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### Structures and Stereochemistry of Pseudolarolides K and L, Novel Triterpene Dilactones from *Pseudolarix Kaempferi*

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## STRUCTURES AND STEREOCHEMISTRY OF PSEUDOLAROLIDES K AND L, NOVEL TRITERPENE DILACTONES FROM *PSEUDOLARIX KAEMPFERI*

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Pseudolarolides K (1) and L (2), two novel triterpenedilactones, were isolated from the seeds of *Pseudolarix kaempferi*, and their structures characterized from spectral data.

**Keywords:** *Pseudolarix kaempferi*; Pseudolarolide K; Pseudolarolide L; Triterpenes

*Pseudolarix kaempferi* Gord. (Pinaceae) is a tall deciduous tree indigenous to middle eastern China. The root bark of *P. kaempferi* is known as "Tu-Jin-Pi" in Chinese folk medicine and has been used for treating skin diseases caused by fungi [1]. Our previous studies on the chemical constituents of *P. kaempferi* revealed many novel di- and tri-terpenes in the root bark [2-7]. Some diterpenes, such as pseudolaric acids A and B, showed antifungal [1], antifertility [8], and cytotoxic activities [9,10]. In the course of our continuing search for novel, potent antitumor agents [11], we recently investigated other parts of this plant and found that the ethereal extract of the seeds showed significant *in vitro* cytotoxicity against KB, A-549, HCT-8, and

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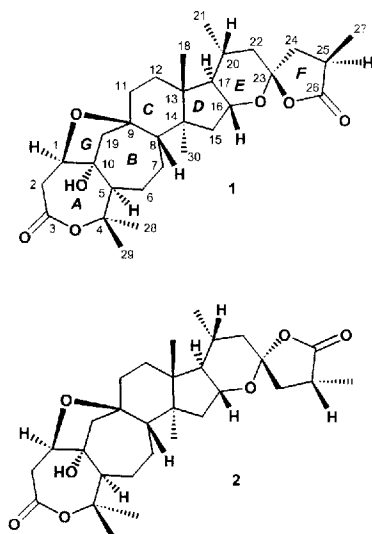


FIGURE 1 Structures of Pseudolarolides K (**1**) and L (**2**).

P-388 cell lines. Bioassay-directed fractionation of the active extract led to the prior isolation and characterization of ten novel triterpene lactones, pseudolarolides A–J, several of these compounds, including pseudolarolides B, H, and I, demonstrated potent cytotoxicity [12–16]. We report herein on the isolation and structural elucidation of two novel triterpene dilactones, pseudolarolide K (**1**) and pseudolarolide L (**2**) (Fig. 1), from this same extract.

## RESULTS AND DISCUSSION

The deoiled ethereal extract of the seeds of *P. kaempferi* was chromatographed on silica gel. The active fractions were further chromatographed and afforded pseudolarolide K (**1**) in 0.0002% yield and pseudolarolide L (**2**) in 0.00025% yield.

Pseudolarolide K (**1**), isolated as a white amorphous solid, has the molecular formula  $C_{30}H_{44}O_7$ , deduced from FAB HRMS peak at  $m/z$  517.3192  $[M + H]^+$  (for  $C_{30}H_{45}O_7$ , calcd 517.3165). The IR (KBr) spectrum indicated that hydroxyl ( $3510\text{ cm}^{-1}$ ),  $\gamma$ -lactone ( $1775\text{ cm}^{-1}$ ), and lactone ( $1710\text{ cm}^{-1}$ ) groups were present in **1**.

The  $^1\text{H}$  NMR spectrum of **1** exhibited signals due to four tertiary methyls [ $\delta$  1.11, 1.14, 1.22, and 1.29 (3H each, s)], two secondary methyls [ $\delta$  0.89, (3H, d,  $J = 6.5\text{ Hz}$ ), 1.24, (3H, d,  $J = 7.0\text{ Hz}$ )], and two low-field protons each attached to a carbon bearing an oxygen function [ $\delta$  4.29, (1H, d,  $J = 4.0\text{ Hz}$ ),

4.15, (1H, dt,  $J = 10.0, 4.5$  Hz)]. The  $^{13}\text{C}$  NMR spectra, DEPT and HETCOR experiments indicated that **1** contained 30 carbon atoms and 43 carbon-bonded hydrogen atoms. The DEPT spectra identified the carbon centers as six methyls, nine methylenes, five simple methines, two oxygenated methines, two quaternary carbons, three oxygenated quaternary carbons, one ketal carbon, and two carbonyls (Table I). These data were consistent with the empirical formula and, coupled with the lack of cyclopropyl group resonances, suggested that **1** was a triterpene possessing a 9,10-seco-cycloartane skeleton in which ring A had been oxidatively cleaved between C3 and C4 to produce a seven-membered lactone ring as also found in pseudolarolides D and I [12–16].

