NITIDASIN, A NOVEL SESTERTERPENOID, FROM THE PERUVIAN FOLK MEDICINE "HERCAMPURI" (*GENTIANELLA NITIDA*)

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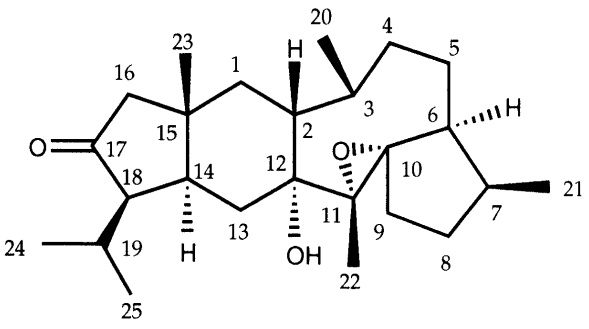
The structure of a novel sesterterpenoid designated as nitidasin (**1**), isolated from the whole plant of *Gentianella nitida*, has been determined by extensive spectroscopic investigation and X-ray analysis.

KEYWORDS *Gentianella nitida*; Gentianaceae; Hercampuri; nitidasin; sesterterpenoid; X-ray analysis

Gentianella nitida (Gentianaceae), a biennial medicinal plant growing in the Andes region, is commonly known as "Hercampuri" or "Hircampure" in Peru. The aqueous extract of the whole plant has been used in traditional Peruvian folk medicine as a remedy for hepatitis, as a cholagogue, and in treatment of obesity.[1] In the course of our chemical investigation of the above medicinal plant, we have isolated a novel sesterterpenoid with a new skeleton designated as nitidasin (**1**) from the dichloromethane extract of the whole plant along with xanthones and phenolic compounds. The structural elucidation of the above compound **1** is reported in this communication.

The MeOH extract (378 g) of the whole plant of *G. nitida* (1 kg) was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-soluble fraction (36.7 g) was subjected to a silica gel column chromatography



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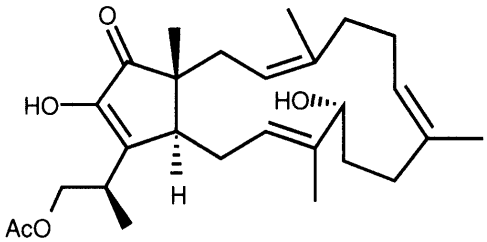

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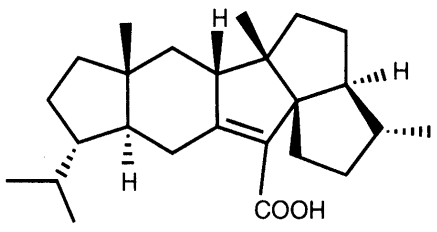

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using a *n*-hexane-EtOAc (5:1) solvent system, followed by reversed-phase low-pressure liquid chromatography (RPLPLC, ULTRA PACK ODS, 11 x 300 mm, Yamazen Co.) with MeOH to yield nitidasin (**1**, 36 mg).

Nitidasin (**1**), in the form of colorless cubes, mp 145-147℃, $[\alpha]_D$ -41.4° (c 0.28, $CHCl_3$), gave a molecular ion at m/z 388 $(M)^+$ in electron-impact ionization (EI) mass spectrometry, and high-resolution EIMS determined the molecular formula $C_{25}H_{40}O_3$ ($[M]^+$ 388.2976, calcd 388.2976). The IR (3423 and 1738 cm^{-1}) spectrum indicated the presence of hydroxyl and carbonyl groups, respectively. The ^{1}H-NMR[2] spectrum of **1** exhibited a hydroxyl proton (δ 2.34) and 39 nonexchangeable protons, including 2 tertiary (δ 0.96 and 1.41) and 4 secondary (δ 0.89, 0.97, 0.98, and 1.00) methyl groups. The ^{13}C-NMR[3] spectrum of **1** displayed 6 methyls, 7 methylenes, 7 methines, and 5 quaternary carbons, including 3 oxygenated carbons (δ 67.29, 75.06, and 79.17) and a carbonyl carbon (δ 220.50). One of the 6 unsaturations were accounted for, thus implying that **1** consisted of a 5-ring system.

Interpretation of the ^{1}H-^{1}H COSY, HOHAHA, and pulse-field gradient (PFG) HMQC spectra of **1** characterized each carbon, except for a carbonyl and 4 quaternary carbons. Further analysis of the PFGHMBC spectrum and carbon skeleton was carried out and the planar structure of nitidasin was described as **1**.

In order to confirm the exact structure of **1**, X-ray crystallographic analysis was undertaken. The crystals of **1** were grown from MeOH solution to obtain colorless cubes. The molecules of **1** are mainly packed by van der Waals forces, and no hydrogen bonding was observed. The molecular structure of **1** is illustrated in Fig. 1.[4] Therefore the relative structure of nitidasin was consequently established as shown in **1**.

