

Pseudolarolides O and P, Two Novel Triterpene Dilactones from *Pseudolarix kaempferi*

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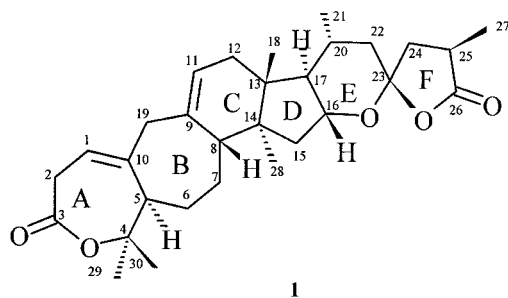
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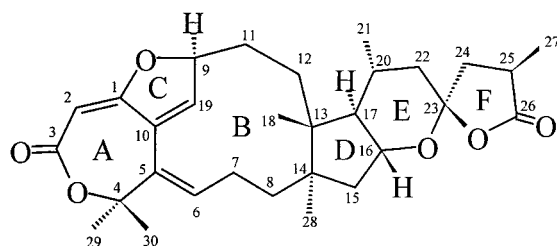
Pseudolarolides O (**1**) and P (**2**), two novel triterpenoids with a cycloartane-type framework, were isolated from the seeds of *Pseudolarix kaempferi* Gord. (Pinaceae). Their structures were elucidated as (16*R*,23*S*,25*R*)-16,23-epoxy-3,4:9,10-disecocycloartan-1(10),9(11)-diene-3(4),26(23)-diolide (**1**), and (9*S*,16*R*,23*S*,25*R*)-1,9:16,23-diepoxy-8,9:9,10-disecocycloartan-1(29),5(6),10(19)-triene-3(4),26(23)-diolide (**2**), respectively, on the basis of spectroscopic techniques and X-ray-diffraction studies.

Introduction. – The root bark of *Pseudolarix kaempferi* Gord. (Pinaceae), a large tree indigenous to Southeast China, is known as ‘Tu-Jin-Pi’ in Chinese folk medicine for the treatment of skin diseases caused by fungi [1]. Many new diterpenes and triterpenes demonstrating potent cytotoxicity have been isolated from this plant since 1982 [2–15]. In the course of our continuing search for novel potent antitumor agents [8–14], we investigated other parts of this plant and found that the CHCl₃ extract of the seeds showed significant cytotoxicity against KB, A-549, HCT-8, and P-388 cell lines *in vitro*. Bio-assay-directed fractionation of active extract has led to the isolation of pseudolarolides O (**1**), and P (**2**), two novel cycloartane-type triterpenoids with 3,4-seco and 9,10-seco skeletons. However, the two compounds showed no bioactivity in the tests. Herein, we report the isolation and structural elucidation of compounds **1** and **2**.

Results and Discussion. – Pseudolarolide O (**1**) was obtained as colorless plates from acetone (mp. 239–240°). Its HR-EI mass spectrum gave the molecular formula C₃₀H₄₂O₅ (*m/z* 482.3018 (C₃₀H₄₂O₅⁺; calc. 482.3032)) with ten degrees of unsaturation. The IR spectrum showed absorption bands for a γ -lactone (1770), together with a seven-membered-ring lactone (1724), and two C=C bond groups (1635 and 1610). The ¹H-NMR spectrum (*Table*) displayed signals due to four tertiary (δ 0.72, 0.88, 1.45, and 1.59) and two secondary (δ 0.89 ($J=7.2$ Hz) and 1.23 ($J=7.2$ Hz) Me groups. The ¹³C-NMR spectrum (*Table*) indicated 30 ¹³C signals, including two lactone C-atoms (δ 179.6 and 171.8), two pairs of trisubstituted C=C bond C-atoms (δ 141.6, 139.8, 119.5 and 115.2), a ketal C-atom (δ 107.3), an O-bearing quaternary C-atom (δ 81.6), and an O-bearing CH C-atom (δ 77.2). This evidence, combined with missing cyclopropane CH₂ signals in the ¹H-NMR spectrum, demonstrated **1** should be a 9,10-secocycloartane-type triterpenoid with a lactone function instead of one of the terminal Me groups of the side chain.



