Triterpenoids from *Schisandra lancifolia* with Anti-HIV-1 Activity

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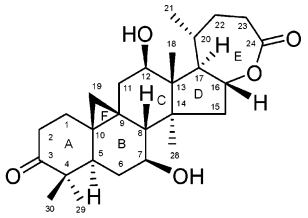
A new trinorcycloartane triterpenoid, lancifodilactone H (**1**), and a new A ring-secocycloartane triterpenoid, lancifoic acid A (**2**), together with a known compound, nigranoic acid (**3**), were isolated from the leaves and stems of *Schisandra lancifolia*. Their structures were elucidated by spectroscopic data analysis. Compound **1** features a biosynthetically modified seven-membered lactone ring, which was confirmed by single-crystal X-ray diffraction. Compounds **1**−**3** exhibited weak anti-HIV-1 activity in vitro.

In our group, previous phytochemical studies on the genus *Schisandra* have reported two bioactive compounds, nigranoic acid[1] and micrandilactone C,[2] which showed anti-HIV-1 activity. As a continuous search for active secondary metabolites, *Schisandra lancifolia* (Rehd. et Wils) A. C. Smith was phytochemically studied, which led to the isolation of lancifodilactones A−G.[3−6] Reinvestigation of the leaves and stems of this plant has resulted in the isolation of a trinorcycloartane triterpenoid, lancifodilactone H (**1**), with a modified seven-membered lactone ring, and a new A ring-secocycloartane triterpenoid, lancifoic acid A (**2**), together with a known compound, nigranoic acid (**3**).[1] In addition, compounds **1**−**3** were tested for their anti-HIV-1 activity. Herein we report the isolation, structure elucidation, and biological activity of compounds **1**−**3**.



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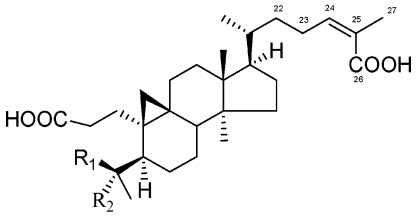

2 $R_1 = OH, R_2 = CH_3$

3 $R_1 = R_2 = CH_2$

A 70% aqueous Me_2CO extract of the stems and leaves of *S. lancifolia* (5.7 kg) was partitioned between H_2O and EtOAc. The EtOAc fraction was condensed under reduced pressure and at low temperature. Silica gel column chromatography followed by further purification by using semipreparative HPLC led to the isolation of compounds **1**−**3**.

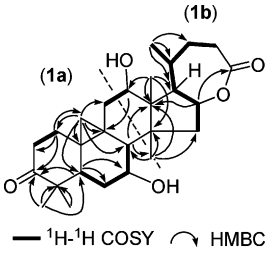

Figure 1. Key HMBC and ^{1}H−^{1}H COSY correlations of **1**.

Lancifodilactone H (**1**) was obtained as optically active white crystals. Its molecular formula, $C_{27}H_{40}O_5$, was established on the basis of HRESIMS analysis ($[M + Na]^+$, *m*/*z* 467.2779) and its ^{1}H and ^{13}C NMR spectra, indicating eight degrees of unsaturation. The IR spectrum of **1** showed an absorption band characteristic of the presence of one or more hydroxyl groups (3483 cm^{-1}). In the ^{1}H NMR spectrum, the two proton AB quartets at δ 0.78 and 0.92 (d, $J = 4.4$ Hz, H-19) due to the characteristic cyclopropyl methylene protons hinted that compound **1** might be derived from cycloartane. Analysis of the ^{13}C NMR and DEPT spectra revealed that **1** contains 27 carbons, including one ester group, one carbonyl group, five methyls, eight methylenes, seven methines, and five quaternary carbons.

