

Lancifodilactone F: A Novel Nortriterpenoid Possessing a Unique Skeleton from *Schisandra lancifolia* and Its Anti-HIV Activity

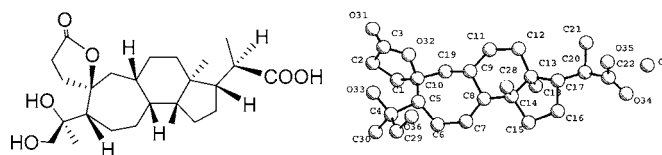
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ABSTRACT



Lancifodilactone F (1)

Lancifodilactone F (1), possessing an unprecedented rearranged pentanortriterpenoid backbone derived from cycloartane, was isolated from the leaves and stems of *Schisandra lancifolia* (Rehd. et Wils) A. C. Smith. Its structure was established by comprehensive NMR and MS spectroscopic analysis, coupled with single-crystal X-ray experiment. Compound 1 exerted minimal cytotoxicity against C8166 cells ($CC_{50} > 200 \mu\text{g/mL}$) and showed anti-HIV activity with $EC_{50} = 20.69 \pm 3.31 \mu\text{g/mL}$ and a selectivity index > 6.62 .

Plants of the genus *Schisandra*, belonging to the economically and medicinally important family Schisandraceae, are known to be a rich source of lignans possessing some beneficial pharmacological effects such as antihepatitis, antitumor, and anti-HIV activities.^{1–3} Recently, some tri-

terpenoids isolated from this genus exhibited anti-HIV activities^{4,5} and inhibitory activities toward cholesterol biosynthesis.^{6–9}

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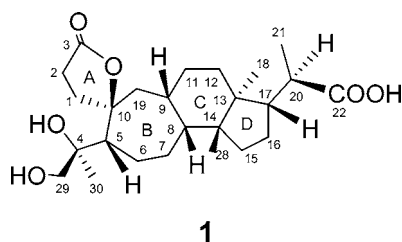
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In our recent phytochemical research on this genus, we reported the isolation and structure elucidation of several highly oxygenated nortriterpenoids with a new skeleton, including micrandilactone A,¹⁰ lancifodilactones A–E,^{11,12} and henridilactones A–D.¹³ Aiming to find potentially bioactive secondary metabolites from this genus, we reinvestigated the leaves and stems of *Schisandra lancifolia* (Rehd. et Wils) A. C. Smith, and a novel triterpene derivative, lancifodilactone F (**1**), was isolated, which featured an unprecedented rearranged pentanortriterpenoid backbone derived from cycloartane. In addition, compound **1** was tested for its cytotoxic and anti-HIV-1 activities. Described in this paper are the structure elucidation and biological activity of compound **1**.



The leaves and stems of *Schisandra lancifolia* were collected in Dali Prefecture of Yunnan Province, China, in August of 2002 and identified by Prof. Su-Gong Wu. The air-dried and powdered stems and leaves (5.7 kg) were extracted with 70% aqueous Me₂CO (4 × 15 L) at room temperature and concentrated in vacuo to give a crude extract (290 g), which was partitioned between H₂O and EtOAc. The EtOAc fraction (101 g) was repeatedly chromatographed on silica gel column. Further purification with preparative HPLC (Agilent 1100 HPLC system; Zorbax SB-C-18, Agilent, 9.4 mm × 25 cm; MeOH–H₂O 40:60) yielded lancifodilactone F (**1**, 25.2 mg).

Lancifodilactone F (**1**)¹⁴ was obtained as a colorless and optically active crystal. Its molecular formula of C₂₅H₄₀O₆ was established on the basis of negative ESIMS, ¹³C NMR, and DEPT NMR spectra and confirmed by HR-ESIMS (found 435.2761, calcd 435.2746). Thus, the structure of **1** possessed six degrees of unsaturation. The ¹H NMR spectrum (Table 1) exhibited four tertiary methyls. The ¹³C and DEPT NMR spectra displayed 25 carbons, including 1 ester group, 1 carboxylic acid group, 4 quaternary carbons (including 2 oxygenated ones), 5 methines, 10 methylenes (including an oxygenated one), and 4 methyls. Considering the fact that

Table 1. ¹H and ¹³C NMR Assignments and Two-Dimensional NMR Correlations of **1**^a

