A Novel Sesterterpenoid, Nitiol, as a Potent Enhancer of IL-2 Gene Expression in a Human T Cell Line, from the Peruvian Folk Medicine "Hercumpuri" (*Gentianella nitida*)

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A novel sesterterpenoid designated as nitiol (1), possessing enhancement activity of IL-2 gene expression in a human T cell line, was isolated from the Peruvian folk medicine "Hercampuri" (*Gentianella nitida*). The structure was elucidated by extensive spectroscopic investigation.

Key words nitiol; *Gentianella nitida*; Hercampuri; Gentianaceae; sesterterpenoid

In a previous paper,[1)] we reported the isolation of a novel sesterterpenoid with a new skeleton designated as nitidasin (**2**) from the dichloromethane extract of the whole plant of *Gentianella* (*G.*) *nitida* (Gentianaceae), a biennial medicinal plant growing in the Andes region and used in traditional Peruvian folk medicine. Commonly known as "Hercampuri" or "Hircampure", it is used as a remedy for hepatitis, as a cholagogue, and in treatment of obesity.[2)] Further investigation of this extract led us to isolate a new sesterterpenoid designated as nitiol (**1**). 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 silica gel column chromatography using a *n*-hexane–EtOAc (5 : 1) solvent system, followed by reversed-phase low-pressure liquid chromatography (RPLPLC, ULTRA PACK ODS, 11×300 mm, Yamazen Co.) with 90% MeOH to yield **1** (25 mg).

Nitiol (**1**), in the form of colorless amorphous, $[\alpha]_D$ −39.8° (c=1.37, $CHCl_3$), gave a molecular ion at *m/z* 356 $(M)^+$ in electron-impact ionization (EI) mass spectrometry, and high-resolution EIMS determined the molecular formula $C_{25}H_{40}O$ ($[M]^+$ 356.3077, Calcd 356.3079). The IR (3422 cm^{-1}) spectrum indicated the presence of hydroxyl group and the UV (238 nm, log ε 3.90) spectrum showed a conjugated diene moiety in the molecule. The ^{1}H-NMR[3)] spectrum of **1** exhibited 39 nonexchangeable protons, including two tertiary (δ 0.92 and 1.76) and three secondary (δ 0.87, 0.90, and 1.13) methyl groups, and three olefinic protons (δ 5.36, 5.58 and 5.63). The ^{13}C-NMR[4)] spectrum of **1** displayed five methyls, eight methylenes, eight methines, and four quaternary carbons, including an oxygenated carbon (δ 59.2) and six olefinic carbons (δ 122.3, 124.3, 131.1, 135.5, 137.1 and 149.8). Three of the 6 unsaturations were accounted for, thus implying that **1** consisted of a 3-ring system. The above data suggested that **1** is a tricyclic sesterterpenoid related to **2**.

Interpretation of the ^{1}H–^{1}H shift correlation spectroscopy (COSY) and heteronuclear multiple quantum coherence (HMQC) spectra of **1** indicated the presence of four partial structures A—D (Fig. 1), except for a quaternary carbon (δ 45.9). The connectivity of each partial structure and the quaternary carbon were determined by the heteronuclear multiple-bond correlation (HMBC) spectrum. The HMBC correlations of H-16 and H_3-23 to C-15 and H_3-23 to C-14 led to the formation of a five-membered ring containing the partial unit A. The other HMBC correlations, as shown by arrows in Fig. 1 allowed us to describe the planar structure of **1**.

The relative stereochemistry of **1** was identified by a nuclear Overhauser exchange spectroscopy (NOESY) spectrum as shown in Fig. 2. The H_3-23 and H-14 showed a cross peak to H-19 and H-18, respectively, and thereby the five-membered ring and the twelve-membered ring fused together with a *trans* relationship. Also the cross peaks observed between H-6 and H-12, H-6 and H_3-20, H-14 and H_2-1α, H-14 and H-12, and H_2-1α and H_3-20 indicated that H-6 and H-14 were both on the same side of **1**. Irradiation of H-6 gave a clear nuclear Overhauser effect (NOE) with H-7 while it gave no NOE with H_3-21. This fact indicated the configuration between H-6 and H-7 was a *cis* relationship. Furthermore, the configurations of the two double bonds (C-2—C-3 and C-11—C-12) were determined as *E* and *Z*, respectively, by ob-

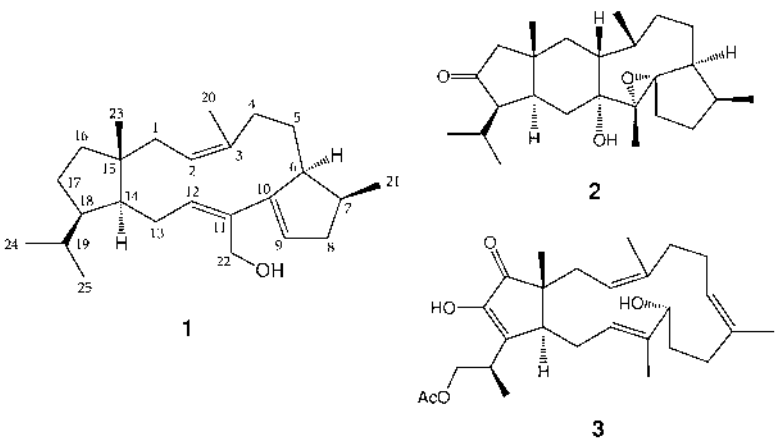

Chart 1

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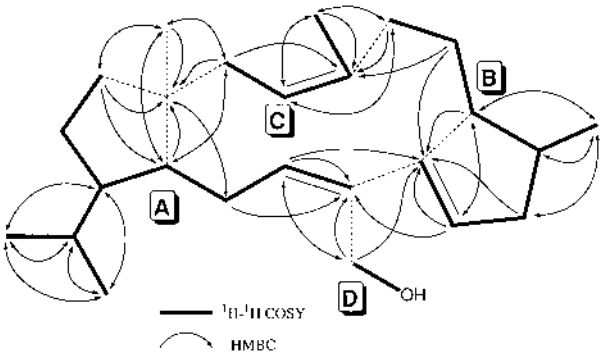

Fig. 1. ^{1}H–^{1}H and Long-Range ^{13}C-^{1}H Correlations of **1**

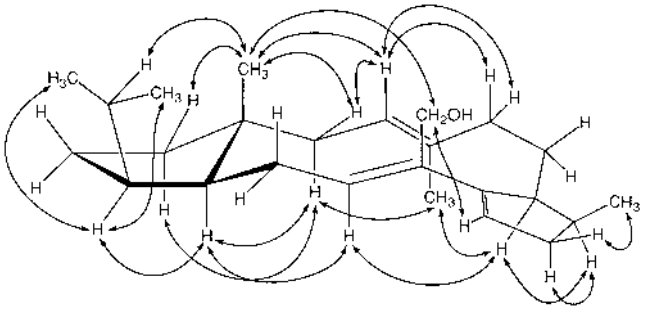

Fig. 2. NOEs of **1**

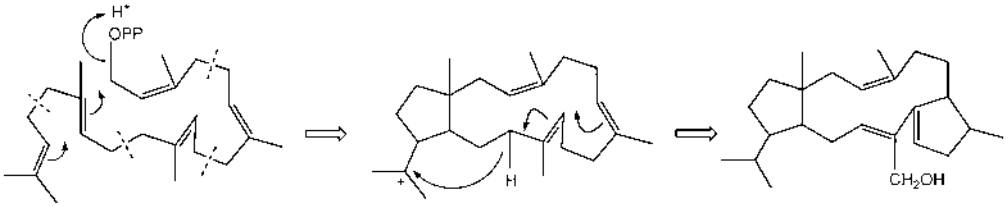

Fig. 3. Possible Biogenesis of **1**

servation of the cross peaks between H-2 and H_2-4, H_3-20 and H_2-1α, H_2-22 and H_3-23, and H-12 and H-14. Thus on the basis of the above spectral data, the structure of nitiol was established as shown in **1**.

Santini *et al*.[5,6] reported the isolation and structure determination of a toxic sesterterpene, fusaproliferin (**3**), from the fungus *Fusarium proliferatum*. The carbon skeleton of **1** is similar to that of **3**. Thus the structure of **1** is thought to be biosynthesized from geranyl farnesyl pyrophosphate as shown in Fig. 3.[7,8]

In our continuing study on intracellular signal transduction mechanisms of human cells, we have been searching for lipophilic low-molecular-weight probes that can easily pass through the cell membrane and have an effect on some signal transduction steps. As part of our search, we assessed the capacity of **1** and **2** to modulate the gene expression of interleukin-2 (IL-2) in Jurkat cells, a human T cell line, by competitive-PCR-based bioassay.[9]

Both compounds dissolved in ethanol were added to the cells at the final concentration of 20 μM. After incubation for 6 h, the IL-2 mRNA level in the nitiol (**1**) treated cells was about three times higher than that in the vehicle (ethanol)-treated cells, while **2** had no significant effect on the IL-2 gene expression. Since **1** has a distinctly different structure from the known modulators of the IL-2 gene expression such as calcineurin inhibitors, it is a possible tool to discover the novel signal transduction pathways guiding the transcription of the IL-2 gene.

References and Notes

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3) ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.87 (d, J=6.2 Hz, H_3-25), 0.90 (d, J=6.3 Hz, H_3-24), 0.93 (s, H_3-23), 1.13 (d, J=6.9 Hz, H_3-21), 1.17 (m, H-5), 1.20 (m, H-17), 1.36 (dt, J=5.1, 11.7 Hz H-16α), 1.45 (dd, J=5.1, 11.7 Hz, H-16β), 1.53 (m, H-19), 1.57 (m, 17-H), 1.61 (m, H-18), 1.72 (t, J=13.0 Hz, H-4), 1.76 (br s, H_3-20), 1.86 (m, H-5), 1.87 (m, H-14), 1.95 (dd, J=4.9, 13.8 Hz, H-1β), 2.11 (m, H-8β), 2.12 (t, H_2-13), 2.17 (dd, J=10.3, 13.8 Hz, H-1α), 2.23 (dd, J=5.5, 13.0 Hz, H-4), 2.31 (m, H-8α), 2.45 (m, H-7), 2.51 (t, J=7.5 Hz, H-6), 4.28 (d, J=12.0 Hz, H_2-22), 4.33 (d, J=12.0 Hz, H_2-22), 5.36 (m, H-2), 5.58 (br t, J=6.0 Hz, H-12), 5.63 (br s, H-9) .
4) ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 14.6 (q, C-21), 19.9 (q, C-20), 22.0 (q, C-24), 22.6 (q, C-25), 23.2 (q, C-23), 24.6 (t, C-13), 27.1 (t, C-5), 28.0 (t, C-17), 28.1 (d, C-19), 36.9 (t, C-4), 38.1 (d, C-7), 38.9 (t, C-8), 43.6 (t, C-1), 44.7 (t, C-16), 45.9 (s, C-15), 47.2 (d, C-6), 48.4 (d, C-14), 53.2 (d, C-18), 59.2 (t, C-22), 122.3 (d, C-9), 124.3 (d, C-2), 131.1 (s, C-11), 135.5 (s, C-3), 137.1 (d, C-12), 149.8 (s, C-10).
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