A New Bislabdane-Type Diterpenoid from the Roots of *Cunninghamia lanceolata*

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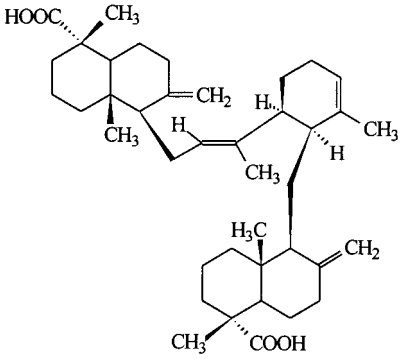
Lanceolatic acid (**1**), a new bislabdane-type diterpenoid, has been isolated from the roots of *Cunninghamia lanceolata*. Its structure was elucidated on the basis of spectroscopic data interpretation and confirmed by single-crystal X-ray diffraction analysis.

The genus *Cunninghamia* (family Taxodiaceae) consists of three species, all of which are found in the People's Republic of China. *Cunninghamia lanceolata* Hook. is a traditional Chinese medicine used for the treatment of hernia, arthritis, and strangury. Its chemical constituents have been examined, and the isolation of flavonoids from the leaves [1−3] and essential oils from the wood[4] have been reported. We now describe the isolation and structure elucidation of a new bislabdane-type diterpenoid (**1**) from the roots of the title plant.

Lanceolatic acid (**1**) was obtained as white needles. The HRFABMS of **1** exhibited a $[MH]^+$ peak at *m/z* 605.4546, corresponding to the molecular formula $C_{40}H_{60}O_4$ (calcd 605.4569). The presence of one or more carboxyl groups (3418, 3250, 1711, 1697 cm^{-1}) and double bonds (1643, 889 cm^{-1}) was indicated by the IR spectrum. The ^1H NMR spectrum showed the presence of six methyl signals and six olefinic protons. The ^{13}C NMR DEPT spectrum revealed 40 carbon signals, composed of six methyls, 16 methylenes, eight methines, and 10 quaternary carbons. Comparison of the spectral data of lanceolatic acid (**1**) with those of cunninghamic acids A and B[5] showed that all these compounds were constructed by a Diels−Alder cycloaddition of two diterpene monomer units. However, there was a marked difference between the newly formed ring C of these compounds, because two chiral centers were formed in lanceolatic acid (**1**) but only one in cunninghamic acids A and B. The number of carbons linked to rings B and C and rings C and D is also different in those compounds. This is due to the difference in the position and numbers of double bonds of these diterpenes. A NOE experiment on **1** showed that the protons at C-19 and the methyl group at C-18 are oriented in a trans arrangement, and the two quaternary methyl groups in rings A and E have a cis relationship. There are two ways in which the Diels−Alder cycloaddition reaction could occur, either head-to-head or head-to-end. Thus, there are two possibilities for the structure of lanceolatic acid (**1**). To resolve the structure and stereochemistry of **1**, a single-crystal X-ray crystallographic study was undertaken.

The structure of (**1**) was solved by direct methods and refined using the NOMCSDP software package.[6] The final reliable factors were $R_f = 0.090$, $R_w = 0.097$, $S = 4.601$, $(\Delta/\sigma)_{max} = 0.048$, $(\Delta\rho)_{min} = -0.33$ e/Å^3, and $(\Delta\rho)_{max} = 0.24$e/Å^3. The bis-diterpenoid as host and pyridine as guest formed a clathrate, and the ratio of host to guest is 1:2. The archival X-ray data have been deposited in the Cambridge Crystallographic Data Centre.[7] The crystallographic structure confirms the link method of lanceolatic acid (**1**) to be head-to-head in Diels−Alder cycloaddtion.



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Experimental Section

General Experimental Procedures. The melting point was determined on a Reichert Nr-229 micromelting point apparatus and is uncorrected. The optical rotation was measured on a Perkin−Elmer 241 polarmeter. The IR spectrum was recorded on Perkin−Elmer 683 infrared spectrometer. NMR spectra were run on a Bruker AM 500 spectrometer with TMS as internal standard. The FABMS were performed on VG-Autospec-3000 instrument.

Plant Material. The roots of *Cunninghamia lanceolata* were collected in September 1997, from Jiujiang, Jiangxi Province, People's Republic of China. The plant material was identified by Professor Ce-Ming Tang, Jiujiang Institute of Forestry, Jiangxi Province, People's Republic of China. A voucher specimen (no. 258) has been deposited in the herbarium of the Department of Botany, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing.

Extraction and Isolation. A crude organic residue was extracted from the dried roots (10 kg) of *C. lanceolata*. The residue was partitioned with petroleum ether, $CHCl_3$, EtOAc, and CH_3COCH_3, respectively. The EtOAc extract (230 g) was subjected to Si gel column chromatography with a gradient petroleum ether−Me_2CO (10:1→ 4:1) system to give lanceolatic acid (**1**) 9 mg.

