

**STRUCTURE AND STEREOCHEMISTRY OF PSEUDOLAROLIDE-I,
A NOVEL CYTOTOXIC PEROXYTRITERPENE DILACTONE FROM
*PSEUDOLARIX KAEMPFERI***

Guo-Fu Chen,^a Zhu-Lian Li,^{a,*} Cheng-Min Tang,^b Xiang He,^b Ke Chen,^c De-Ji Pan,^a
Chang-Qi Hu,^c Donald R. McPhail,^d Andrew T. McPhail,^{d,*} and Kuo-Hsiung Lee^{c,*}

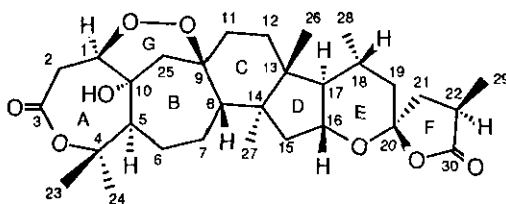
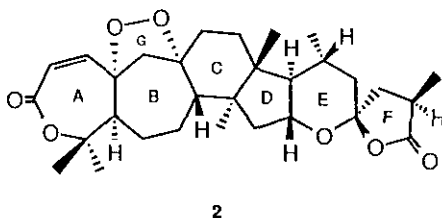
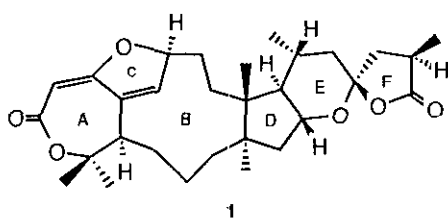
- a. Department of Chemistry of Natural Drugs, School of Pharmacy, Shanghai Medical University, Shanghai 200032, People's Republic of China
- b. Shanghai Institute of Materia Medica, Academia Sinica, Shanghai 200032, People's Republic of China
- c. Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, U.S.A.
- d. Department of Chemistry, Duke University, Durham, North Carolina 27706, U.S.A.

Abstract - A novel cytotoxic peroxytriterpene dilactone, pseudolarolide I, has been isolated from the seeds of *Pseudolarix kaempferi*, and its structure and stereochemistry have been established from spectral data in conjunction with a single-crystal X-ray analysis.

The root bark of *Pseudolarix kaempferi* (Pinaceae), known as "Tu-Jin-Pi" in Chinese folk medicine, has been used for the treatment of skin diseases caused by fungi.¹ A variety of structurally novel di- and triterpenes have been isolated recently from *P. kaempferi*² and some of them, such as pseudolaric acids-A and -B, have been found to demonstrate potent cytotoxicity.³ Our recent investigations on other parts of this same plant for further novel cytotoxic antitumor compounds revealed that the ethereal extract of the seeds showed significant *in vitro* cytotoxicity against KB tissue culture cells. This extract has already been shown to contain two novel triterpene dilactones, viz. pseudolarolide-E (1)⁴ and pseudolarolide-H (2).⁵ We report herein on the isolation and structural characterization of a further novel cytotoxic⁶ triterpene dilactone, pseudolarolide-I (3), from this same extract.

Purification of the ethereal extract of the seeds (1.8 kg) of *P. kaempferi* by silica gel column chromatography, employing benzene-acetone (8:1) as eluant, afforded 240 mg (0.0133% yield) of pseudolarolide-I (**3**) in the form of needles from ethanol [mp 203-205 °C, R_f 0.3 [SiO₂ plate, benzene-acetone (5:1)]; uv (EtOH) $\lambda_{\max}$ 205(log ϵ 3.28) nm; cd [c 1.2 mmol/l, MeOH, $\Delta\epsilon$ (nm) -2.08 (215°, broad)]; ir, ¹H- and ¹³C-nmr spectral data for **3** are provided as a footnote.⁹

