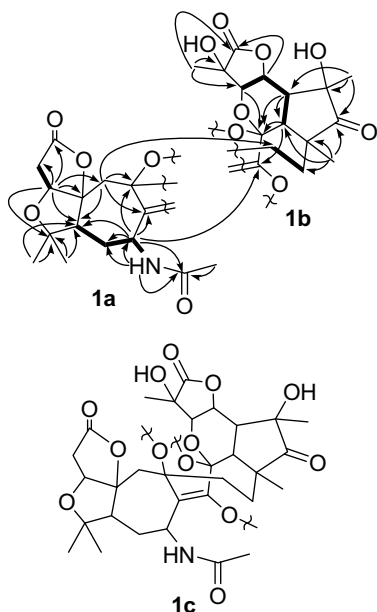


Fig. 2. Fragments and key COSY (---), and HMBC (—) correlations of **1**.

The relative stereochemistry of **1** was determined by ROESY correlations, together with a 1D NMR spectroscopic data comparison with that of spenadilactone B (**7**). The configuration of the acetamide group at C-7 was deduced to be β -oriented from the ROESY correlation of H-5/H-7. The β -orientation of the hydroxyl group at C-25 was determined by the ROESY correlation of CH₃-27/H-22. Further ROESY correlations of H-19 α /H₂-11, CH₃-18/H-14, CH₃-18/CH₃-21, CH₃-18/H-22, H-14/H-22, CH₃-21/H-22, H-23/H-24 suggested that other chiral centers in **1** were the same as those of **7**.

In addition, a computer-generated 3D structure was obtained by CHEM 3D ULTRA V 8.0, with MM₂ force-field calculations for energy minimization (Xiao et al., 2006b). The calculated interatomic distances between H-5/H-7 (2.394 Å), CH₃-27/H-22 (2.340 Å), H-19 α /H₂-11 (2.306 Å), CH₃-18/H-14 (2.449 Å), CH₃-18/CH₃-21 (2.375 Å), CH₃-18/H-22 (2.974 Å), H-14/H-22 (2.234 Å), CH₃-21/H-22 (2.461 Å), H-23/H-24 (2.296 Å) are all less than 4.00 Å (Fig. 3). This further supported the well-defined ROESY correlations observed for each of these proton pairs.

Sphenasin A (**2**) gave a quasi-molecular ion peak at m/z 449 [M+Na]⁺ in its positive ESIMS spectrum and was assigned a molecular formula of C₂₇H₃₈O₄, which was confirmed by HRESIMS (found [M+Na]⁺ 449.2231, calcd 449.2238) and NMR spectroscopic data (Table 1). Obvious in the ¹H NMR spectrum were five methyl signals due to four tertiary methyls and a secondary methyl. The ¹³C NMR and DEPT spectra of **2** exhibited signals for 27 carbons. Careful investigation of the ¹H and ¹³C NMR spectroscopic data of **2** established that it was quite similar to that reported for lancifodilactone H (**8**) (Xiao et al., 2006c), which was isolated from *Schisandra lancifolia*. Analysis of HMBC and ¹H-¹H COSY spectra of **2** showed that the two compounds have same carbon backbone and ring system (Fig. 4). The differences between **2** and **8** were two olefinic carbons signals assigned to C-1 and C-2 appeared and C-12 was not oxidized in **2**. This was confirmed by the HMBC correlations of H-1 (δ_H 6.79, d, J = 10.1 Hz) and H-2 (δ_H 6.16, d, J = 10.1 Hz) with C-3 (δ_C 203.9) and H-18 (δ_H 0.99, s) with C-12 (δ_C 31.7). In addition, the HMBC correlation of H-16 (δ_H 4.72) with C-24 (δ_C 175.2) suggested that there is a seven-membered lactone ring in **2**. The relative stereochemistry of **2** was deduced by ROESY correlations and finally determined by X-ray diffraction experiment (Fig. 5).

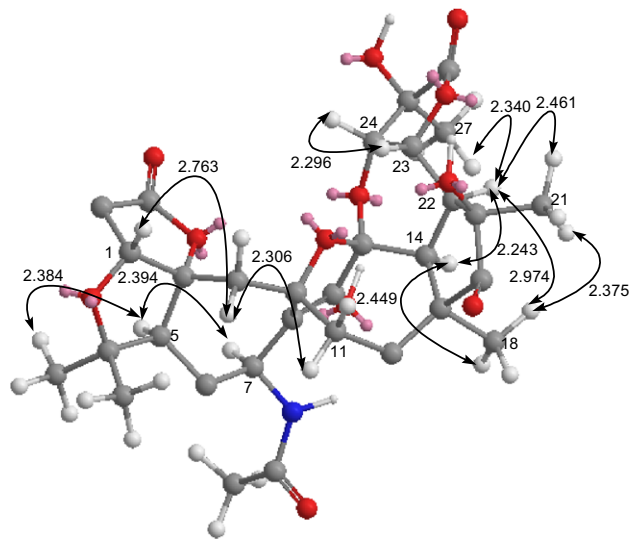


Fig. 3. Key ROESY correlations of **1** and corresponding interatomic distance (Å); some protons are hidden for conciseness.