position	δ _H (mult, <i>J</i> , Hz)	δ _C (mult)	HMBC (H–C)	NOESY (H–H)
1α	2.76 (overlap)	30.4 (t)	2, 3, 10	1β
1β	2.84 (m)		2, 3, 10	2β, 29, 30
2α	1.83 (m)	30.1 (t)	1, 3, 10	1α, 2β
2β	3.29 (m)		1, 3, 10	1β, 2α, 5, 29, 30
3		177.6 (s)		
4		76.0 (s)		
5	2.54 (br d, 7.8)	51.0 (d)	4, 6, 7, 10, 29, 30	7, 6β, 8, 9, 29, 30
6α	1.31 (overlap)	25.7 (t)	7, 8	19α
6β	1.78 (m)		4, 7, 8, 10	5, 29
7	1.97 (2H, m)	31.9 (t)		
8	1.46 (overlap)	48.2 (d)	9, 28	5, 28
9	1.38 (overlap)	31.5 (d)	8, 11, 12	5, 19β
10		92.1 (s)		
11	1.67 (2H, overlap)	33.0 (t)	13, 19	28
12	1.43 (2H, m)	33.1 (t)	11, 18	
13		45.8 (s)		
14		49.1 (s)		
15α	1.28 (overlap)	33.8 (t)	13, 16	18
15β	1.16 (overlap)		13, 17, 28	15α, 16β, 28
16α	1.66 (overlap)	26.7 (t)		16β
16β	2.14 (m)		17	15β, 16α, 28
17	2.30 (m)	48.6 (d)	13, 18, 20	21, 28
18	0.85 (3H, s)	15.2 (q)	12, 13, 14, 17	15α, 20, 21
19α	1.70 (d, 15.4)	52.4 (t)	8, 9, 10	6α, 19β
19β	1.92 (d, 15.4)		8, 9	9, 15α
20	2.73 (m)	43.9 (d)	17, 21, 22	18, 21
21	1.34 (3H, d, 6.8)	17.7 (q)	17, 20, 22	17, 18, 20
22		179.3 (s)		
28	0.77 (3H, s)	16.7 (q)	13, 14, 15	8, 11, 15β, 16β, 17
29	3.75 (d, 10.8)	70.1 (t)	4, 5, 30	1β, 2β, 5, 6β, 30
	3.90 (d, 10.8)			
30	1.49 (3H, s)	22.7 (q)	4, 5, 29	2β, 5, 29

^a Data were recorded in C₅D₅N on Bruker AM-400 MHz (¹H, ¹³C) and Bruker DRX-500 MHz spectrometers (COSY, HMBC, NOESY); chemical shifts (δ) are expressed in parts per million with reference to the most downfield signal 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 ¹³C, respectively.

the chemical constituents isolated from the genus *Schisandra* mainly belong to lignans and triterpenes,^{1–9} it is reasonable to presume that **1** was derived from triterpene. Since the NMR spectra of **1** were quite distinctive from those of the known triterpene skeleton, we first established the possible structure by extensive analysis of two-dimensional NMR spectral data, and the still uncertain structure details were established by single-crystal X-ray analysis.

In the HMBC spectrum, the proton signals at δ 1.49 (s, H-30) and 3.75, 3.90 (d, *J* = 10.8 Hz, H-29) showed correlations with C-4 and C-5. Both the signals at δ 2.54 (br d, *J* = 7.8 Hz, H-5) and 1.78 (m, H-6β) showed cross-peaks with C-4, C-7, and C-10. Furthermore, signals at δ 2.84 (m, H-1β), 1.83, and 3.29 (m, H-2) showed correlations

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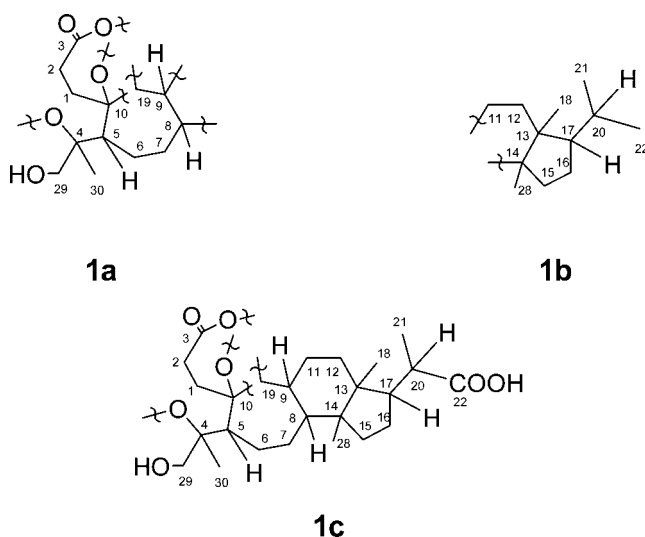
(14) Lancifodilactone F (**1**): white crystals, mp 189–190 °C; [α]_D^{25.7} +28.07 (c 0.095, MeOH); UV (MeOH) λ_{max} (lg ε) 206 (3.27) nm; IR (KBr) ν_{max} 3435, 2933, 1738, 1635, 1451, 1385, 1266, 1180, 1046, 932 cm^{–1}; NMR can be found in Table 1; negative ESIMS *m/z* (rel intensity) 435 (100, [M – H][–]); HR-ESIMS found 435.2761, calcd for C₂₅H₃₉O₆ 435.2746.

