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

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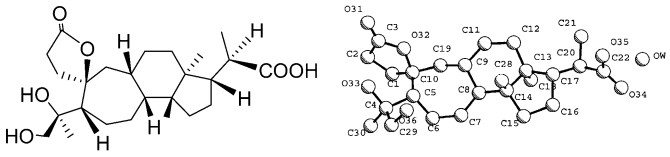
## ABSTRACT




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 μg/mL) and showed anti-HIV activity with $EC_{50}$ = 20.69 ± 3.31 μg/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 triterpenoids isolated from this genus exhibited anti-HIV activities[4,5] and inhibitory activities toward cholesterol biosynthesis.[6−9]

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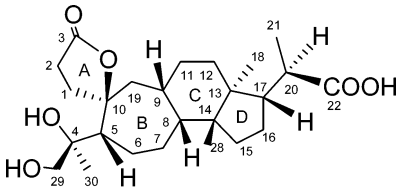
In our recent phytochemical research on this genus, we reported the isolation and structure elucidation of several highly oxygenated nortriterpenoids with a new skeleton, including micrandilactone A,[10] lancifodilactones A−E,[11,12] and henridilactones A−D.[13] Aiming to find potentially bioactive secondary 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**.




**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 $Me_2CO$ (4 × 15 L) at room temperature and concentrated in vacuo to give a crude extract (290 g), which was partitioned between $H_2O$ 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−$H_2O$ 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 $C_{25}H_{40}O_6$ was established on the basis of negative ESIMS, $^{13}C$ 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 $^{13}C$ 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 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.

**Table 1.** $^1H$ and $^{13}C$ NMR Assignments and Two-Dimensional NMR Correlations of **1**[a]

| position | $\delta_H$ (mult, $J$, Hz) | $\delta_C$ (mult) | HMBC (H−C) | NOESY (H−H) |
|---|---|---|---|---|
| 1α | 2.76 (overlap) | 30.4 (t) | 2, 3, 10 | 1$\beta$ |
| 1$\beta$ | 2.84 (m) | | 2, 3, 10 | 2$\beta$, 29, 30 |
| 2α | 1.83 (m) | 30.1 (t) | 1, 3, 10 | 1α, 2$\beta$ |
| 2$\beta$ | 3.29 (m) | | 1, 3, 10 | 1$\beta$, 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, 6$\beta$, 8, 9, 29, 30 |
| 6α | 1.31 (overlap) | 25.7 (t) | 7, 8 | 19α |
| 6$\beta$ | 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, 19$\beta$ |
| 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 |
| 15$\beta$ | 1.16 (overlap) | | 13, 17, 28 | 15α, 16$\beta$, 28 |
| 16α | 1.66 (overlap) | 26.7 (t) | | 16$\beta$ |
| 16$\beta$ | 2.14 (m) | | 17 | 15$\beta$, 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, 19$\beta$ |
| 19$\beta$ | 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, 15$\beta$, 16$\beta$, 17 |
| 29 | 3.75 (d, 10.8) | 70.1 (t) | 4, 5, 30 | 1$\beta$, 2$\beta$, 5, 6$\beta$, 30 |
| | 3.90 (d, 10.8) | | | |
| 30 | 1.49 (3H, s) | 22.7 (q) | 4, 5, 29 | 2$\beta$, 5, 29 |

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

In the HMBC spectrum, the proton signals at $\delta$ 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 $\delta$ 2.54 (br d, $J$ = 7.8 Hz, H-5) and 1.78 (m, H-6$\beta$) showed cross-peaks with C-4, C-7, and C-10. Furthermore, signals at $\delta$ 2.84 (m, H-1$\beta$), 1.83, and 3.29 (m, H-2) showed correlations

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(14) Lancifodilactone F (**1**): white crystals, mp 189−190 °C; $[\alpha]^{25.7}_D$ +28.07 (*c* 0.095, MeOH); UV (MeOH) $\lambda_{max}$ (lg $\epsilon$) 206 (3.27) nm; IR (KBr) $\upsilon_{max}$ 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 $C_{25}H_{39}O_6$ 435.2746.