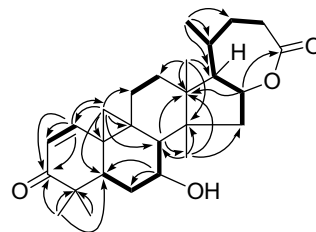


Fig. 4. Key COSY (---), and HMBC (—) correlations of **2**.

Since some nortriterpenoids isolated from *Schisandra* genus are reported to possess anti-HIV activities (Li et al., 2005a; Xiao et al., 2006c), compounds **1**, **3–6** were tested for cytotoxicity assay against C8166 cells (CC₅₀), and anti-HIV-1 activity evaluated by the inhibition assay for the cytopathic effects of HIV-1_{IIIB} (EC₅₀), using AZT as a positive control (EC₅₀ = 0.0034 μ g/mL and CC₅₀ > 200 μ g/mL) (Wang et al., 2004). Compounds **1**, **3–6** showed weak anti-HIV-1 activity with EC₅₀ values of 29.5, 15.5, 17.2, 19.0 and 20.5 μ g/mL, therapeutic index values (CC₅₀/EC₅₀) of 6.68, 8.78, 9.25, 10.1 and 8.56, respectively. In addition, compound **1** was further evaluated for its cytotoxicity against Hela and HepG2 cell lines, using the same bioassay method as previously described

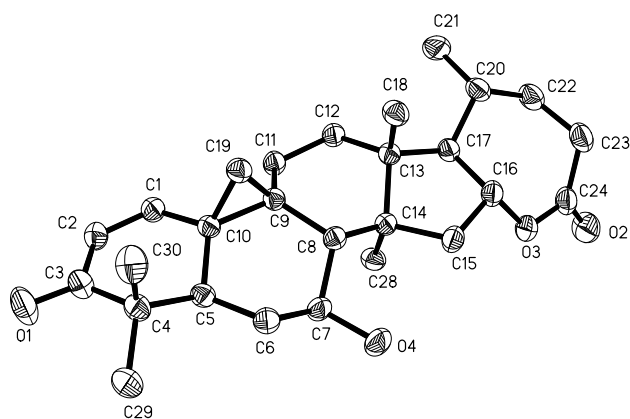


Fig. 5. X-ray structure of **2** showing the relative configuration.

(Monks et al., 1991), but showed no obvious inhibitory activities with IC_{50} values more than 100 $\mu\text{g/mL}$. Compound **2** was not tested for bioactivities due to its limited mass.

3. Conclusions

Two new minor nortriterpenoids, together with four known lignans, were isolated from the leaves and stems of *S. spheanthra*. This was the first report of an acetamide-bearing highly oxygenated nortriterpenoid, spenadilactone C (**1**), from *Schisandra* genus. Additionally, another new nortriterpenoid, named spenasin A (**2**), featured a biosynthetically modified seven-membered lactone ring missing C-25, C-26 and C-27 in its molecule. This discovery expands the library of natural occurring nortriterpenoids from the *Schisandra* genus. In the anti-HIV-1 activity assays, all tested compounds showed weak activity with therapeutic index values >6.5 .

4. Experimental

4.1. General experimental 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 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 (CC) 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-C₁₈, 9.4 mm \times 25 cm, column. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H₂SO₄ in EtOH.

