Structure and Stereochemistry of Pseudolarolide E, a Novel Triterpene Dilactone from *Pseudolarix kaempferi*

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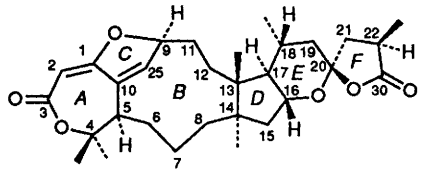
The structure and stereochemistry of pseudolarolide E, a novel triterpene dilactone isolated from the seeds of *Pseudolarix kaempferi*, have been established from spectral data and single-crystal *X*-ray analysis.

The discovery of pseudolaric acids A and B, which were previously isolated from the root bark of *Pseudolarix kaempferi* (Pinaceae), as potent cytotoxic agents,[1,2] prompted our continuing search for further novel cytotoxic antitumour compounds from other parts of this same plant. The ethereal extract of the seeds of *P. kaempferi* was found to show significant *in vitro* cytotoxicity against HCT-8 colon carcinoma cells.† We report herein on the isolation and structural characterization of a novel triterpene dilactone, pseudolarolide E (**1**).

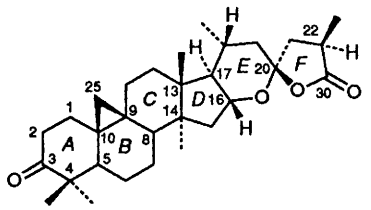
Pseudolarolide E (**1**) {$C_{30}H_{42}O_6$, m.p. 209—211 °C, $[\alpha]_D$ +2.5° (*c* 0.5, 95% EtOH)}, was isolated in 0.011% yield from the active ethereal extract of the seeds of *P. kaempferi* by silica gel chromatography. Its IR (KBr) spectrum showed bands at 1770 and 1753 (saturated γ-lactone), 1667 (conjugated unsaturated lactone), 1640 and 1612 cm^{-1} (double bond). That (**1**) contained a conjugated unsaturated system was revealed by its UV spectrum [λ_{max} 292.8 nm, (log ε 4.21)]. The ^1H NMR spectrum (400 MHz, $CDCl_3$) indicated the presence of six methyl groups [δ 0.52, 1.01, 1.41, 1.43 (each 3H, s); 0.90 (3H, d, *J* 6.5 Hz); and 1.18 (3H, d, *J* 7.2 Hz)], two low-field protons attached to carbon atoms bearing an oxygen function [δ 3.86 (1H, dt, *J* 7.2, 10.4 Hz, 16-H) and 5.49 (1H, m, 9-H)], and two olefinic protons [δ 5.46 (1H, d, *J* 1 Hz, 2-H)‡ and 6.49 (1H, d, *J* 1 Hz, 25-H)]. The ^{13}C NMR spectrum (25.1 MHz, $CDCl_3$, DEPT experiment) contained signals in the low-field region for two lactonic carbonyl carbon atoms [δ 179.2 (C-30) and 170.3 (C-3)], four olefinic carbon atoms [δ 166.1 (s, C-1), 142.3 (d, C-25), 136.7 (s, C-10), and 92.7 (d, C-2)], one ketal carbon atom [δ 106.4 (C-20)], and three carbon atoms bearing oxygen functions [δ 88.7 (d, C-9), 79.4 (s, C-4), and 74.5 (d, C-16)]. Of the latter, the signal at δ 74.5 was assigned to C-16

† *In vitro* cytotoxicity was assayed by Dr. J. J. Chang, Department of Laboratory Animal Medicine, School of Medicine, UNC-CH, according to the protocol described in ref. 1. Detailed evaluation of (**1**) as a selective cytotoxic agent is in progress.

‡ The doublet for H-2 is due to long-range coupling between H-2 and H-25.



(1)



(2)

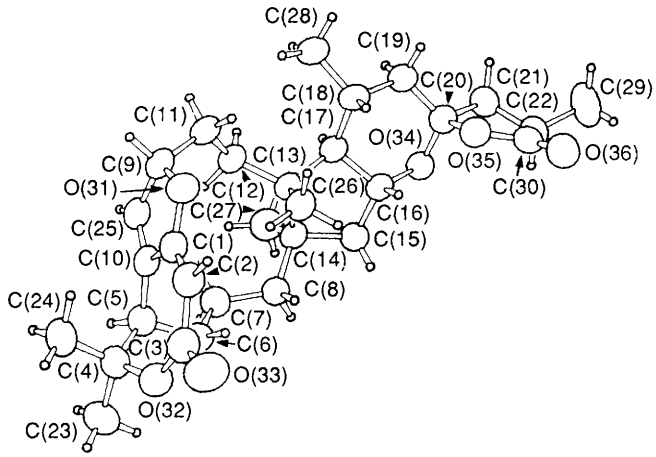


Figure 1. Structure and solid-state conformation of pseudolarolide E (**1**); small circles represent hydrogen atoms.