The mass spectral fragment at  $m/z$  139 ( $\text{C}_8\text{H}_{11}\text{O}_2$ ), which is characteristic of the spiro E and F rings in pseudolarolides, and comparison of the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra indicated that **1** possessed the same side chain found in pseudolarolides A–J [12–16]. Strong fragments at  $m/z$  307 (100%) and 289 (63%) {arising from  $[\text{M} - \text{side chain and C-13, C-16, C-17, and C-18}]^+$  and  $(307 - \text{H}_2\text{O})$ } indicated that the remaining two oxygen atoms and a hydroxyl group were located in ring A, B, or C. From the  $^1\text{H}$  and  $^{13}\text{C}$  spectral data, the hydroxyl oxygen and a second oxygen atom were assigned in rings A and B as shown. These assignments were confirmed by long-range HETCOR and  $^1\text{H}-^1\text{H}$  COSY experiments. Thus, both Me-28 ( $\delta$  1.29) and Me-29 ( $\delta$  1.11) protons showed two-bond correlation to the carbon signal at  $\delta$  84.7 (C-4) and three-bond correlation to the signal at  $\delta$  61.1 (C-5). Furthermore, H<sub>2</sub>-19 ( $\delta$  1.78 and 1.91) correlated with the signals at  $\delta$  37.5 (C-11, 3-bond), 55.3 (C-8, 3-bond), 61.1 (C-5, 3-bond), 76.1 (C-9, 2-bond), 80.7 (C-1, 3-bond), and 97.7 (C-10, 2-bond), and two methylene ( $\delta$  2.62 and 2.74) protons adjacent to a carbonyl showed long-range correlations only to the carbonyl ( $\delta$  173.8, C-3, 2-bond), C-1 (80.7, 2-bond), and C-10 ( $\delta$  97.7, 3-bond). These data supported the presence of a seven-membered lactone A ring, formed by oxidative cleavage between C-3 and C-4. The other  $^1\text{H}$  NMR signals were assigned from  $^1\text{H}-^1\text{H}$  COSY data. One of the two low-field protons was attached to an oxygen-bearing carbon and appeared as a doublet of triplets (*dt*); this proton was assigned to H-16 based upon a comparison with the NMR spectra of other pseudolarolides. 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 C-3 carbonyl moiety. This evidence, coupled with biogenetic consideration and the comparison of the  $^{13}\text{C}$  NMR spectrum with those of pseudolarolides I and J, led to the conclusion that C-9 must be connected to C-1 through an oxygen bridge with the hydroxyl group located at C-10.

TABLE I  $^1\text{H}$  and  $^{13}\text{C}$  NMR data and long-range HETCOR correlations of pseudolarolides K (1) and L (2)

Position	1		2		Long-range HETCOR correlation (C#)
	$\delta_{\text{H}}$ [mult, $J$ (Hz)] <sup>a</sup>	$\delta_{\text{C}}$ (mult) <sup>b</sup>	$\delta_{\text{H}}$ [mult, $J$ (Hz)] <sup>a</sup>	$\delta_{\text{C}}$ (mult) <sup>b</sup>	
1	4.29 (d, 4.0)	80.7 (d)	4.29 (d, 4.0)	80.7 (d)	3
2	2.62 (d, 18.0)	36.8 (t)	2.63 (d, 18.0)	36.8 (t)	1, 3, 10
	2.74 (dd, 18.0, 4.0)		2.74 (dd, 18.0, 4.0)		3
3	---	173.8 (s)		173.8 (s)	
4	---	84.7 (s)		84.7 (s)	
5	1.90 (m)	61.1 (d)	1.90 (m)	61.1 (d)	1, 4, 7, 10, 19, 28, 29
6	1.74 (m)	23.5 (t)	1.75 (m)	23.5 (t)	5, 8, 10
7	1.50 (m)	29.9 (t)	1.50 (m)	29.9 (t)	
	1.87 (m)		1.88 (m)		5, 6, 8, 9, 14
8	2.17 (dd, 11.5, 3.5)	55.3 (d)	2.18 (dd, 11.5, 3.5)	55.3 (d)	6, 7, 9, 14,
9	---	76.1 (s)		76.1 (s)	
10	---	97.7 (s)		97.7 (s)	
11	1.76 (m)	37.5 (t)	1.76 (m)	37.5 (t)	9, 12, 19
12	1.65 (m)	30.1 (t)	1.65 (m)	30.1 (t)	18
13	---	43.4 (s)		43.4 (s)	
14	---	49.6 (s)		49.6 (s)	
15	1.33 (dd, 13.8, 4.5)	42.1 (t)	1.33 (dd, 13.0, 4.5)	42.1 (t)	13, 14, 16, 30
	1.87 (dd, 13.8, 10.0)		1.90 (dd, 13.0, 10.0)		8, 14, 17, 30

