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STRUCTURE AND STEREOCHEMISTRY OF PSEUDOLAROLIDE-H, A NOVEL PEROXY TRITERPENE DILACTONE FROM *PSEUDOLARIX KAEMPFERI*

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Summary: A novel peroxy triterpene dilactone, pseudolarolide-H, has been isolated from the seeds of *Pseudolarix kaempferi*, and its structure and stereochemistry have been established from spectral data and single-crystal X-ray analysis.

"Tu-Jin-Pi", the root bark of *P. kaempferi* (Pinaceae), has been used as a folk medicine in China for the treatment of skin diseases caused by fungi. Discovery of a variety of structurally novel di- and tri-terpenes, including potent cytotoxic pseudolaric acid-A and -B,[1,2] prompted our continuing search for further novel cytotoxic antitumor compounds from other parts of this same plant. The ethereal extract of the seeds of *P. kaempferi* yielded the novel triterpene dilactone pseudolarolide-E(**1**).[3] We report herein on the isolation and structural characterization of pseudolarolide-H (**2**), a novel peroxy triterpene dilactone from this same extract.

Pseudolarolide-H (**2**) was isolated in 0.0044% yield from the active ethereal extract of the seeds of *P. kaempferi* by silica gel chromatography. Compound **2** [$C_{30}H_{42}O_7$; calc. (M^+) m/z 514.2930, found 514.2949] crystallized as colorless prisms, m.p. 218-221 °C (acetone). Its i.r. [ν_{max} (KBr) cm^{-1}] spectrum revealed the presence of a γ-lactone (1170), an α,β-unsaturated lactone (1692), and a double bond (1642). The ^1H n.m.r. spectrum (400MHz, $CDCl_3$) of **2** contained signals for two mutually coupled olefinic protons [δ 6.02, 1H, d, J = 12.5 Hz (H-1); δ 5.92, 1H, d, J = 12.5 Hz (H-2)] and six methyl groups of which two were bonded to quaternary oxygen-bearing carbon atoms (δ 1.41, 3H, s; δ 1.37, 3H, s), two were attached to quaternary carbon atoms (δ 0.84, 3H, s; δ 1.15, 3H, s), and two were located at tertiary carbon centers (δ 1.23, 3H, d, J = 7.2 Hz; δ 0.88, 3H, d, J = 6.6 Hz). This evidence, coupled with the lack of any

cyclopropyl group signals, suggested that **2** was a triterpene possessing a 9,10-seco-cycloartane skeleton wherein ring A had suffered an oxidative cleavage between C(3) and C(4) to form a seven-membered lactone ring similar to that in **1**, which was isolated previously from this same extract. The mass spectrum of **2** revealed a fragment at m/z 482.3045 ($C_{30}H_{42}O_5$) originating from a molecular ion resulting from loss of O_2 and characteristic of the presence of a peroxyl group in the parent compound. An AB doublet for the C(25) methylene group protons occurred in the low-field region (δ 2.73, 1H, d, J = 12.4 Hz; δ 2.15, 1H, d, J = 12.4 Hz) due to the effect of the peroxyl bridge. These data suggested that the peroxyl moiety was located between C(9) and C(10). The α-orientation of the peroxyl bridge was deduced from nOe difference spectra: thus, irradiation of the H-5 signal (δ 2.93) did not cause any nOe of H-25β whereas irradiation of the CH_3-C(23) signal (δ 1.41) did produce an nOe with H-25β, indicating that the latter lay close to the axial β-oriented CH_3-C(23).

The constitution of rings C, D, E, and F, was derived from biogenetic considerations and comparison of the ^{1}H n.m.r. and mass spectral data for **2** with those of pseudolarolide-A (**3**)[4] and -D (**4**),[4] as well as **1**.

The complete structure and stereochemistry of **2** were established unequivocally by X-ray crystallographic analysis.[5] A view of the solid-state conformation is provided in Figure 1. Bond lengths and angles are in accord with expectations.