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

| compound | cytotoxicity $CC_{50}$ ($\mu$g/mL)[a] | anti-HIV-1 activitiy $EC_{50}$ ($\mu$g/mL) | selectivity index $CC_{50}/EC_{50}$ |
|---|---|---|---|
| lancifodilactone F (**1**) | >200 | 20.69 ± 3.31 | >6.62−8.34 |

[a] Minimal cytotoxicity against C8166 cells when $CC_{50}$ > 200 ($\mu$g/mL).

with C-3 and C-10. These, along with two proton spin systems deduced from $^1$H−$^1$H 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

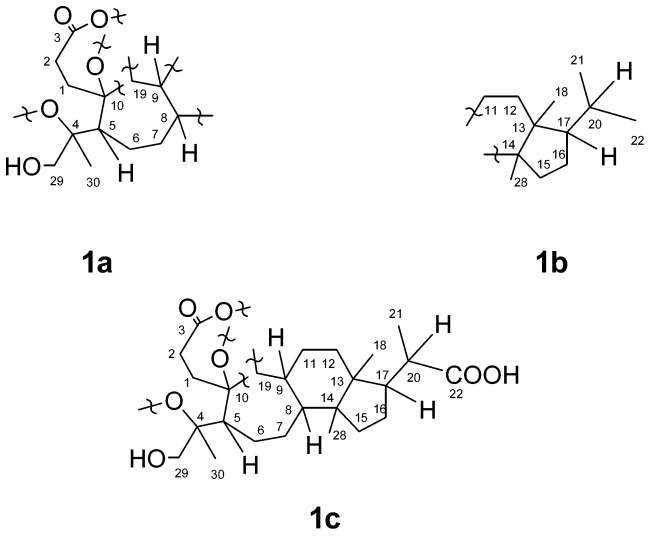


**Figure 1.** Structural fragments of **1**.

also showed the following correlations: a methyl singlet resonance at $\delta$ 0.85 (s, H-18) showing cross-peaks with C-12, C-13, C-14, and C-17; another proton signal at $\delta$ 0.77 (s, H-28) correlating with C-13, C-14, and C-15; and an H-11 signal at $\delta$ 1.67 showing a cross-peak with C-13. In addition, HMBC correlations observed from H-21 at $\delta$ 1.34 (d, $J$ = 6.8 Hz) to C-17, C-20, and C-22, and from H-17 at $\delta$ 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 $^1$H−$^1$H 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 $\delta$ 1.46 with C-28, and H-9 at $\delta$ 1.38 with C-11 and C-12, together with $^1$H−$^1$H COSY correlation between H-9 at $\delta$ 1.38 and H-11 at $\delta$ 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. Fortunately, after many attempts with different solvents, a single crystal of compound **1** was finally obtained from $Me_2CO$−MeOH (1:1), and an X-ray crystallographic analysis was realized (Figure 2)[15] that clarified the still uncertain structural details.

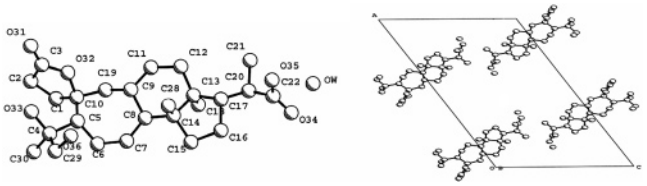

**Figure 2.** X-ray structure of **1** showing the relative configuration.

The relative stereochemistry of **1** was established by X-ray analysis, together with NOESY experiment. Stereochemically, C-18 was biogenetically $\alpha$, and C-28 was in $\beta$-orientation. The $\beta$-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 ($CC_{50}$) 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 ($EC_{50}$).[17] Compound **1** exerted minimal cytotoxicity against C8166 cells ($CC_{50}$ > 200 $\mu$g/mL) and showed anti-HIV activity with

(15) Crystallographic data for **1**: $C_{25}H_{40}O_6$, $M$ = 436.59, monoclinic, space group $C2$, $a$ = 29.832 (3) Å, $b$ = 5.941(1) Å, $c$ = 15.108(1) Å, $\beta$ = 115.27(1)°, $V$ = 2421.4(4) Å$^3$, $Z$ = 4, $d$ = 1.225 g/cm$^3$, crystal dimensions 0.15 × 0.20 × 0.50 mm were used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator ($\omega$-2$\theta$ scans, $2\theta_{max}$ = 50.0°), Mo K$\alpha$ 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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$EC_{50} = 20.69 \pm 3.31$ $\mu$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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