On the basis of the detailed analysis of its HMQC, ^{1}H−^{1}H COSY, and HMBC NMR spectroscopic data, two substructures were established for **1** as follows. Substructure **1a** was elucidated starting from the following HMBC correlations (Figure 1): two methyl proton signals at δ 1.15 (Me-29) and 1.06 (Me-30) with C-3, C-4, and C-5; signals of a methylene at δ 0.78 and 0.92 (each 1H, d, $J = 4.4$ Hz, H-19) with C-1, C-5, C-8, C-9, C-10, and C-11; signals of H-1 (δ 1.52 and 1.85) with C-2, C-3, and C-19, and the signal of H-5 (δ 1.94) with C-3, C-6, and C-7. The above evidence, 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, established substructure **1a**. Further analysis of the HMBC spectrum also showed the following correlations: a methyl singlet resonance at δ 1.38 (Me-18) exhibited cross-peaks with C-12, C-13, C-14, and C-17; the methyl proton signals at δ 1.35 (Me-28) exhibited correlations with C-13, C-14, and C-15; a methyl doublet resonance at δ 1.46 (d, $J = 7.4$ Hz, Me-21) correlated with C-17, C-20, and C-22; and another proton signal at δ 4.88 (H-16) correlated with C-12. These

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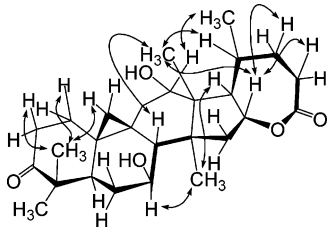

Figure 2. Key ROESY correlations of **1**.

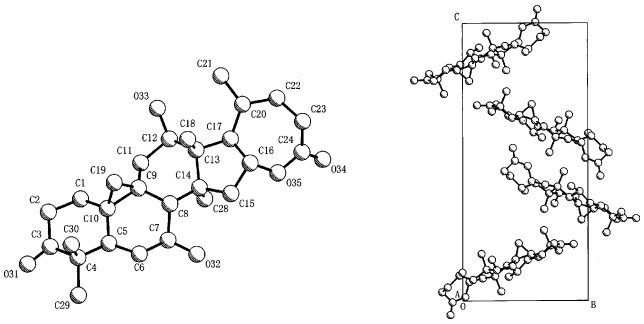

Figure 3. X-ray structure of **1** showing the relative configuration.

facts, combined with a proton spin system deduced from the ^{1}H−^{1}H COSY spectrum, H-15/H-16/H-17/H-20/H-21/H-22/H-23, were used to determine the presence of substructure **1b** (Figure 1). Furthermore, HMBC cross-peaks of H-12 (δ 4.17, br d, J = 5.9 Hz) with C-9, and H-8 (δ 1.86, d, J = 9.5 Hz) with C-13 and C-28, along with the ^{1}H−^{1}H COSY correlations between H-11 (δ 1.52 and 2.71) and H-12, required direct connectivities of C-11 with C-12, and C-8 with C-14, and permitted substructures **1a** and **1b** to be joined to produce the planar structure of **1**.

The relative stereochemistry of **1** was established by an X-ray crystallographic analysis (Figure 3), together with a ROESY NMR experiment. On a biogenetic basis, Me-18 was assigned as β-oriented, while Me-21 and Me-28 were α-oriented. The ROESY correlations of H-7 with Me-28, H-17 with Me-28, H-12 with Me-21, and H-16 with Me-18 demonstrated that H-7, H-12, and H-17 were α-oriented, while H-16 possesses the β-orientation. Thus, compound **1** was established as 3-oxo-7β,12β-dihydroxy-25, 26, 27-trinorcycloartan-24,16α-olide.

Lancifoic acid A (**2**), obtained as white crystals, was found to possess a molecular formula of $C_{30}H_{48}O_5$, as evidenced by the HRESIMS at *m*/*z* 488.3393 (calcd 488.3399). The IR spectrum of **2** showed absorption bands for a hydroxyl group (3444 cm^{-1}) and two carbonyl bands, one of them indicative of a carboxylic acid (1706, 3040−2881 cm^{-1}) and the second band at 1679 cm^{-1} of an α,β-unsaturated carbonyl group. The presence of the two carbonyl groups was further supported by ^{13}C NMR spectroscopic data at δ 177.1 (C-3), 170.8 (C-26), 142.9 (C-24), and 128.7 (C-25) and accounted for three of the total seven degrees of unsaturation required by the molecular formula, and implied that **2** contains four rings. In the ^{1}H NMR spectrum, the two proton AB quartets typical for a cyclopropyl methylene at δ 0.59 and 0.80 (each 1H, d, J = 4.1 Hz, H-19) hinted that **2** is also derived from cycloartane. Initial observation found that the spectroscopic data of **2** were very similar to those of nigranoic acid (**3**).[1] Detailed comparison of 1D NMR data of **2** with those of **3** indicated that the only difference between those compounds is that the olefinic carbons at C-4 and C-29 in **3** are replaced by an oxygenated quaternary carbon and a methyl group in **2**, respectively. This was confirmed by the HMBC correlations of the proton signal at δ 1.42 (H-30) with resonances at δ 75.2 (C-4), 48.9 (C-5), and 26.9 (C-29). A hydroxyl group at C-4 of **2** was deduced from the IR spectrum and the molecular formula. The obvious ROESY correlation of H-24 with H-27, together with chemical shift comparison of C-24 and C-27 with those of **3**, determined the double bond to be in a *Z* configuration.

