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-seccycloartane 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 a trinorcycloartane triterpenoid, lancifodilactone H (1), with a modified seven-membered lactone ring, and a new A ring-seccycloartane triterpenoid, lancifoic acid A (2), together with a known compound, nigranoic acid (3). 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.

Figure 1. Key HMBC and 1H−1H COSY correlations of 1.

A 70% aqueous Me₂CO extract of the stems and leaves of *S. lancifolia* (5.7 kg) was partitioned between H₂O 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.

Lancifodilactone H (1) was obtained as optically active white crystals. Its molecular formula, C₇H₄₆O₅, was established on the basis of HRESIMS analysis ([M + Na]⁺, m/z 467.2779) and its ¹H and ¹³C 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 ¹H 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 ¹³C NMR and DEPT spectra revealed that 1 contains 27 carbons, including one ester group, one carbyl group, five methyls, eight methylenes, seven methines, and five quaternary carbons.

On the basis of the detailed analysis of its HMCO, ¹H−¹H 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 its ¹H−¹H 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
facts, combined with a proton spin system deduced from the 1H–1H 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 1.52 and 2.71) and H-16 with Me-18 demonstrated that H-7, H-12, and H-17 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-trinorocycloartan-24, 16-olide.

Lanocaric acid A (2), obtained as white crystals, was found to possess a molecular formula of C_{30}H_{46}O_{6}, as evidenced by the HRESIMS at m/z 488.3399 (calcd 488.3399). The IR spectrum of 2 showed absorption bands for a hydroxyl group (3444 cm\(^{-1}\)) and 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 nigranic 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-20) 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.

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HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18, 9.4 mm × 25 cm, column. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H2SO4 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 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 portion (101 g) was chromatographed on a silica gel column eluting with CHCl3-Me2CO (1:0, 9:1, 8:2, 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 H2O as the mobile phase, to yield compounds 1 (8 mg) and 2 (30 mg).

**Lancifodiactone H (1):** white prisms; mp 211–212 °C; [α]D26 −6.0 (c 0.2, MeOH); UV (MeOH) λmax (log e) 200 (3.66) nm; IR (KBr) νmax 3483, 2955, 2929, 1724, 1699, 1639, 1452, 1383, 1277, 1035, 1016, 576 cm−1; 1H and 13C NMR data, see Table 1; positive ESIMS m/z [M + Na]+ 467; HRESIMS m/z [M + Na]+ 467.2779 (calcd 467.2773 for C27H40O5Na).

**Lancifoidic acid A (2):** white crystals; mp 153–154 °C; [α]D26 +98.8 (c 0.7, MeOH); UV (MeOH) λmax (log e) 218 (3.27) nm; IR (KBr) νmax 3444, 3040, 2953, 2881, 1706, 1697, 1642, 1549, 1378, 1290, 1378, 1222, 1152, 1082, 938, 780, 611 cm−1; 1H and 13C NMR data, see Table 1; negative ESIMS m/z 487 [M – H]; HRESIMS m/z 488.3393 (calcd 488.3399 for C26H40O5).

**Crystallographic Data for 1.** C27H40O5, M = 444.61, monoclinic, space group P21/c, a = 8.006(1) Å, b = 11.604(1) Å, c = 25.699(1) Å, V = 2386.7(4) Å3, Z = 4, d = 1.237 g/cm3, crystal dimensions 0.20 × 0.30 × 0.80 mm, measured on a MAC’ DIP-2030K diffractometer with a graphite monochromator (ω scans, 2θmax = 50.0°). Mo Kα radiation. The total number of independent reflections measured was 2628, of which 2091 were observed (|F| ≥ 3σ|F|2). Final indices: R = 0.0544, wR = 0.0536 (w = 1/σ2|F|2). The crystal structure (1) was solved by direct methods using SHELX-86 and expanded using difference Fourier techniques, refined by the program NONCSDP 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 (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).

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

**References and Notes**