1



2

On the basis of ten degrees of unsaturation, **1** must have a hexacyclic structure, indicating that two new rings as the D ring were formed through the side chain as well. The quaternary C-atom signal at δ 107.3 indicated the existence of the spiro E and F rings [10]. Two C=C bond groups were positioned as C(1)=C(10), and C(9)=C(11), on the basis of the CH₂(19) signals (δ 2.99 and 2.71, each *d*, *J* = 14.3 Hz) and the possible biogenic pathway. The characteristic chemical shift of C(4) of general cycloartane-type triterpenes was upshifted to δ 81.6, implying that the other lactone group was derived from C(3) and its O-atom connected to C(4).

The postulated gross structure was further supported by the detailed analysis of the HMQC, ¹H,¹H-COSY, and HMBC spectra. The HMBC and HMQC spectra disclosed the long-range correlations signals for C(3)/H–C(1), CH₂(2) and Me(30); C(26)/H–C(25), CH₂(24), and Me(27); C(13)/H–C(8), H–C(11), CH₂(12), CH₂(13), and H–C(17); C–(23)/H–C(16), H–C(20), Me(21), CH₂(22), and CH₂(24); and CH₂(19)/C(1), C(10), C(5), C(9), and C(11), which confirmed the presence of a seven-membered A ring containing a lactone, and the spiro E and F rings. Thus, the gross structure of pseudolarolide O was defined as **1**. Its relative configuration was determined by the X-ray-diffraction analysis¹⁾. A view of the solid-state conformation and its packing arrangement in crystals are shown in *Fig. 1*. Therefore, **1** was established as (16*R*,23*S*,25*R*)-16,23-epoxy-3,4:9,10-disecocycloartan-1(10),9(11)-diene-3(4),26(23)-diolide.

¹⁾ The crystallographic data have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC-189276 (**1**) and No. CCDC-196935 (**2**). Copies of data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB21EZ UK (fax: + 44(1223)336033; e-mail: deposit@ccdc.ac.uk).

Table. ^1H - (400 MHz) and ^{13}C - (100 MHz) NMR Data of **1** and **2** (CDCl_3 , δ [ppm])

Position	$\delta(\text{H})$ of 1	$\delta(\text{H})$ of 2	$\delta(\text{C})$ of 1	$\delta(\text{C})$ of 2
1	5.26 (<i>d</i> , $J = 7.0$)	–	119.5 (<i>d</i>)	166.4 (<i>s</i>)
2	α : 3.38 (<i>dd</i> , $J = 19.5, 0.6$) β : 3.24 (<i>dd</i> , $J = 19.5, 7.0$)	5.51 (<i>s</i>)	37.5 (<i>t</i>)	92.3 (<i>d</i>)
3	–	–	171.8 (<i>s</i>)	171.8 (<i>s</i>)
4	–	–	81.6 (<i>s</i>)	79.0 (<i>s</i>)
5	2.13 (<i>dd</i> , $J = 3.6, 1.7$)	–	55.4 (<i>d</i>)	133.0 (<i>s</i>)
6	α : 2.31 (<i>ddd</i> , $J = 10.5, 4.6, 1.7$) β : 1.32 (<i>ddd</i> , $J = 10.5, 3.6, 1.3$)	6.33 (<i>dd</i> , $J = 10.1, 6.8$)	30.9 (<i>t</i>)	134.0 (<i>d</i>)
7 α	1.43 (<i>m</i>)	2.79 (<i>m</i>)	27.2 (<i>t</i>)	23.8 (<i>t</i>)
7 β	1.60 (<i>m</i>)	1.98 (<i>m</i>)		
8	1.98 (<i>m</i>)	α : 1.47 (<i>m</i>) β : 1.24 (<i>m</i>)	46.0 (<i>d</i>)	35.8 (<i>t</i>)
9	–	5.61 (<i>dd</i> , $J = 5.8, 0.8$)	139.8 (<i>s</i>)	89.0 (<i>d</i>)
10	–	–	141.6 (<i>s</i>)	133.0 (<i>s</i>)
11	5.25 (<i>m</i>)	α : 2.28 (<i>m</i>) β : 2.34 (<i>m</i>)	115.2 (<i>d</i>)	31.3 (<i>t</i>)
12 α	2.01 (<i>m</i>)	2.17 (<i>m</i>)	36.2 (<i>t</i>)	33.3 (<i>t</i>)
12 β	1.81 (<i>m</i>)	1.58 (<i>m</i>)		
13	–	–	42.6 (<i>s</i>)	44.8 (<i>s</i>)
14	–	–	47.6 (<i>s</i>)	46.7 (<i>s</i>)
15 α	1.26 (<i>dd</i> , $J = 14.1, 5.2$)	1.32 (<i>dd</i> , $J = 14.0, 7.0$)	38.4 (<i>t</i>)	44.0 (<i>t</i>)
15 β	1.80 (<i>dd</i> , $J = 14.1, 10.7$)	1.63 (<i>dd</i> , $J = 14.0, 10.4$)		
16	4.10 (<i>dt</i> , $J = 10.7, 5.2$)	3.83 (<i>td</i> , $J = 10.5, 5.9$)	77.2 (<i>d</i>)	74.7 (<i>d</i>)
17	1.45 (<i>t</i> , $J = 10.4$)	1.38 (<i>t</i> , $J = 10.3$)	54.2 (<i>d</i>)	59.5 (<i>d</i>)
18	0.72 (<i>s</i> , 3 H)	0.59 (<i>s</i> , 3 H)	16.6 (<i>q</i>)	17.2 (<i>q</i>)
19	α : 2.71 (<i>d</i> , $J = 14.3$) β : 2.99 (<i>d</i> , $J = 14.3$)	6.77 (<i>s</i>)	47.4 (<i>t</i>)	142.7 (<i>d</i>)
20	2.08 (<i>m</i>)	2.04 (<i>m</i>)	30.2 (<i>d</i>)	29.9 (<i>d</i>)
21	0.89 (<i>d</i> , $J = 7.2, 3$ H)	0.93 (<i>d</i> , $J = 6.4, 3$ H)	19.3 (<i>q</i>)	20.0 (<i>q</i>)
22 α	1.38 (<i>dd</i> , $J = 14.0, 11.7$)	1.36 (<i>dd</i> , $J = 14.1, 11.6$)	44.4 (<i>t</i>)	44.6 (<i>t</i>)
22 β	1.90 (<i>dd</i> , $J = 14.0, 3.9$)	1.87 (<i>dd</i> , $J = 14.1, 4.1$)		
23	–	–	107.3 (<i>s</i>)	106.6 (<i>s</i>)
24 α	2.39 (<i>dd</i> , $J = 12.9, 8.4$)	2.35 (<i>dd</i> , $J = 12.9, 8.4$)	42.9 (<i>t</i>)	42.7 (<i>t</i>)
24 β	1.70 (<i>dd</i> , $J = 12.9, 11.6$)	1.68 (<i>dd</i> , $J = 12.9, 11.5$)		
25	2.93 (<i>m</i>)	2.90 (<i>m</i>)	34.2 (<i>d</i>)	34.2 (<i>d</i>)
26	–	–	179.6 (<i>s</i>)	179.5 (<i>s</i>)
27	1.23 (<i>d</i> , $J = 7.2, 3$ H)	1.22 (<i>d</i> , $J = 7.3$)	15.0 (<i>q</i>)	15.0 (<i>q</i>)
28	0.88 (<i>s</i> , 3 H)	1.07 (<i>s</i> , 3 H)	20.7 (<i>q</i>)	23.0 (<i>q</i>)
29	1.59 (<i>s</i> , 3 H)	1.52 (<i>s</i> , 3 H)	26.2 (<i>q</i>)	26.0 (<i>q</i>)
30	1.45 (<i>s</i> , 3 H)	1.63 (<i>s</i> , 3 H)	29.1 (<i>q</i>)	27.2 (<i>q</i>)

The molecular formula $\text{C}_{30}\text{H}_{40}\text{O}_6$ for pseudolarolide P (**2**) was deduced from the HR-EI mass spectrum (m/z 496.2825 ($\text{C}_{30}\text{H}_{40}\text{O}_6^+$; calc. 496.2825)). A *Liebermann-Burchard* test gave a dark brown color. The UV absorptions at λ_{max} 294.5 nm ($\log \varepsilon$ 3.86), and 231.5 nm ($\log \varepsilon$ 4.03) revealed the presence of a long conjugated unsaturated system. The IR bands showed the existences of a γ -lactone (1770 and 1759 cm^{-1}) and a conjugated unsaturated lactone (1672 cm^{-1}), and C=C bond (1643 and 1610 cm^{-1}) groups. The ^{13}C - and ^1H -NMR spectra (Table) displayed 30 C-atom and 40 C-bonded H-atom signals, which were attributed to two lactones (δ 179.5 and 171.8), three couples of trisubstituted C=C bonds (δ 166.4, 142.7, 134.0, 133.0, 133.0, and 92.3), a

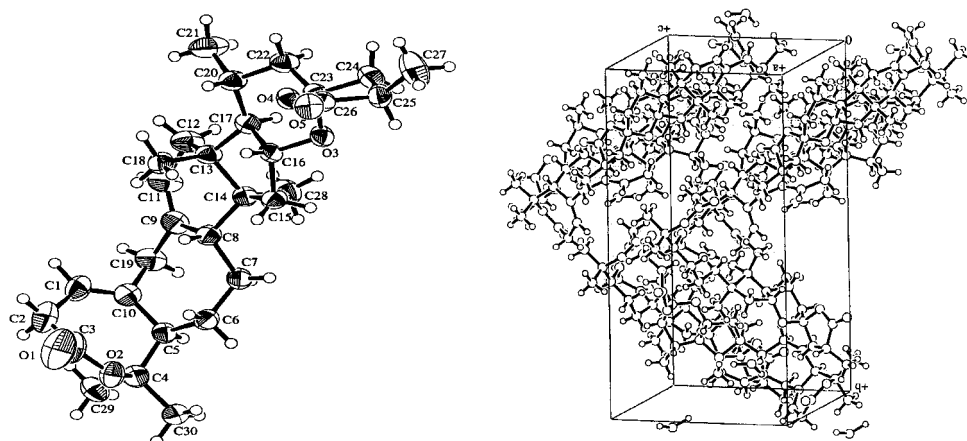


Fig. 1. The ORTEP view of **1** and its packing arrangement in crystals

ketal (δ 106.6), two O-bearing CH groups (δ 89.0 and 74.7), and an O-bearing quaternary C-atom (δ 79.0), and 18 upfield C-atoms (six Me, seven CH_2 , three CH groups, and two quaternary C-atoms).

Comparison of the NMR data of **2** with those of **1** indicated the presence of the same side chains (D, E, and F rings), which was further supported by the fragment peak at m/z 139 in the EI mass spectrum [10]. Detailed analysis of the HMQC, ^1H , ^1H -COSY, and HMBC spectra revealed the existences of an eleven-membered B ring with two pairs of conjugated C=C bonds, as well as a seven-membered A ring with an unsaturated lactone. As shown in Fig. 2, the ^1H , ^1H -COSY spectrum allowed to build two isolated spin systems, $-\text{CH}_2\text{CH}_2\text{CH}(\text{O})-$ and $-\text{CH}_2\text{CH}_2\text{CH}=\text{C}-$, which are connected with a conjugated C=C bonds system (δ 142.7, 134.0, 133.0, and 133.0), together with the two quaternary C-atoms of the D ring, C(13) and C(14), by means of those of HMBC-crossed points. Similarly, the seven-membered A ring was elucidated on the basis of the HMBC correlations and the probable biogenetic pathway. A dihydrofuran ring was constructed as ring C based on the inspection of δ (C(9)) (89.0) and δ (C(1)) (166.4) with the molecular formula. Accordingly, the planar structure of **2** was derived. In contrast to that of **1**, **2** had a novel structure obtained by the cleavage of

