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

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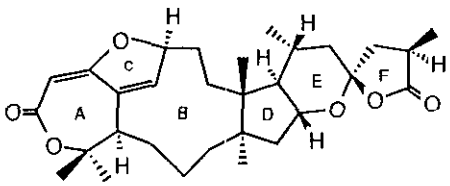
Abstract - A novel cytotoxic peroxytriterpene dilactone, pseudolarolide I, has been isolated from the seeds of *Pseudolarix kaempferi*, and its structure and stereochemistry have been established from spectral data in conjunction with a single-crystal *X*-ray analysis.

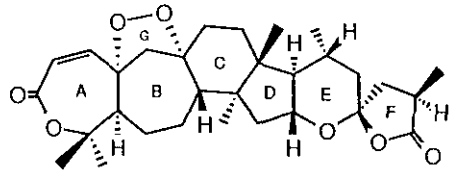
The root bark of *Pseudolarix kaempferi* (Pinaceae), known as "Tu-Jin-Pi" in Chinese folk medicine, has been used for the treatment of skin diseases caused by fungi.[1] A variety of structurally novel di- and triterpenes have been isolated recently from *P. kaempferi*[2] and some of them, such as pseudolaric 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 cytotoxic[6] 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 [SiO_2 plate, benzene-acetone (5:1)]; uv (EtOH) λ_{max} 205(logε 3.28) nm; cd [c 1.2 mmol/l, MeOH, $\Delta\varepsilon$(nm) -2.08 (215°, broad)]]; ir, ^{1}H- and ^{13}C-nmr spectral data for **3** are provided as a footnote.[9]

The molecular formula for **3**, $C_{30}H_{44}O_8$, was deduced from hrms and eims peaks at *m/z* 514.2934 (M^+ - H_2O, 1.6%, for $C_{30}H_{42}O_7$, 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 ^{1}H 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 C_3 and C_4 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%) - O_2] and (M^+ - H_2O - O_2), respectively] which were characteristic of the presence of a peroxyl moiety in the parent compound. A hrms fragment at *m/z* 139.0728 (26.8%, $C_8H_{11}O_2$) was typical for rings E and F of pseudolarolides. The other ^{1}H nmr signals, which were assigned by use of the ^{1}H-^{1}H COSY technique, included an AB doublet (CH_2-25) which appeared in the low-field region, due to the effect of the peroxyl bridge. One of the two low-field protons, which was attached to carbon bearing an oxygen function, was assigned to H_{16} based upon a comparison with the nmr spectrum of **1**. The other low-



1



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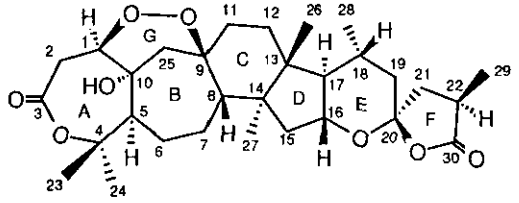

3

field proton was ascribed to H-1, due to the formation of an ABX system through coupling to a methylene group adjacent to the C_3 carbonyl moiety. The foregoing evidence, coupled with the co-occurence of **1** and **2**, **3**, in which all contain a seven-membered lactone ring A, led to the conclusion that C_9 must be connected to C_1 through a peroxyl bridge with the hydroxyl group located at C_{10}. 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 **3** with corresponding data for **1** and **2**.

An *X*-ray crystallographic analysis[10] 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 C_8-C_9 and C_{11}-C_{12} 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 O_{35}-H_{35}...O_{31} hydrogen bond [O...O = 3.039(2) Å].

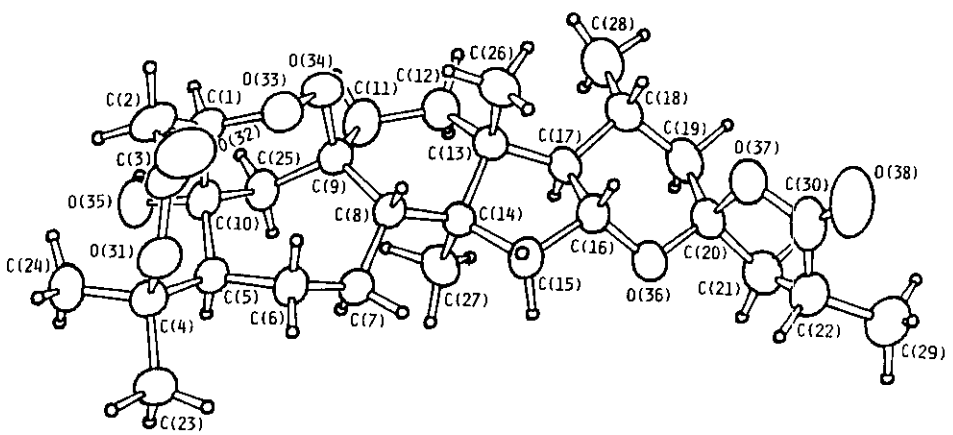

Figure 1. Structure and solid-state conformation of pseudolarolide-1 (**3**); small circles represent hydrogen atoms.

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6. Pseudolarolide-I showed significant cytotoxicity against KB cells with ED_{50} = 0.37 μg/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 Hz and 4.8 Hz, H-1), 4.07 (1H, td, J = 10.4 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 Hz and 7.3 Hz, H-2), 2.36 (1H, dd, J = 12.8 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 Hz, H-25), 2.07 (1H, m, H-18), 1.89 (1H, dd, J = 13.8 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 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 Hz and 4.9 Hz, H-15), 1.22 (3H, d, J = 7.3 Hz, H-29), 1.02 (3H, s, H-27), 1.00 (3H, s, H-26), and 0.84 (3H, d, J = 6.4 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: $C_{30}H_{44}O_8$ (**3**), M =532.68, orthorhombic, space group $P2_12_12_1$, a = 8.090(1) Å, b = 50.299(3) Å, c = 6.887(1) Å (from 25 orientation reflections, 41° < θ < 47°), V = 2802.5(9) $Å^3$, Z = 4, $d_{calcd.}$ = 1.262 g cm^{-3}, μ(Cu-Kα) = 7.0 cm^{-1}. Intensity data ($+h,+k,+l$; 3346 reflections; θ_{max} = 75°) were recorded on an Enraf-Nonius CAD-4 diffractometer (Cu-Kα radiation, λ = 1.5418 Å; graphite monochromator; ω-2θ 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 <0.02σ) at R = 0.034 (R_w = 0.047, GOF = 1.34) over 2762 reflections with I > 3.0σ(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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