Lanceolatic Acid (1): colorless needles (C_5H_5N); mp 208210 °C; $[\alpha]^{18}_D$ −30.2° (*c* 0.12, C_5H_5N); IR (KBr) ν_{max} 3418, 3250, 1711, 1697, 1643, 889 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) δ 5.34 (1H, d, $J = 1.5$ Hz, H-19), 5.21 (1H, t, $J = 4.1$ Hz, H-14), 4.98 (2H, s, H-33, H-38), 4.88 (1H, s, H-37), 4.73 (1H, s, H-33), 2.49 (1H, dd, $J = 2.4$, 12.4 Hz, H-5), 2.35 (1H, dd, $J = 2.3$, 12.4 Hz, H-25), 1.83 (3H, s, H-36), 1.61 (3H, s, H-35), 1.43 (3H,

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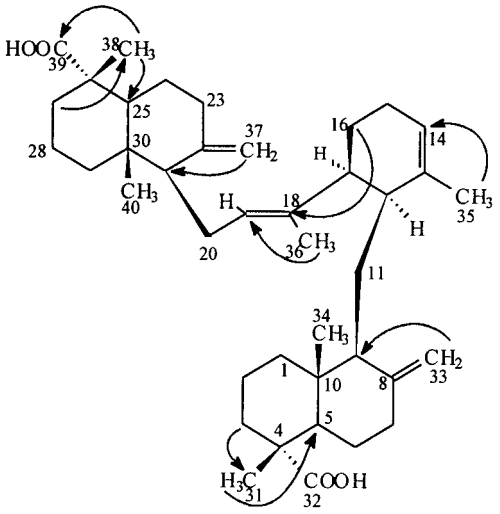

Figure 1. HMBC spectrum of lanceolatic acid (**1**) in C_5D_5N.

s, H-38), 0.93 (3H, s, H-34), 0.82 (3H, s, H-40);^{13}C NMR (125 MHz, C_5D_5N) δ 181.2 (s, C-32), 180.0 (s, C-39), 148.7 (s, C-8, C-22), 139.3 (s, C-18), 137.3 (s, C-13), 125.3 (d, C-14), 121.9 (d, C-19), 108.4 (t, C-33), 107.6 (t, C-37), 58.1 (d, C-9), 54.1 (d, C-21), 50.9 (d, C-25), 50.1 (d, C-5), 47.7 (s, C-4, C-26), 46.2 (d, C-17), 40.2 (s, C-30), 39.2 (t, C-27), 39.0 (s, C-10), 38.8 (d, C-7,C-12), 38.4 (t, C-3), 38.1 (t, C-23), 37.8 (t, C-29), 37.7 (t, C-1), 27.2 (t, C-24), 27.1 (t, C-6), 26.3 (t, C-15), 24.0 (t, C-20), 23.8 (q, C-36), 23.3 (t, C-11), 21.7 (t, C-16), 19.2 (t, C-2), 19.1 (t, C-28), 17.6 (q, C-35), 17.5 (q, C-38), 17.4 (q, C-31), 15.1 (q, C-34), 14.6 (q, C-40); FABMS *m*/*z* 605 $[MH]^+$, 559 (15), 383 (20), 317 (54), 21 (85), 105 (76), 81 (100); HRFABMS *m*/*z* $[M^+ + 1]$ 605.4546 ($C_{40}H_{60}O_4$, calcd 605.4569) (Figure 1).

Single-Crystal X-ray Structure Determination. A crystal of lanceolatic acid (**1**), $C_{40}H_{60}O_4$, with approximate dimensions 0.1 × 0.4 × 1.4 mm^3 was selected for X-ray crystallographic analysis. The X-ray intensity data were measured on a MAC Science DIP 2030k Image Plate equipped with graphite monochromate using Mo Kα (λ = 0.710 73 Å) radiation. Intensity data were collected at a detector to crystal distance of 10 cm, using the $\omega-2\theta$ scan technique ($2\theta_{max}$ = 50°) at a constant speed of 1.2°/min. Weak reflections were rescanned

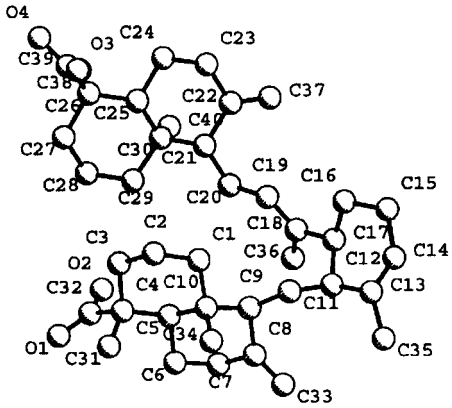

Figure 2. Molecular structure of lanceolatic acid (**1**) with crystallographic numbering scheme.

a maximum of two times. The unit cell was monoclinic with space group $P2_1$. Accurate cell parameters are as follows: a = 7.687(1) Å; b = 11.499(3) Å; c = 25.784(5) Å; V = 2266.8(8) Å^3; Z = 2, D_{calc} = 1.118 g·cm^{-3}. There were 3570, of which 2048 were observed, and the positions of 38 atoms were obtained directly from an *E*-map, and the positions of the other 18 nonhydrogen atoms were obtained by the least squares and difference Fourier processes. The positions of all hydrogen atoms were obtained by geometric calculation and the difference Fourier method (Figure 2).

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References and Notes

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(7) A full list of crystallographic data and parameters for lanceolatic acid (**1**) has been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

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