Table 2. Summary of Cytotoxicity and Anti-HIV-1 Activity of Compound 1

compound	cytotoxicity CC ₅₀ (μg/mL) ^a	anti-HIV-1 activity EC ₅₀ (μg/mL)	selectivity index CC ₅₀ /EC ₅₀
lancifodilactone 1	>200	20.69 ± 3.31	>6.62–8.34

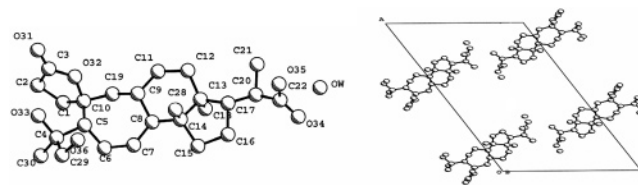
^a Minimal cytotoxicity against C8166 cells when CC₅₀ > 200 (μg/mL).

with C-3 and C-10. These, along with two proton spin systems deduced from ¹H–¹H COSY correlations, H-1/H-2 and H-5/H-6/H-7/H-8/H-9/H-19, led to the establishment of partial structure **1a** (Figure 1). Interpretation of HMBC data

**Figure 1.** Structural fragments of **1**.

also showed the following correlations: a methyl singlet resonance at δ 0.85 (s, H-18) showing cross-peaks with C-12, C-13, C-14, and C-17; another proton signal at δ 0.77 (s, H-28) correlating with C-13, C-14, and C-15; and an H-11 signal at δ 1.67 showing a cross-peak with C-13. In addition, HMBC correlations observed from H-21 at δ 1.34 (d, J = 6.8 Hz) to C-17, C-20, and C-22, and from H-17 at δ 2.30 to C-13 and C-18, suggested that C-17 is directly connected to C-20. The above evidence, coupled with two proton spin systems deduced from ¹H–¹H COSY correlations, H-11/H-12 and H-15/H-16/H-17/H-20, determined the existence of partial structure **1b** (Figure 1). Furthermore, HMBC cross-peaks of H-8 at δ 1.46 with C-28, and H-9 at δ 1.38 with C-11 and C-12, together with ¹H–¹H COSY correlation between H-9 at δ 1.38 and H-11 at δ 1.67, required direct connections of C-9 to C-11 and of C-8 to C-14 and permitted fragments **1a** and **1b** to be joined to get **1c** (Figure 1). Since C-3, C-4, and C-10 were fully substituted carbons and the NMR spectra, including two-dimensional NMR spectra, did not provide sufficient information to elucidate the pattern of connection of C-3, C-4, C-10, and C-19, further solid evidence such as X-ray diffraction was necessary. Fortu-

nately, after many attempts with different solvents, a single crystal of compound **1** was finally obtained from Me₂CO–MeOH (1:1), and an X-ray crystallographic analysis was realized (Figure 2)¹⁵ that clarified the still uncertain structural details.

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

The relative stereochemistry of **1** was established by X-ray analysis, together with NOESY experiment. Stereochemically, C-18 was biogenetically α , and C-28 was in β -orientation. The β -orientations of H-5, H-8, H-9, and H-17 were established by the NOESY correlations (Table 1) between H-5 and H-8, between H-8 and H-28, between H-9 and H-5, and between H-17 and H-28.

Compound **1** was tested for cytotoxicity in an assay against C8166 cells (CC₅₀) using the MTT method as reported previously,¹⁶ and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC₅₀).¹⁷ Compound **1** exerted minimal cytotoxicity against C8166 cells (CC₅₀ > 200 μg/mL) and showed anti-HIV activity with

(15) Crystallographic data for **1**: C₂₅H₄₀O₆, M = 436.59, monoclinic, space group C2, a = 29.832 (3) Å, b = 5.941 (1) Å, c = 15.108 (1) Å, β = 115.27 (1)°, V = 2421.4 (4) Å³, Z = 4, d = 1.225 g/cm³, crystal dimensions 0.15 × 0.20 × 0.50 mm were used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator (ω -2 θ scans, $2\theta_{\max}$ = 50.0°), Mo K α radiation. The total number of independent reflections measured was 2460, of which 2005 were observed ($|F|^2 \geq 3\sigma(|F|^2)$). Final indices: R_f = 0.048, R_w = 0.051 ($w = 1/\sigma(|F|^2)$). The crystal structure (**1**) was solved by direct methods using SHELX-86 (Sheldrich, G. M.; University of Gottingen: Gottingen, Germany, 1985) and expanded using difference Fourier techniques, refined by the program and method NOMCSDP (Lu, Y.; Wu, B. M. *Chin. Chem. Lett.* **1992**, 3, 637–640) and full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited at the Cambridge Crystallographic Data Centre (deposition number: CCDC 254747). Copies of these data can be obtained free of charge via the Internet at 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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$EC_{50} = 20.69 \pm 3.31 \mu\text{g/mL}$ and a selectivity index in the range of 6.62–8.34.

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Supporting Information Available: One- and two-dimensional NMR spectra of lancifodilactones F (**1**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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