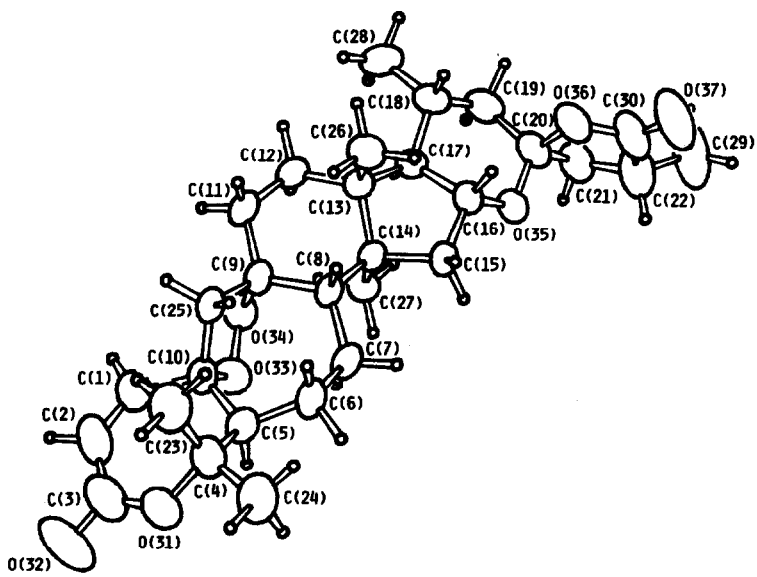
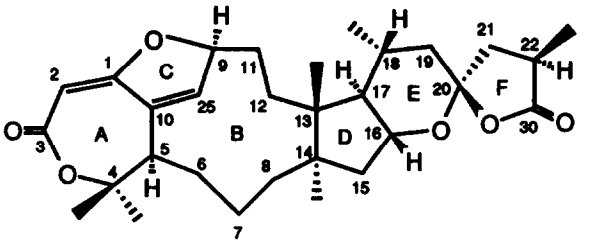
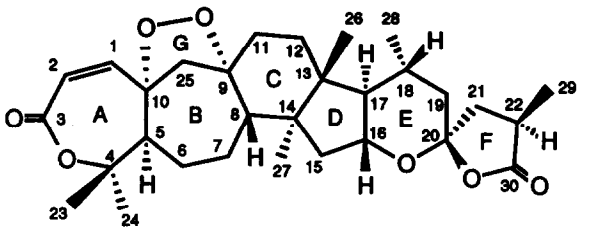


Figure 1. Structure and solid-state conformation of pseudolarolide-H (**2**); small circles represent hydrogen atoms.



1



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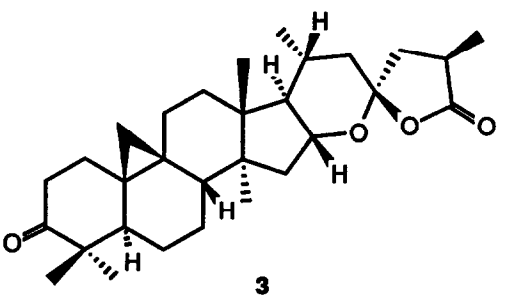

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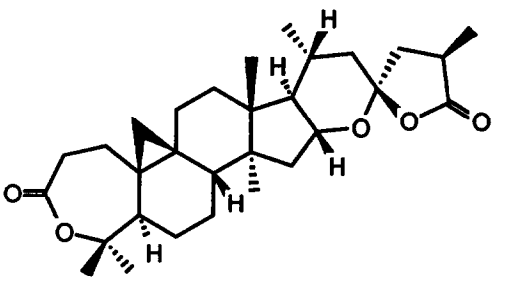

4

α,β-Unsaturated ε-lactone ring A has a conformation characterized by three adjacent small torsion angles and, by analogy with the cyclopentane and cyclohexane half-chair counterparts it also may be described as a half-chair form in which the approximate C_2-axis of symmetry passes through C(2) and the mid-point of the C(4)-C(5) bond. Endocyclic torsion angles for ring B are related by an approximate C_2 symmetry axis passing through C(5) and the mid-point of the C(8)-C(9) bond, and it has a twist-chair form. Six-membered rings C and E are both in flattened chair conformations. Five-membered rings D and F have envelope forms in which C(13) and C(21), respectively, are the out-of-plane atoms whereas ring G is in a half-chair conformation with its C_2 symmetry axis passing through C(10).

The co-occurrence of **2** and **4** in the same plant suggests that **2** might be derived biosynthetically from **4** through peroxidation accompanied by C(9)-C(10) bond cleavage.

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References and Notes

1. Z. L. Li, K. Chen, D. J. Pan, and G. Y. Xu, *Acta Chimica Sinica* ,1989, **47**, 258.
2. D. J. Pan, Z. L. Li, C. Q. Hu, K. Chen, J. J. Chang, and K. H. Lee, *Planta Medica*, in press.
3. G. F. Chen, Z. L. Li, K. Chen, C. M. Tang, X. He, D. J. Pan, C. Q. Hu, D. R. McPhail, A. T. McPhail, and K. H. Lee, *J. Chem. Soc., Chem. Commun.*, submitted.
4. Data for compounds **3** and **4**, which were also isolated from this same extract, will be presented in detail elsewhere.
5. *Crystal data.* $C_{30}H_{42}O_7$ (**2**), M = 514.67, orthorhombic, space group $P2_12_12_1$, a = 18.169(1), b = 19.489(1), c = 7.792(1) Å (from 25 orientation reflections, $42° < \theta < 48°$), V = 2759.1(6) Å^3, Z = 4, D_c = 1.239 g cm^{-3}, μ(Cu-$K\alpha$ radiation, λ = 1.5418 Å) = 6.7 cm^{-1}; crystal dimensions: 0.20 x 0.20 x 0.40 mm. Intensity data ($+h,+k,+l$; θ_{max} = 75°; 3215 reflections) were recorded on an Enraf-Nonius CAD-4 diffractometer (Cu-$K\alpha$ radiation, 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; fixed H contributions) converged at R = 0.053 (R_w = 0.075) over 2558 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 parameters, bond lengths and angles for **2** have been deposited at the Cambridge Crystallographic Data Center.

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