4.2. Plant material

The leaves and stems of *S. spheanthra* were collected in August 2006 from Maoxian county of Sichuan province, PR China. The specimen was identified by Prof. Xi-Wen Li. A voucher specimen, No. KIB 2006-08-12, has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

4.3. Extraction and isolation

Air-dried and powdered stems and leaves (4.5 kg) were extracted with Me₂CO–H₂O (7:3, v/v, 4 \times 5 L) at room temperature and concentrated *in vacuo* to give a crude extract (192.0 g), which was partitioned between H₂O and EtOAc. The EtOAc part (97.0 g) was subjected to silica gel CC eluting with CHCl₃–CH₃OH (1:0, 9:1, 8:2, 2:1 and 0:1) to afford fractions I–V. Fraction II (20.4 g) was applied to a silica gel column eluted with petroleum ether–EtOAc (9:1, 8:2, 2:1 and 0:1) to afford four fractions. Fraction II-2 (2.3 g) was further subjected to Sephadex LH-20 CC (CH₃OH) to give nine fractions. Then, Fraction II-2-3 (70 mg) yielded compound **1** (7.3 mg) by semi-preparative HPLC (CH₃OH:H₂O, 42:58) and fraction II-2-4 (40 mg) yielded compound **2** (3.1 mg) by semi-preparative HPLC (CH₃OH:CH₃CN:H₂O, 10:35:55). Fraction III (17.5 g) was chromatographed on silica gel eluting with petroleum ether–(CH₃)₂CO (9:1, 8:2, 3:1, 2:1 and 0:1) to afford five fractions. Then, fraction III-3 (1.5 g) was applied to a Sephadex LH-20 CC (CH₃OH) to give seven fractions. Finally, fraction III-3-4 (100 mg) afforded compounds **3**

(15.0 mg) and **4** (25.0 mg) by semi-preparative HPLC (CH₃OH:H₂O, 40:60) and fraction III-3-6 (40 mg) yielded compounds **5** (6.1 mg) and **6** (7.8 mg) by semi-preparative HPLC (CH₃OH:H₂O, 45:55).

4.3.1. Sphenadilactone C (**1**)

White powder; $[\alpha]_D^{25} = +59.1$ (c 0.26, CH₃OH); UV (CH₃OH): λ_{max} (log ϵ) 210 (3.05) nm; IR (KBr): ν_{max} 3441, 2925, 2854, 1776, 1745, 1630, 1377, 1187, 1110 and 1012 cm^{−1}; for ¹H and ¹³C NMR spectroscopic data, see Table 1; positive ESIMS: $m/z = 640$ [M+Na]⁺; HR-ESIMS: $m/z = 640.2382$ [M+Na]⁺ (calcd. 640.2369 for C₃₁H₃₉NO₁₂Na).

4.3.2. Sphenasin A (**2**)

White prism; mp 198–199 °C; $[\alpha]_D^{26} = 5.1$ (c 0.19, CH₃OH); UV (CH₃OH) λ_{max} (log ϵ) 207 (3.14) nm; IR (KBr) ν_{max} 3443, 2935, 2917, 1728, 1689, 1633, 1442, 1373, 1287, 1025, 1012 and 575 cm^{−1}; for ¹H and ¹³C NMR spectroscopic data, see Table 1; positive ESIMS: $m/z = 449$ [M+Na]⁺; HR-ESIMS: $m/z = 449.2231$ [M+Na]⁺ (calcd. 449.2238 for C₂₇H₃₈O₄Na).

4.4. X-ray crystallographic analysis of **2**

Crystal data of **2**: C₂₇H₃₈O₄, $M = 426.6$, monoclinic, space group $P2_12_12_1$, $a = 7.974(1)$ Å, $b = 11.730(1)$ Å, $c = 24.646(1)$ Å, $V = 2305.3(4)$ Å³, $Z = 4$, $d = 1.229$ g/cm³, crystal dimensions 0.30 \times 0.50 \times 0.70 mm, measured on a MAC DIP-2030K diffractometer with a graphite monochromator (ω scans, $2\theta_{\text{max}} = 50.0^\circ$), Mo K α radiation. The total number of independent reflections measured was 2395, of which 2292 were observed ($|F|^2 \geq 3\sigma|F|^2$). Final indices: $R_1 = 0.0393$, $wR_2 = 0.1109$ ($W = 1/\sigma|F|^2$), $S = 1.005$. The crystal structure (**2**) was solved by direct methods using SHELX-86 (Sheldrich, 1997) and expanded using difference Fourier techniques, refined by the program and method NOMCSDP (Lu and Wu, 1992) and full-matrix least-squares calculations. Crystallographic data for the structure of **2** has been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 649266). Copies of those 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].

4.5. Bioactivity assay

The cytotoxicity assay against C8166 cells (CC₅₀) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC₅₀) (Wang et al., 2004). The cytotoxicity against Hela and HepG2 cell lines was tested using the same bioassay method as previously described (Monks et al., 1991).

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Appendix A. Supplementary material

Supplementary data (one- and two-dimensional NMR spectra and crystallographic data of spenadilactone C (**1**) and spenasin

A (2)) associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2008.09.010.

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