The Isolation and Structural Elucidation of Four Novel Triterpene Lactones, Peudolarolides A, B, C, and D, from Pseudolarix kaempferi

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J. Nat. Prod., **1993**, 56 (7), 1114-1122• DOI: 10.1021/np50097a015 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 7, pp. 1114-1122, July 1993

THE ISOLATION AND STRUCTURAL ELUCIDATION OF FOUR NOVEL TRITERPENE LACTONES, PSEUDOLAROLIDES A, B, C, AND D, FROM *PSEUDOLARIX KAEMPFERI*

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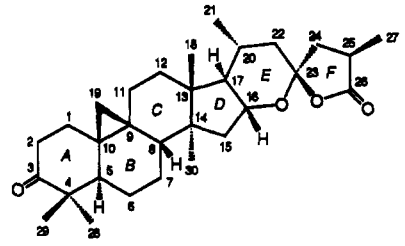
ABSTRACT.—Four novel triterpene lactones, pseudolarolides A [**1**], B [**2**], C [**3**], and D [**4**], were isolated from the seeds of *Pseudolarix kaempferi*. Their structures and stereochemistry were elucidated from spectral data. Compound **2** shows potent cytotoxicity against three human cancer cell lines, KB (nasopharyngeal), A-549 (lung), and HCT-8 (colon), and against a murine leukemia cell line (P-388) with ED_{50} values of 0.49, 0.67, 0.73, and 0.79 μg/ml, respectively.

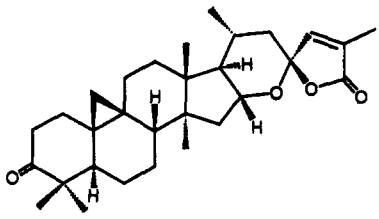
The root bark of *Pseudolarix kaempferi* Gord. (Pinaceae), a plant indigenous to eastern China, is known as "Tu Jin Pi" in Chinese folk medicine and has been used to treat skin diseases caused by fungi (1). Many novel terpenes have been isolated from "Tu Jin Pi," including four diterpene acids, two glucosides, and one triterpene acid (2). Some of these compounds, such as pseudolaric acids A and B, demonstrate antifungal (1), antifertility (3), and cytotoxic (4,5) activity. Recently, we have screened other parts of this plant for novel cytotoxic antitumor compounds. The Et_2O extract of the seeds showed significant in vitro cytotoxicity against KB, A-549, HCT-8, and P-388 cell lines. Bioassay-directed fractionation of this active extract has now led to the isolation and characterization of four novel triterpene lactones, pseudolarolides A, B, C, and D. The second triterpene is a potent cytotoxic principle. In addition, three biogenetically related compounds, pseudolarolides E (6), H (7), and I (8), were isolated and have been reported recently. We report herein on the structural elucidation and cytotoxic activity of pseudolarolides A, B, C, and D.

RESULTS AND DISCUSSION

Pseudolarolide A [**1**], obtained as plates (mp 257–259°), has the molecular formula $C_{30}H_{44}O_4$ as found from its hrms (*m/z* 468.3221). A Lieberman-Burchard test gave a yellow-brown color. The ir spectrum showed the presence of γ-lactone (1775 cm^{-1}) and six-membered ring ketone (1702 cm^{-1}) groups. The ^1H-nmr spectrum (Table 1) showed signals for four tertiary (δ 1.03, 1.06, 1.08, and 1.08) and two secondary (δ 0.88 and 1.23) methyls and a cyclopropyl methylene (δ 0.57 and 0.83). The ^{13}C-nmr (Table 2), DEPT, and HETCOR spectra indicated that **1** contains 30 carbon atoms and 44 carbon-bonded hydrogen atoms. The multiplicity of the carbon atoms was determined by the DEPT experiment. Four low-field signals correspond to two carbonyl carbons (δ 216.2 and 179.6), one ketal carbon (δ 107.3), and one oxygenated methine (δ 77.4); the high-field region showed six methyl, ten methylene, five methine, and five quaternary carbons.



1



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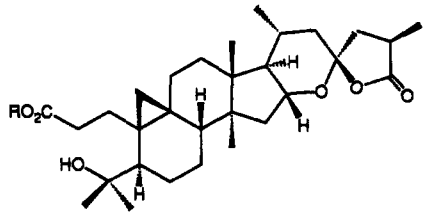
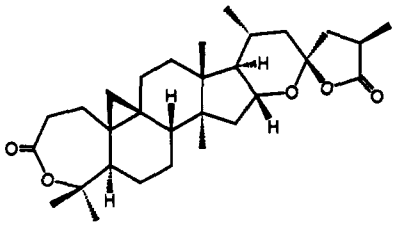


3 R=Me
5 R=H



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These data are consistent with the hrms empirical formula and suggested that **1** was a triterpene with a cycloartane skeleton (9).

The ketone was assigned to ring A due to the presence of a mass spectral fragment at *m/z* 330 $[M-C_9H_{14}O]^+$ (Scheme 1) originating from loss of ring A plus C-6 (10). This placement was also suggested by the similarity of the ^{13}C-nmr spectra of **1** and the known 3-oxocycloartanes (11). A long-range HETCOR experiment showed correlations between this carbonyl and the methyls at C-4 and further confirmed assignment of the ketone carbonyl to C-3.

Three oxygen atoms then remained to be assigned. A γ-lactone would account for two oxygens, while a ketal would also contain two oxygens. Thus, one oxygen atom must be shared between the two groups (O-C-O_2C). The low-field methine in the ^{1}H- and ^{13}C-nmr spectra (δ_H 4.09 and δ_C 77.4), together with the absence of an hydroxyl absorption in the ir spectrum, indicated a CH-O-C ether bond. The three oxygens must then be linked in the order CH-O-C-O_2C. The hrms fragment at *m/z* 139.0741 ($C_8H_{11}O_2$, Scheme 1) can be attributed to a cycloartane side chain at C-17 (12) containing two oxygen atoms. