Structure and Stereochemistry of Pseudolarolide J, a Novel Nortriterpene Lactone from *Pseudolarix kaempferi*

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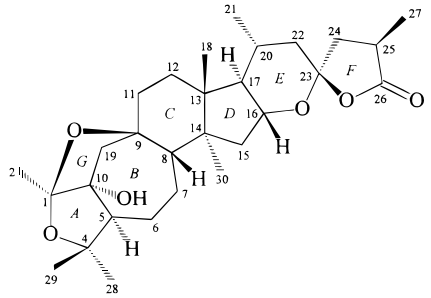
Pseudolarolide J (**1**), a novel nortriterpene lactone, has been isolated from the seeds of *Pseudolarix kaempferi* and structurally characterized from spectral data and X-ray crystallographic analysis.

The root bark of *Pseudolarix kaempferi* Gord. (Pinaceae), a plant indigenous to eastern China, is known as "Tu-Jin-Pi" in Chinese folk medicine and has been used for treatment of skin diseases caused by fungi.[1] Many novel di- and triterpenes have been isolated from this source.[2] Some of these compounds, such as pseudolaric acids A and B, have been found to demonstrate potent cytotoxicity.[3] In the course of our continuing search for novel, potent antitumor agents,[4] we recently investigated other parts of this plant and found that the ethereal extract of the seeds showed significant *in vitro* cytotoxicity against KB, A-549, HCT-8, and P-388 cell lines. Bioassay-directed fractionation of the active extract has led to the isolation and characterization of seven novel triterpene lactones, pseudolarolides A−I, of which pseudolarolides B and I showed potent cytotoxic activity.[5−8] We report herein on the isolation and structural elucidation of a novel nortriterpene lactone, pseudolarolide J (**1**), from this extract.



Pseudolarolide J (**1**)

The deoiled ethereal extract of the seeds of *P. kaempferi* was chromatographed on silica gel. The active fractions were further chromatographed and afforded pseudolarolide J (**1**) in 0.000 15% yield. Pseudolarolide J (**1**), isolated as colorless prisms, has the molecular formula $C_{29}H_{44}O_6$ deduced from FAB HRMS and EIMS peaks at *m/z* 511.3035 $[M + Na]^+$ (98.6%, for $C_{29}H_{44}O_6Na$, calcd 511.3035) and 489 $[M + H]^+$, respectively. The IR (KBr) spectrum indicated that hydroxyl (3510 cm^{-1}) and γ-lactone (1770 cm^{-1}) groups were present in **1**. The ^{1}H-NMR spectrum of **1** exhibited signals due to five tertiary methyls [δ 0.91, 1.22, 1.35, 1.40, and 1.47 (3H each, s)], three of which were attached to oxygen-bearing carbon atoms, two secondary methyls [δ 0.88, (3H, d, J = 6.5 Hz), 1.25, (3H, d, J = 7.0 Hz)], and one low-field proton attached to a carbon bearing an oxygen function [δ 4.21, (1H, dt, J = 4.0, 10.5 Hz). The ^{13}C-NMR spectrum revealed that **1** contained two ketal carbons (δ 113.2 and 107.5) and four oxygen-bearing carbons (δ 78.0, 85.0, 85.3, and 92.1) in addition to those for one carbonyl (δ 179.5) and seven methyls (δ 14.9, 16.0, 19.2, 22.2, 24.4, 24.6, and 31.4). These data, coupled with the lack of cyclopropyl group resonances, suggested that **1** was a triterpene possessing a 9,10-secocycloartane skeleton.

The mass spectrum fragment at *m/z* 139 ($C_8H_{11}O_2$, 40), which is characteristic of the spiro E and F rings in pseudolarolides, and comparison of the ^{1}H-NMR and ^{13}C-NMR spectra with those of pseudolarolides A−I[5−8] indicated that **1** possesses same side chain. Strong fragments at *m/z* 307 (27.2) and 289 (21.3) (arising from $[M -$ side chain and C-16 and C-17$]^+$ and $(307 - H_2O)$) indicated the loss of a carbon and presence of a hydroxyl group in the parent compound.