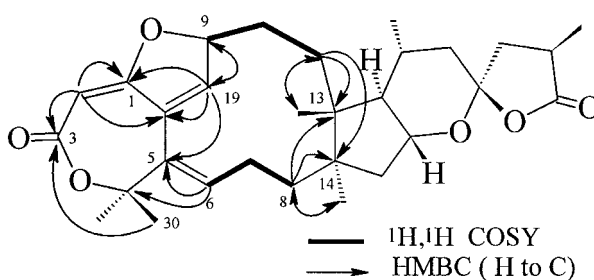
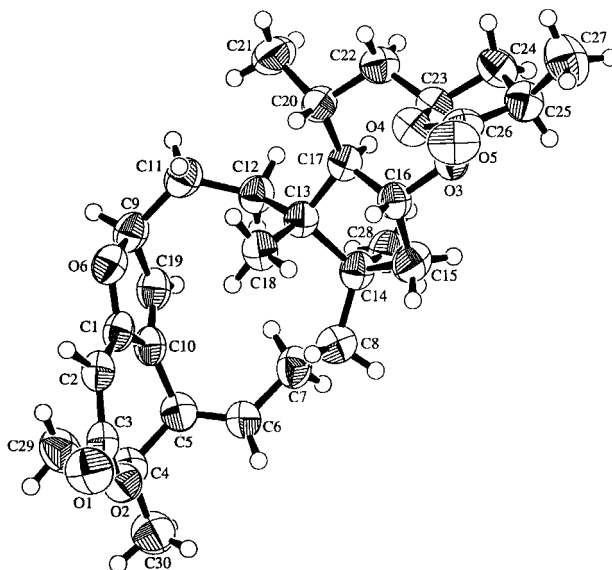


Fig. 2. ^1H -, ^1H COSY and selected HMBC correlations of **2**

Fig. 3. The ORTEP view of **2**

the C(8)–C(9) bond and formation of one additional dihydrofuran cycle. The complete structure and configuration of **2** were established unequivocally by a single-crystal X-ray analysis (Fig. 3). Therefore, pseudolarolide P (**2**) was established as (9*S*,16*R*,23*S*,25*R*)-1,9 : 16,23-diepoxy-8,9 : 9,10-disecocycloartan-1(2),5(6),10(19)-triene-3(4),26(23)-diolide.

Experimental Part

General. Column chromatography (CC): silica gel (200–300, 400 mesh) from Qingdao Haiyang Chemical Group Co., China. M.p.: Fisher-John; uncorrected. $[\alpha]_D^{20}$: Perkin-Elmer 241 MC polarimeter. IR Spectra: Nicolet Magna 750 FT-IR spectrophotometer, KBr pellets; $\tilde{\nu}$ in cm^{-1} . NMR Spectra: Bruker AMX-400 instrument, CDCl_3 as solvent with TMS as an internal standard; chemical shifts δ in ppm, J in Hz. EI-MS: MAT-95 spectrometer; 70 eV; m/z (rel. int. in %).

Plant Material. The seeds of *P. kaempferi* were collected at Changle and Nanshan Forest Center, Zhejiang Province, P.R. China, and identified by Dr. X. Q. Ma of our institute. A voucher specimen is deposited at the herbarium of our institute.

Extraction and Isolation. The lowest-polarity lipidic material was removed from the seeds of *P. kaempferi* (17 kg) with petroleum ether. The defatted seeds were extracted with CHCl_3 (3×3 l). After filtration, CHCl_3 was removed under reduced pressure to give the crude material (1.2 kg). This crude material was chromatographed on silica-gel column under low pressure with petroleum ether/acetone 100:0 to 0:100. Fractions of petroleum ether/acetone 6:1 and petroleum ether/acetone 3:1 were further purified by flash chromatography with petroleum ether/ Et_2O 3:1, and petroleum ether/ Et_2O 1:1 to afford pseudolarolide O (**1**; 660 mg, 0.0039% yield) and pseudolarolide P (**2**; 740 mg, 0.0043% yield), respectively.