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

	1		**2**	
position	δ_H	δ_C	δ_H	δ_C
1 α	1.52 (m)	33.4 t	2.63 (m), 3.26 (m)	25.7 t
1 β	1.85 (m)			
2 α	2.65 (m)	37.5 t	1.95 (m), 3.84 (m)	33.5 t
2 β	2.31 (m)			
3		214.6 s		177.1 s
4		49.9 s		75.2 s
5	1.94 (dd, 4.2, 11.3)	47.3 d	2.19 (dd, 5.7, 11.5)	48.9 d
6 α	1.32 (m)	32.5 t	0.57 (m)	28.6 t
6 β	1.93 (m)		1.75 (m)	
7	3.77 (m)	69.8 d	2.11 (m)	27.4 t
8	1.86 (d, 9.5)	54.1 d	1.41 (m)	46.0 d
9		20.5 s		21.8 s
10		25.7 s		27.0 s
11 α	1.51 (br d, 16.2)	40.7 t	2.29 (m)	26.3 t
11β	2.70 (dd, 5.9, 16.2)			
12	4.17 (br d, 5.9)	70.7 d	1.60 (m)	36.3 t
13		48.5 s		45.2 s
14		53.7 s		49.3 s
15 α	2.72 (dd, 7.5, 11.7)	46.3 t	1.48 (m)	33.4 t
15 β	2.48 (br d, 11.7)		1.68 (m)	
16	4.88 (m)	84.6 d	1.76 (m)	27.2 t
17	1.95 (br d, 3.5)	60.4 d	1.55 (m)	52.7d
18	1.38 (s)	12.7 q	0.98 (s)	18.4 q
19 α	0.78 (d, 4.4)	28.5 t	0.59 (d, 4.1)	31.4 t
19 β	0.92 (d, 4.4)		0.80 (d, 4.1)	
20	1.84 (m)	35.7 d	1.50 (m)	36.4 d
21	1.46 (d, 7.4)	22.0 q	0.94 (d, 6.1)	19.8 q
22 α	1.35 (m)	34.4 t	1.14 (m), 1.53 (m)	32.0 t
22 β	1.89 (m)			
23 α	2.73 (m)	34.5 t	2.78 (m), 2.86 (m)	26.9 t
23 β	2.94 (m)			
24		175.5 s	6.05 (t, 7.1)	142.9 d
25				128.7 s
26				170.8 (s)
27			2.14 (s)	21.6 q
28	1.35 (s)	20.1 q	0.91 (s)	20.1 q
29	1.15 (s)	22.6 q	1.44 (s)	26.9 q
30	1.06 (s)	20.7 q	1.42 (s)	32.0 q

[a] Data were recorded in C_5D_5N on Bruker AM-125 (^{13}C NMR) and AM-500 (^{1}H NMR) MHz; chemical shifts (δ) are expressed in ppm with reference to the most downfield signal of C_5D_5N (δ 8.71 ppm) for ^{1}H and to the center peak of the most downfield signal of C_5D_5N (δ 149.9 ppm) for ^{13}C, respectively.

Accordingly, the structure of **2** was established as 3,4-secocycloarta-4-hydroxy-24(*Z*)-en-3,26-dioic acid and has been accorded the trivial name lancifoic acid A.