16	4.15 (dt, 10, 4.5)	77.5 (d)	4.23 (dt, 10, 4.5)	78.0 (d)	13, 16, 18
17	1.52 (t, 10.0)	56.4 (d)	1.53 (t, 10.0)	56.3 (d)	12, 13, 14, 17
18	1.22	18.8 (q)	1.23	18.8 (q)	5, 8, 9, 10
19	1.78 (d, 14.5)	46.4 (t)	1.78 (d, 14.5)	46.4 (t)	1, 5, 8, 9, 10, 11
20	1.91 (d, 14.5)		1.91 (d, 14.5)		
21	2.13 (m)	30.4 (d)	2.12 (m)	30.2 (d)	
22	0.89 (d, 6.5)	19.2 (q)	0.89 (d, 6.5)	19.1 (q)	17, 20, 22
23	1.93 (dd, 14.0, 4.0)	44.4 (t)	1.95 (dd, 14.0, 4.0)	44.9 (t)	17, 20, 23
24	1.41 (dd, 14.0, 11.5)		1.40 (dd, 14.0, 4.0)		20, 21, 24
25		107.5 (s)		109.0 (s)	
26	2.40 (dd, 13.0, 8.5)	42.8 (t)	2.32 (dd, 13.4, 9.7)	42.0 (t)	23, 25, 26
27	1.72 (dd, 13.0, 13.0)		1.96 (dd, 13.4, 13.0)		22, 23, 25
28	2.94 (m)	34.2 (d)	2.71 (m)	35.3 (d)	
29		179.6 (s)		179.0 (s)	
30	1.24 (d, 7.0)	14.9 (q)	1.35 (d, 7.5)	17.1 (q)	24, 25, 26
	1.29 (s)	30.1 (q)	1.30 (s)	30.1 (q)	4, 5, 29
	1.11 (s)	24.2 (q)	1.12 (s)	24.2 (q)	4, 5, 28
	1.14 (s)	23.1 (q)	1.15 (s)	23.1 (q)	8, 13, 14, 15

<sup>a</sup>Data were recorded at 300 MHz.

<sup>b</sup>Data were recorded at 75 MHz, multiplicity was determined by a DEPT experiment. Assignments were made from HETCOR, COSY, and NOESY studies.

The stereochemistry in rings A and B was determined by 2D  $^1\text{H}$  NOE (phase-sensitive NOESY) and 1D NOE difference experiments. In the phase-sensitive NOESY experiment, H-1 ( $\delta$  4.29) showed a strong nOe response to H-19b ( $\delta$  1.91) as well as to H-11 ( $\delta$  1.76). This result established that H-1, H-11, and H-19b have the same orientation. Furthermore, a strong nOe response was also observed between the protons of Me-30 ( $\delta$  1.14) and H-19a ( $\delta$  1.78). The stereochemical nature of rings C, D, E, and F was also determined by a phase-sensitive NOESY experiment and by comparison of the spectral data ( $^1\text{H}$  and  $^{13}\text{C}$  NMR, MS) for **1** with corresponding data for other pseudolarolides [12–16]. From the above evidence, the structure of **1** was elucidated to be as shown.

Pseudolarolide L (**2**), isolated as a white amorphous solid, also has the molecular formula  $\text{C}_{30}\text{H}_{44}\text{O}_7$ , deduced from an FAB HRMS peak at  $m/z$  517.3187  $[\text{M} + \text{H}]^+$  (for  $\text{C}_{30}\text{H}_{45}\text{O}_7$ , calcd 517.3165). In addition, from the IR and NMR spectral data, compounds **2** and **1** have identical functional groups and numbers and types of carbons. This correspondence suggested that **2** possessed the same skeleton found in **1**, and that compounds **2** and **1** are isomers.