Recently, Santini *et al.*[5-6] reported the isolation and structure determination of a toxic sesterterpene, fusaproliferin (**2**), from the fungus *Fusarium proliferatum*. On the other hand,

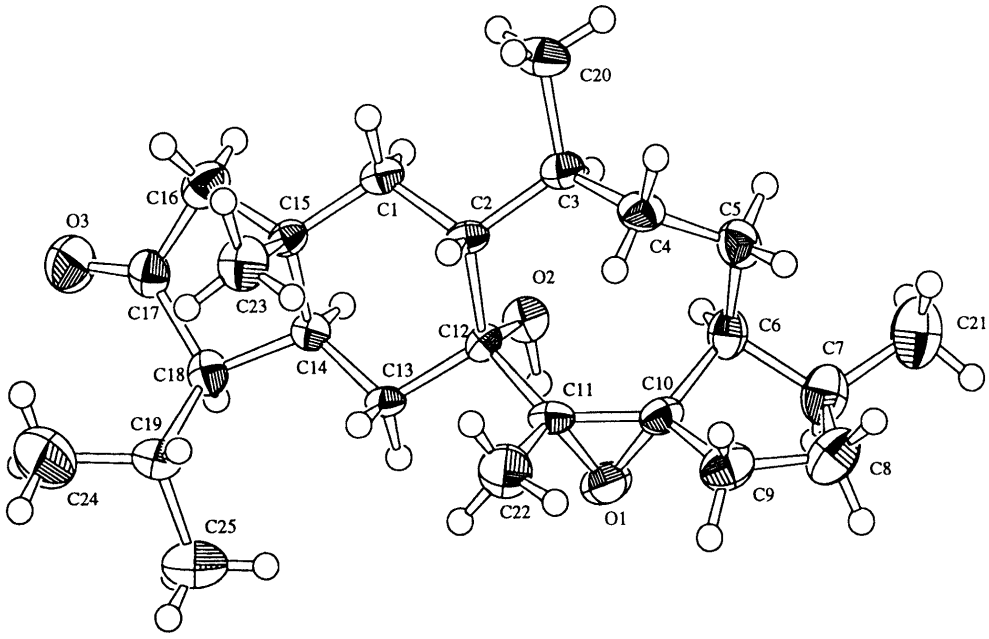

Fig. 1. Perspective View of the Crystal Structure of **1**

retigeranic acid (**3**) was isolated from the lichens of the *Lobaria retigera* group.[7] The carbon skeleton of nitidasin (**1**) is similar to that of the above compounds. Thus the structure of **1** is considered to derive from the precursor geranyl farnesol *via* a similar biosynthetic pathway as proposed by Kaneda *et al.* for retigeranic acid.[7]

Nitidasin (**1**) is the first example of a sesterterpenoid with a new ring skeleton. A large number of xanthones and flavonoids have been isolated from Gentianaceae family, while **1** is the first isolation of a sesterterpenoid.

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2) ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.89 (d, *J*=5.5 Hz, 20-H_3), 0.96 (s, 23-H_3), 0.97 (d, *J*=6.1 Hz, 25-H_3), 0.98 (d, *J*=6.1 Hz, 21-H_3), 1.00 (d, *J*=6.2 Hz, 24-H_3), 1.12 (m, 5-H), 1.26 (m, 1 and 8-H), 1.32 (m, 4-H), 1.41 (s, 22-H_3), 1.44 (m, 5-H), 1.46 (m, 9-H), 1.55 (m, 3-H), 1.56 (m, 2-H), 1.65 (br.d, *J*=12.5 Hz, 13α-H), 1.76 (br. d, *J*=13.5 Hz, 1-H), 1.87 (m, 8-H), 1.88 (m, 18 and 19-H), 1.98 (d, *J*=17.2 Hz, 16-H), 2.06 (m, 9-H), 2.10 (d, *J*=17.2 Hz, 16-H), 2.17 (t, *J*= 12.5 Hz, 13β-H), 2.34 (s, 12-OH), 2.40 (m, 7-H), 2.46 (m, 6-H), 2.55 (m, 14-H).

3) ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 14.89 (q, C-21), 16.34 (q, C-22), 20.11 (q, C-23), 21.16 (q, C-24), 23.08 (q, C-20), 24.67 (t, C-5), 25.32 (q, C-25), 26.56 (t, C-8), 28.35 (d, C-19), 32.13 (d, C-3), 32.22 (t, C-9), 34.20 (t, C-13), 34.83 (t, C-4), 36.00 (d, C-7), 39.11 (s, C-15), 41.70 (d, C-14), 41.84 (t, C-1), 42.18 (d, C-6), 42.35 (d, C-2), 55.77 (t, C-16), 55.83 (d, C-18), 67.29 (s, C-11), 75.06 (s, C-12), 79.17 (s, C-10), 220.50 (s, C-17).

4) Crystal data for **1**: $C_{25}H_{40}O_3$, M = 388.59, orthorhombic, space group $P2_12_12_1$, a = 13.634(4), b = 25.607(7), c = 6.466(5) Å, V = 2257 (1) Å^3, Z = 4, D_C = 1.143 g·cm^{-3}, F(000) = 856, Cu-Kα X-radiation (graphite monochromator), λ = 1.54178Å. Diffraction intensities were collected from a crystal of dimensions 0.70 x 0.60 x 0.50 mm on a Rigaku AFC-7 4-circle diffractometer. Of the total 1995 reflections (complete for 2θ < 120.2°), 1898 satisfied the criterion F > 3σ(F) and only these were used in the solution and refinement of the structure. The structure was solved by direct methods using SAPI91[A] and expanded using Fourier techniques (DIRDIF 92).[B] The final refinement was done by the full-matrix least-squares method. Anisotropic thermal parameters were used for all nonhydrogen atoms and the hydrogen atoms were fixed. The refinement converged at R 0.075.

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