The anti-HIV activity was indicated as potencies of compounds **1**−**3** in preventing the cytopathic effects of HIV-1 in C8166 cells, and cytotoxicity was measured in parallel with the determination of antiviral activity, using AZT as a positive control (EC_{50} = 0.0034 μg/mL and CC_{50} > 200 μg/mL). Compound **1** showed anti-HIV-1 activity with an EC_{50} of 16.6 μg/mL and exerted minimal cytotoxicity against C8166 cells (CC_{50} > 200 μg/mL). The EC_{50} values of compounds **2** and **3** were 16.2 and 10.3 μg/mL, and the CC_{50} values were 104.9 and 88.0 μg/mL, respectively.

Experimental Section

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 the spectra. IR spectroscopy was performed with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers. Unless otherwise specified, chemical shift (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 was performed with silica gel (200−300 mesh, Qing-dao Marine

Chemical, Inc., Qingdao, People's Republic of China). Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm × 25 cm, column. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The leaves and stems of *S. lancifolia* were collected in Dali Prefecture, Yunnan Province, People's Republic of China, in August 2002, and were identified by Prof. Su-Gong Wu. A voucher specimen (No. KIB 2002-08-11) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. 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 portion (101 g) was chromatographed on a silica gel column eluting with $CHCl_3$−Me_2CO (1:0, 9:1, 8:2, 2:1, 1:1, and 0:1) to afford fractions I−V. Fraction II was purified by repeated chromatography over silica gel (200−300 mesh) and recrystallization to yield compound **3** (20 mg). Fraction III was chromatographed sequentially over silica gel and Sephadex LH-20 and finally purified by semipreparative HPLC, with 45% MeOH in H_2O as the mobile phase, to yield compounds **1** (8 mg) and **2** (30 mg).

Lancifodilactone H (1): white prisms; mp 211−212 °C; $[\alpha]_D^{26}$ −6.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 200 (3.66) nm; IR (KBr) ν_{max} 3483, 2955, 2929, 1724, 1699, 1639, 1452, 1383, 1277, 1035, 1016, 576 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive ESIMS *m*/*z* $[M + Na]^+$ 467; HRESIMS *m*/*z* $[M + Na]^+$ 467.2779 (calcd 467.2773 for $C_{27}H_{40}O_5Na$).

Lancifoic acid A (2): white crystals; mp 153−154 °C; $[\alpha]_D^{26}$ +98.8 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (3.27) nm; IR (KBr) ν_{max} 3444, 3040, 2953, 2881, 1706, 1697, 1642, 1459, 1378, 1290, 1378, 1222, 1152, 1082, 938, 780, 611 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; negative ESIMS *m*/*z* 487 $[M - H]^-$; HRESIMS *m*/*z* 488.3393 (calcd 488.3399 for $C_{30}H_{48}O_5$).

Crystallographic Data for 1. $C_{27}H_{40}O_5$, $M = 444.61$, monoclinic, space group $P2_12_12_1$, $a = 8.006(1)$ Å, $b = 11.604(1)$ Å, $c = 25.691(1)$ Å, $V = 2386.7(4)$ Å^3, $Z = 4$, $d = 1.237$ g/cm^3, crystal dimensions 0.20 × 0.30 × 0.80 mm, measured on a MAC DIP-2030K diffractometer with a graphite monochromator (ω scans, $2\theta_{max} = 50.0°$), Mo Kα radiation. The total number of independent reflections measured was 2628, of which 2091 were observed ($|F|^2 \geq 3\sigma|F|^2$). Final indices: $R_F = 0.054$, $R_w = 0.053$ ($w = 1/\sigma|F|^2$). The crystal structure (**1**) was solved by direct methods using SHELX-86[7] and expanded using difference Fourier techniques, refined by the program NOMCSDP[8] and full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 281118). Copies of the 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].

Anti-HIV-1 Assay. The cytotoxicity assay against C8166 cells (CC_{50}) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}).[9]

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Supporting Information Available: Detailed anti-HIV-1 activity testing method and 1D and 2D NMR spectra of lancifodilactone H (**1**) and lancifoic acid A (**2**). These materials are available free of charge via the Internet at http://pubs.acs.org.

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