Careful comparison of the proton and carbon resonances of **2** with those of **1** revealed that the signals in rings A, B, C, and D were identical, while some signals in rings E and F were different. Thus, **2** and **1** should have the same partial structures and the same stereochemistry in rings A, B, C, and D. However, we postulated that the stereochemistry of C-23 was inverted from the *S* configuration in **1** to the *R* configuration in **2**. In support of this hypothesis, we found that the H-24a and H-24b signals were shifted upfield from  $\delta$  2.40 in **1** to  $\delta$  2.32 in **2** and downfield from  $\delta$  1.72 in **1** to  $\delta$  1.96 in **2**, respectively. Also, the Me-27 signal was also shifted downfield  $\Delta$  0.11 ppm; and the H-25 signal was shifted upfield  $\Delta$  0.23 ppm, due to the inversion of the C-23 configuration. In the  $^{13}\text{C}$  NMR spectrum, the carbon resonances of C-22, C-23, C-25, and C-27 were shifted downfield  $\Delta$  0.5,  $\Delta$  1.5,  $\Delta$  1.1, and  $\Delta$  2.2 ppm, respectively, and the signals of C-24 and C-26 were shifted upfield  $\Delta$  0.8 and  $\Delta$  0.6 ppm, also due to this inversion. These spectral differences effectively supported the assignment of the stereochemistry of C-23. This assignment was further confirmed by a phase-sensitive NOESY experiment. A strong nOe response was observed between H-16 ( $\delta$  4.23) and H-24b ( $\delta$  1.96) and between Me-18 ( $\delta$  1.23) and H-20 ( $\delta$  2.12). Furthermore, H-25 ( $\delta$  2.71) showed a strong nOe response with H-24a ( $\delta$  2.32); while H-24a showed a strong nOe response with H-22 $\beta$  ( $\delta$  1.40). From the above evidence, the structure of **2** was elucidated to be as shown.

## EXPERIMENTAL SECTION

*General Experimental Procedures* The melting point was taken on a Fischer-Johns apparatus and is uncorrected. The IR spectra were measured as KBr pellet using a Perkin-Elmer 1320 spectrophotometer. NMR spectra were recorded on a Bruker AC 300, or on a Bruker AMX 300, in CDCl<sub>3</sub> using TMS as internal standard. Both FAB HRMS and EIMS measurements were taken on a VG-70-250 SEQ mass spectrometer. Aldrich silica-gel 60 (5–25 μ) was used for cc and Kieselgel 60 F<sub>254</sub>, 0.25 mm was used for TLC. Pseudolarolides K and L were detected by spraying with 50% H<sub>2</sub>SO<sub>4</sub> solution containing 1% anisaldehyde in 95% EtOH, followed by heating.

*Plant Material* The seeds of *P. kaempferi* were collected at Chang-Le Forest Centre, Zhejiang Province, China. A voucher specimen is deposited at the School of Pharmacy, Shanghai Medical University, Shanghai, China.

*Extraction and Isolation* The seeds of *P. kaempferi* (2 kg) were pulverized and extracted with EtOH. After evaporation of the EtOH, the ethanolic extract was extracted with hexane, Et<sub>2</sub>O, and EtOAc, consecutively. The ethereal extract (85 g) was applied to silica gel CC under low pressure and eluted with hexane–Et<sub>2</sub>O (100:0 to 0:100). Fractions of hexane–Et<sub>2</sub>O (1:1) were combined and further purified by flash chromatography with hexane–Et<sub>2</sub>O (1:1) as eluent to afford 4 mg (0.0002% yield) of pseudolarolide K (**1**) and 5 mg (0.00025% yield) of pseudolarolide L (**2**).

*Pseudolarolide K (1)* White amorphous solid. <sup>1</sup>H and <sup>13</sup>C NMR data see Table I; FABMS *m/z* [MH]<sup>+</sup> 517 (74), 499 (43), 307 (100), 289 (63), 139 (5).

*Pseudolarolide L (2)* White amorphous solid. <sup>1</sup>H and <sup>13</sup>C NMR data see Table I; FABMS *m/z* [MH]<sup>+</sup> 517 (74), 499 (43), 307 (100), 289 (63), 139 (5).

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