Data of 1: Colorless plates from acetone. M.p. 239–240°. R_f (Et_2O) 0.88. $[\alpha]_D^{20} = -23.4^\circ$ ($c = 0.820$, CHCl_3). CD ($c = 0.096$, EtOH , $\Delta\epsilon$ [nm]): 596.4 (195), -1460.4 (205), 1437.5 (sh., 220), 1712.7 (229), -84.1 (263), 56.6 (292). UV: 190.5 (3.82), 194.0 (3.86), 199.0 (4.01). IR: 2960, 2940, 2926, 1770, 1724, 1635, 1610, 1452, 1375, 1360, 1283, 1217, 1178, 1115, 1078, 964, 918, 887, 731, 611. ^1H - and ^{13}C -NMR: see the Table. EI-MS: 482 (10, M^+), 438 (33), 424 (100), 410 (8), 409 (24), 396 (74), 381 (13), 351 (10), 325 (11), 315 (11), 294 (5), 267 (9), 199 (9), 157

(11), 145 (12), 139 (57), 133 (11), 131(11), 119 (14), 107 (19), 105 (16), 91(16). HR-EI-MS: 482.3018 ($C_{30}H_{42}O_5^+$; calc. 482.6628).

X-Ray Crystal-Structure Analysis of 1. $C_{30}H_{42}O_5 \cdot H_2O$ (M_r 500.67), monoclinic $P2_12_12_1$ (#19): $a = 12.680(4)$ Å, $b = 20.688(4)$ Å, $c = 10.799(2)$ Å, $Z = 4$, $V = 2832(1)$ Å³, $D_{\text{calc}} = 1.174$ g/cm³, $R = 0.056$, $R_w = 0.067$. From a crystal of size $0.20 \times 0.20 \times 0.30$ mm, 2083 reflections were measured on a *Rigaku AFC7R* diffractometer with *MoK α* radiation (graphite monochromator $\lambda = 0.71069$ Å) at 273 ± 1 K. The structure was solved by direct methods and expanded by *Fourier* techniques. The non-H-atoms were refined anisotropically. The H-atoms were included but not refined. Drawings of the molecule were achieved with ORTEP.

Data of 2: Colorless prisms from petroleum ether/Et₂O 3:1. M.p. 235–237°. R_f (Et₂O) 0.25. $[\alpha]_D^{20} = +15.7^\circ$ ($c = 0.665$, CHCl₃). CD ($c = 0.017$, EtOH, $\Delta\epsilon$ [nm]): 19853.6 (203), –29564.6 (234), 7596.2 (296). UV: 206.0 (4.14), 231.5 (4.03), 294.5 (3.86). IR: 2987, 2935, 1770, 1759, 1672, 1643, 1610, 1458, 1380, 1273, 1213, 1136, 1082, 974, 895, 762. ¹H- and ¹³C-NMR: see the *Table*. EI-MS: 496 (29, M⁺), 481(15), 480 (35), 478 (16), 470 (11), 454 (38), 453 (32), 452 (100), 437 (22), 412 (14), 409 (10), 408 (12), 394 (24), 369 (12), 368 (11), 292 (16), 287 (13), 257 (20), 215 (18), 201 (24), 189 (21), 187 (41), 175 (42), 174 (51), 173 (45), 161 (32), 159 (41), 147 (37), 145 (31), 139 (89), 135 (21), 133 (31), 131 (33), 121 (33), 119 (29), 107 (37), 105 (32). HR-EI-MS: 496.2825 ($C_{30}H_{40}O_6^+$; calc. 496.6464).

X-Ray Crystal-Structure Analysis of 2. $C_{30}H_{40}O_6$ (M_r 496.64), monoclinic $P212121$ (#19): $a = 11.986(4)$ Å, $b = 21.854(4)$ Å, $c = 10.312(2)$ Å, $Z = 4$, $V = 2701(1)$ Å³, $D_{\text{calc}} = 1.221$ g/cm³, $R = 0.048$, $R_w = 0.059$. From a crystal of size $0.20 \times 0.20 \times 0.30$ mm, 2859 reflections are measured on a *Rigaku AFC7R* diffractometer with *MoK α* radiation (graphite monochromator $\lambda = 0.71069$ Å) at 273 ± 1 K. The structure was solved by direct methods and expanded by *Fourier* techniques. The non-H-atoms were refined anisotropically. The H-atoms were included but not refined. Drawings of the molecule were achieved with ORTEP.

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