based on comparison of its chemical shift with that for the corresponding centre in (**2**).§ Thus, the low-field doublet at δ 88.7 must be assigned to C-9 which is allylic in nature. The foregoing data, when considered along with the fact that (**1**) contains an αβ,γδ-conjugated ketone system for which the C-1 resonance is located at low field (δ 166.1, *vide supra*), led to the conclusion that C-1 must be connected to C-9 through an oxygen bridge. The presence of the seven-membered lactone ring *A* was deduced from biogenetic considerations,[3] whereas the constitution of the fused *D*/*E*/*F*-ring moiety was derived from comparison of spectral data (^{1}H and ^{13}C NMR, mass) for (**1**) with those for (**2**).

The complete structure and stereochemistry of (**1**) were established unequivocally by *X*-ray crystallographic analysis.¶ A view of the structure is presented in Figure 1. Bond lengths and angles are, in general, close to expected values.‖ Lactone ring *A* has a very flattened twist-chair conformation.** Cycloundecene ring *B* adopts a conformation which is similar to that calculated by Anet and Rawdah[4] for the cycloundecane [121412] transition state.** Ring *C* is essentially planar, ring *D* is intermediate between half-chair and envelope forms, while rings *E* and *F* have chair and envelope conformations, respectively.

The co-occurrence of (**1**) and (**2**) in the same plant suggests that (**1**) might be derived biosynthetically from (**2**) through extensive oxidation accompanied by C-8—C-9 and C-9—C-10 bond cleavage.

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§ Compound (**2**) was also isolated from this extract. Data for (**2**) will be presented in detail elsewhere.

¶ *Crystal data* for (**1**): $C_{30}H_{42}O_6$, M = 498.67, orthorhombic, space group $P2_12_12_1$, a = 12.005(1), b = 21.972(2), c = 10.256(1) Å (from 25 orientation reflections, 42° < θ < 48°), U = 2705.3(7) Å^3, Z = 4, D_c = 1.224 g cm^{-3}, μ(Cu-K_α radiation, λ = 1.5418 Å) = 6.4 cm^{-1}; crystal size: 0.28 × 0.28 × 0.40 mm. One octant of intensity data was recorded on an Enraf-Nonius CAD-4 diffractometer (Cu-K_α radiation, graphite monochromator, ω–2θ scans, θ_{max} = 75°, 3147 reflections). The crystal structure was solved by direct methods (MULTAN11/82). Full-matrix least-squares refinement [$\Sigma w\Delta^2$ minimized; $w = 1/\sigma^2(|F_o|)$, $\Delta = (|F_o| - |F_c|)$] of atomic positional and thermal parameters (anisotropic C, O; isotropic H) converged at R = 0.034 (R_w = 0.047) for 2649 reflections with $I > 3.0\sigma(I)$. Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

‖ Strain is, however, reflected in elongation of several bonds involving the tetrasubstituted C-13 and C-14 centres, and is especially evident in the C-13—C-14 distance of 1.608(3) Å.

** Endocyclic torsion angles (ω_{ij}, σ ± 0.2—0.4°) around the bonds between atoms *i* and *j* in ring *A* ($\omega_{1,2}$ 2.3, $\omega_{2,3}$ −6.2, $\omega_{3,32}$ −19.4, $\omega_{32,4}$ 63.6, $\omega_{4,5}$ −77.4, $\omega_{5,10}$ 51.5, $\omega_{10,1}$ −14.5) are related by an approximate C_2 symmetry axis passing through C-2 and the mid-point of the C-4—C-5 bond; the flattened twist-chair conformation characterized by these torsion angles may alternatively be described as a 'half twist-chair' form by analogy with the cyclopentane and cyclohexane half-chair forms. For ring *B*, the torsion angles ($\omega_{5,6}$ −61.9, $\omega_{6,7}$ 142.4, $\omega_{7,8}$ −140.8, $\omega_{8,14}$ 73.5, $\omega_{14,13}$ −88.3, $\omega_{13,12}$ 149.0, $\omega_{12,11}$ −98.4, $\omega_{11,9}$ −15.8, $\omega_{9,25}$ 118.1, $\omega_{25,10}$ −166.5, $\omega_{10,5}$ 92.5°) are related by an approximate mirror plane of symmetry passing through C-11 and the mid-point of the C-6—C-7 bond (corresponding values for the [121412] transition state of cycloundecane are: −60, 122, −141, 76, −105, 163, −94, 0, 90, −170, 112°).