From biogenetic considerations, the lost carbon, the second ketal group, and the hydroxyl group were assigned in ring A as shown. These assignments were confirmed by an HMBC experiment. Thus, both Me-28 (δ 1.40) and Me-29 (δ 1.35) protons showed two-bond correlation to the carbon signal at δ 85.3 (C-4) and three-bond correlation to the signal δ 54.5 (C-5); H_2-19 correlated with the signals at δ 39.4 (C-11, three-bond), 44.0 (C-8, three-bond), 54.5 (C-5, three-bond), 85.0 (C-9, two-bond), 92.1 (C-10, two-bond), and 113.2 (ketal, three-bond); the lowest-field methyl (δ 1.49) only showed long-range correlations to the ketal (δ 113.2, two-bond) and C-10 (δ 92.1, three-bond). These data suggested that ring A had suffered an oxidative cleavage between C-3 and C-4 to form a carboxylic group at C-3 followed by loss of CO_2, leading to assignments of the lowest-field methyl to C-2 and the ketal to C-1. An AX doublet for the C-19 methylene occurred in the low-field region [δ 3.16 (1H, d, J = 13.5 Hz) and 1.73 (1H, d, J = 13.5 Hz)] due to the effect of the oxygen bridge. This evidence, coupled with the comparison of ^{13}C NMR spectrum with that of pseudolarolide I, led to the

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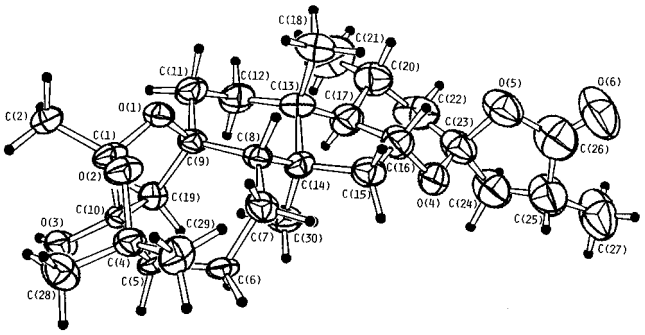

Figure 1. ORTEP diagram (40% probability ellipsoids) showing the crystallographic atom numbering scheme and solid-state conformation of pseudolarolid J (**1**); small filled circles represent hydrogen atoms.

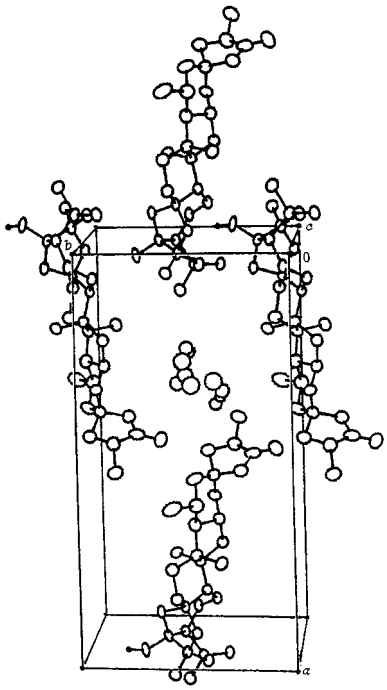

Figure 2. Packing arrangement in crystals of **1**·Et_2O. Small filled circles indicate hydroxyl hydrogen atoms. The cluster of atoms in the cavity formed by hydrogen-bonded molecules of **1** represents the severely disordered Et_2O molecule.

conclusion that C-9 must be connected to C-1 through an oxygen bridge with the hydroxyl group located at C-10.

The stereochemistry in rings A and B was determined by 2D ^{1}H NOE (phase-sensitive NOESY) and 1D NOE difference experiments. The nature of rings C, D, E, and F was derived from biogenetic considerations and comparison of the spectral data (^{1}H and ^{13}C NMR, MS) for **1** with corresponding data for other pseudolarolides.[5−8]

The complete structure and stereochemistry of **1** were established unequivocally by a single-crystal X-ray analysis of the ether solvate. A view of the solid-state conformation is presented in Figure 1. Figure 2 shows the packing of molecules of **1** and the severely disordered ether in the crystal. Molecules of **1** related by the 2_1 screw axis along *b* are associated in a head-to-head manner by an O-H···O [O-3···O-1 = 2.846(6) Å] hydrogen bond, creating a large cavity in which the ether molecules reside [shortest intermolecular distances: **1**···Et_2O = 3.36(4) Å; Et_2O···Et_2O = 4.08(5) Å between atoms in clusters related by unit translation along *c*].