The molecular formula for **3**, C₃₀H₄₄O₈, was deduced from hrms and eims peaks at m/z 514.2934 ($M^+ - H_2O$, 1.6%, for C₃₀H₄₂O₇, calcd 514.2930) and 532 (M^+ , 1.6%), respectively. The ir spectrum indicated that the hydroxyl group and two lactonic carbonyls, one of which was a γ -lactone, were present in **3**. The ¹H nmr spectrum contained signals for six methyl groups, of which two were attached to oxygen-bearing quaternary carbon atoms (δ 1.51, 3H, s, and 1.70, 3H, s), two were located at quaternary carbon atoms (δ 1.02, 3H, s, and 1.00, 3H, s), and two were bonded to tertiary carbon atoms (δ 1.22, 3H, d, $J = 7.3$ Hz, and δ 0.84, 3H, d, $J = 6.4$ Hz). These data, coupled with the lack of any cyclopropyl group resonances, suggested that **3** was a triterpene possessing a 9,10-seco-cycloartane skeleton in which ring A had suffered oxidative cleavage between C₃ and C₄ to produce a seven-membered lactone ring similar to those in **1** and **2**. The mass spectrum of **3** revealed fragments at m/z 450 (4.3%) and 482 (5%) [arising from ($M^+ (m/z$ 532, 1.6%) - O₂) and ($M^+ - H_2O - O_2$), respectively] which were characteristic of the presence of a peroxy moiety in the parent compound. A hrms fragment at m/z 139.0728 (26.8%, C₈H₁₁O₂) was typical for rings E and F of pseudolarolides. The other ¹H nmr signals, which were assigned by use of the ¹H-¹H COSY technique, included an AB doublet (CH₂-25) which appeared in the low-field region, due to the effect of the peroxy bridge. One of the two low-field protons, which was attached to carbon bearing an oxygen function, was assigned to H₁₆ based upon a comparison with the nmr spectrum of **1**. The other low-



field proton was ascribed to H-1, due to the formation of an ABX system through coupling to a methylene group adjacent to the C₃ carbonyl moiety. The foregoing evidence, coupled with the co-occurrence of **1** and **2**, **3**, in which all contain a seven-membered lactone ring A, led to the conclusion that C₉ must be connected to C₁ through a peroxy bridge with the hydroxyl group located at C₁₀. The nature of rings C, D, E, and F was derived from biogenetic considerations and comparison of the spectral data (¹H- and ¹³C-nmr, ms) for **3** with corresponding data for **1** and **2**.

An X-ray crystallographic analysis¹⁰ established unequivocally the complete structure and stereochemistry of **3**. A view of the structure is provided in Figure 1. Bond lengths and angles are in accord with expectations. Seven-membered rings A and B have chair conformations. Six-membered rings C and E are in chair forms whereas ring C, with two small endocyclic torsion angles about the C₈-C₉ and C₁₁-C₁₂ bonds, has a 1,3-diplanar form. Cyclopentane ring D is in a half-chair conformation while γ-lactone ring F has an envelope form. In crystals of **3**, molecules related by unit translation along *c* are linked via an O₃₅-H₃₅...O₃₁ hydrogen bond [O...O = 3.039(2) Å].

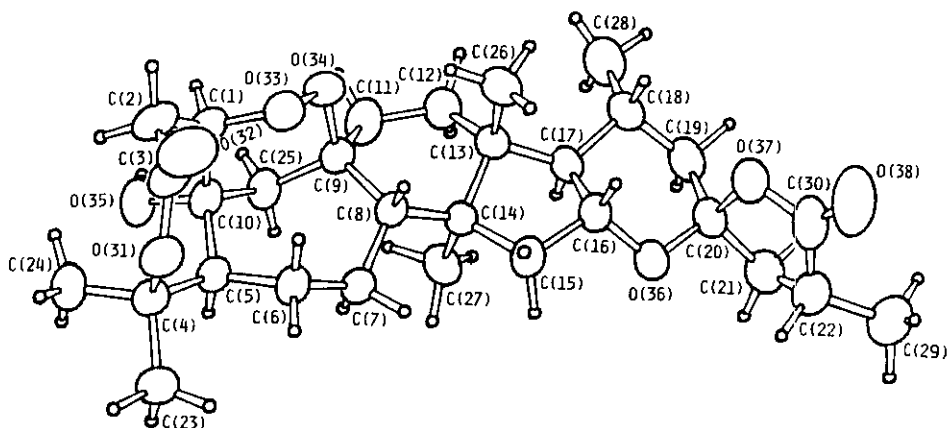


Figure 1. Structure and solid-state conformation of pseudolarolide-I (**3**); small circles represent hydrogen atoms.