Experimental Section

General Experimental Procedures. The melting point was taken on a Fischer-Johns apparatus and is uncorrected. The IR spectrum was measured as a KBr pellet using a Perkin-Elmer 1320 spectrophotometer. ^{1}H-NMR and ^{1}H−^{1}H COSY spectra were recorded on a Bruker AC 300, while ^{13}C-NMR, HMQC, HMBC, and phase-sensitive NOESY spectra were measured on a Bruker AMX 300 in $CDCl_3$ using TMS as internal standard. Both FAB HRMS and EIMS measurements were taken on a VG-70-250 SEQ mass spectrometer. Aldrich silica gel 60 (5−25 μm) was used for column chromatography, and Kieselgel 60 F_{254}, 0.25 mm was used for TLC. Pseudolarolide J was detected by spraying with 50% H_2SO_4 solution containing 1% anisaldehyde in 95% EtOH, followed by heating.

Plant Material. The seeds of *P. kaempferi* were collected at Chang-Le Forest Centre, Zhejiang Province, China. A voucher specimen is deposited at the School of Pharmacy, Shanghai Medical University, Shanghai, China.

Extraction and Isolation. The seeds of *P. kaempferi* (2 kg) were pulverized and extracted with EtOH. After evaporation of the EtOH, the ethanolic extract was extracted with hexane, Et_2O, and EtOAc, consecutively. The ethereal extract (85 g) was applied to Si gel CC under low pressure and eluted with hexane−Et_2O (100:0 to 0:100). Fractions of hexane−Et_2O (1:1) were combined and further purified by flash chromatography with hexane−Et_2O (1:1) as eluent to afford 3 mg (0.000 15%yield) of pseudolarolide J (**1**).

Pseudolarolide J (1): colorless crystals (from Et_2O); mp 198−200 °C; ^{1}H-NMR (300 MHz) δ 1.49 (3H, s, H-2), 2.02 (1 H, dd, J = 4, 14 Hz; H-5), 1.55 (1 H, m, H-6α), 2.15 (1 H, m, H-6β), 1.90 (1 H, m, H-7α), 2.00 (1 H, m, H-7β), 1.99 (1 H, m, H-8), 1.53 (1 H, ddd, J = 1.5, 4.5, 14 Hz; H-11α), 2.26 (1 H, ddd, J = 6, 14, 14 Hz; H-11β), 1.42 (1 H, ddd, J = 4.5, 1.5, 14 Hz; H-12α), 1.68 (1 H, ddd, J = 4.5, 14, 14 Hz; H-12β), 1.25 (1 H, dd, J = 5, 13 Hz; H-15α), 1.78 (1 H, dd, J = 10.5, 13 Hz; H-15β), 4.21 (1 H, dt, J = 4, 10.5 Hz; H-16), 1.46 (1 H, t, J = 10 Hz; H-16), 0.91 (3H, s, H-18), 3.16 (1 H, d, J = 13.5 Hz; H-19a), 1.73 (1 H, d, J = 13.5 Hz; H-19b), 2.11 (1 H, m, H-20), 0.88 (3 H, d, J = 6.5 Hz; H-21), 1.40 (1 H, dd, J = 12, 14 Hz; H-22α), 1.92 (1 H, dd, J = 4, 14 Hz; H-22β), 2.40 (1 H, dd, J = 8.5, 13 Hz; H-24α), 1.73 (1 H, t, J = 13 Hz; H-24β), 2.94 (1 H, m, H-25), 1.25 (3 H, d, J = 7 Hz; H-27), 1.40 (3H, s, H-28), 1.35 (3H, s, H-29), 1.22 (3H, s, H-30); ^{13}C-NMR (75 MHz) δ 113.2 (s, C-1), 24.6 (q, C-2), 85.3 (s, C-4), 54.5 (d, C-5), 23.6 (t, C-6), 25.3 (t, C-7), 44.0 (d, C-8), 85.0 (s, C-9), 92.1 (s, C-10), 39.4 (t, C-11), 29.7 (t, C-12), 44.6 (s, C-13), 50.4 (s, C-14), 39.5 (t, C-15), 78.0 (d, C-16), 54.7 (d, C-17), 16.0 (q, C-18), 49.2 (t, C-19), 30.7 (d, C-20), 19.2 (q, C-21), 44.7 (t, C-22), 107.5 (s, C-23), 42.8 (t, C-24), 34.1 (d, C-25), 179.5 (s, C-26), 14.9 (q, C-27), 31.4 (q, C-28), 24.4 (q, C-29), 22.2 (q, C-30).

X-ray Crystal Structure Analysis of 1·Et_2O. Crystal data: $C_{29}H_{44}O_6$·$C_4H_{10}O$, MW 562.79, monoclinic, space group $P2_1$(C_2^2), No. 4, a = 20.764(6) Å, b = 10.571-(2) Å, c = 7.488(2) Å, β = 96.05(1)°, V = 1634(1) Å^3, Z = 2, D_{calcd} = 1.143 g cm^{-3}, μ(Cu Kα: radiation, λ = 1.5418 Å) = 6.0 cm^{-1}; crystal dimensions: 0.02 × 0.32 × 0.60 mm.

Oscillation and Weissenberg photographs yielded preliminary unit-cell parameters and space group information. Intensity data ($+h,+k, \pm l$; $\theta_{max} = 50°$; 3285 nonequivalent reflections) were recorded on an Enraf-Nonius CAD-4 diffractometer [Cu Kα radiation, incident beam graphite monochromator; $\omega - 2\theta$ scans; scanwidth $(1.20 + 0.14 \tan \theta)°$]. The intensities of four strong reference reflections, monitored every 2 h during data collection, showed significant intensity loss (33%). In addition to the usual corrections for Lorentz and polarization effects, an empirical absorption correction [T_{max}: T_{min}(rel) = 1.00:0.84, based on the ϕ-dependency of the intensities of several reflections with χ *ca.* 90°] and a linear decay correction were also made to the data. The space group was established from the systematic absences: $0k0$ when $k \neq 2n$, and the fact that **1** is chiral. Refined unit-cell parameters were derived from the diffractometer setting angles for 25 reflections ($36° < \theta < 40°$) widely separated in reciprocal space. Only 1877 reflections with $I > 2.0\sigma(I)$ from the weakly diffracting crystal were retained for the analysis.

The crystal structure was solved by direct methods (MULTAN11/82). Approximate coordinates for the nonhydrogen atoms of **1** were derived in part from an *E*-map and from a series of weighted F_0 and difference Fourier syntheses phased successively by an increasing number of atoms. Positional and temperature factor parameters (first isotropic and then anisotropic) of these atoms were adjusted by means of several rounds of full-matrix least-squares calculations during which $\Sigma w\Delta^2$ [$w = 1/\sigma^2(|F_o|)$, $\Delta = (|F_o| - |F_c|)$] was minimized. A difference Fourier synthesis then revealed several peaks corresponding to a severely disordered ether solvent molecule for which all of the atoms were included with 50% site occupancy, and their positional and isotropic thermal parameters were also refined during the subsequent least-squares cycles. Hydrogen atoms, other than those of the disordered solvent molecule, were incorporated at their calculated positions during the later iterations. Parameter refinement converged (max shift:esd = 0.03) at $R = 0.091$ (Rw = 0.114) [R (= $\Sigma||F_o| - |F_c||/\Sigma|F_o|$); Rw [= $\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$; GOF = $[\Sigma w\Delta^2/(N_{obsvns} - N_{param})]^{1/2} = 2.23$]. A final difference Fourier synthesis contained no usual features [$\Delta\rho$(e·Å^3) = 0.48 (max); −0.30 (min)].[9]

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For structure−factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from the literature.[10]

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