ACKNOWLEDGEMENT

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6. Pseudolarolide-I showed significant cytotoxicity against KB cells with $ED_{50} = 0.37 \mu\text{g/ml}$. *In vitro* cytotoxicity was assayed by Dr. J. J. Chang, Department of Laboratory Animal Medicine, School of Medicine, University of North Carolina at Chapel Hill, by a literature procedure.^{7,8}
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9. The ir and nmr spectral data for **3** are described below:
 Ir (KBr) ν_{max} : 3510, 1775, 1710, 1450, 1377, 1357, 1276, 1210, 1075, 1025, 970, and 885 cm^{-1} .
 ^1H Nmr (400 MHz, CDCl_3): δ 4.11 (1H, dd, $J = 7.3 \text{ Hz}$ and 4.8 Hz , H-1), 4.07 (1H, td, $J = 10.4 \text{ Hz}$ and 4.9 Hz , H-16), 3.23 (1H, dd, $J = 14.5$ and 4.8 Hz , H-2), 2.90 (1H, m, H-22), 2.86 (1H, dd, $J = 14.5 \text{ Hz}$ and 7.3 Hz , H-2), 2.36 (1H, dd, $J = 12.8 \text{ Hz}$ and 8.5 Hz , H-21), 2.21 (1H, s, OH-10), 2.17 (1H, m, H-5), 2.09 (1H, m, H-8), 2.09 (1H, d, $J = 13.7 \text{ Hz}$, H-25), 2.07 (1H, m, H-18), 1.89 (1H, dd, $J = 13.8 \text{ Hz}$ and 3.9 Hz , H-19), 1.84 (1H, m, H-15), 1.70 (1H, m, H-21), 1.70 (3H, s, H-23), 1.57 (1H, d, $J = 13.7 \text{ Hz}$, H-25), 1.51 (3H, s, H-24), 1.44 (1H, m, H-17), 1.39 (1H, m, H-19), 1.26 (1H, dd, $J = 12 \text{ Hz}$ and 4.9 Hz , H-15), 1.22 (3H, d, $J = 7.3 \text{ Hz}$, H-29), 1.02 (3H, s, H-27), 1.00 (3H, s, H-26), and 0.84 (3H, d, $J = 6.4 \text{ Hz}$, H-28). ^{13}C Nmr (25 MHz, CDCl_3 , DEPT experiment): δ 181.39 (C_{30}), 171.50 (C_3), 109.25 (C_{20}), 87.08 (C_{10}), 85.80 (C_9), 84.46 (C_1), 79.31 (C_{16}), 75.03 (C_4), 58.52 (C_5), 57.99 (C_{17}), 53.48 (C_{25}), 52.10 (C_8), 50.12 (C_{14}), 46.40 (C_2), 45.21 (C_{13}), 44.88 (C_{19}), 43.40 (C_{21}), 37.22 (C_{15}), 36.18 (C_{22}), 35.56, 32.19, 30.12, 28.69 (C_6 , C_7 , C_{11} , C_{12}), 33.92, 32.19 (C_{23} , C_{24}), 24.28 (C_{27}), 21.12 (C_{26}), 20.41 (C_{28}), and 16.99 (C_{29}).
10. Crystal data: $\text{C}_{30}\text{H}_{44}\text{O}_8$ (**3**), $M = 532.68$, orthorhombic, space group $P2_12_12_1$, $a = 8.090(1) \text{ \AA}$, $b = 50.299(3) \text{ \AA}$, $c = 6.887(1) \text{ \AA}$ (from 25 orientation reflections, $41^\circ < \theta < 47^\circ$), $V = 2802.5(9) \text{ \AA}^3$, $Z = 4$, $d_{\text{calcd.}} = 1.262 \text{ g cm}^{-3}$, $\mu(\text{Cu-K}\alpha) = 7.0 \text{ cm}^{-1}$. Intensity data ($+h, +k, +l$; 3346 reflections; $\theta_{\text{max}} = 75^\circ$) were recorded on an Enraf-Nonius CAD-4 diffractometer (Cu-K α radiation, $\lambda = 1.5418 \text{ \AA}$; graphite monochromator; ω -2 θ scans). The crystal structure was solved by direct methods (MULTAN11/82). Full-matrix least-squares refinement of atomic parameters (anisotropic C, O; isotropic H) converged (max. shift $< 0.02\sigma$) at $R = 0.034$ ($R_w = 0.047$, GOF = 1.34) over 2762 reflections with $I > 3.0\sigma(I)$. Atomic positional and thermal parameters, bond lengths and angles for **3** have been deposited with the Cambridge Crystallographic Data Centre.

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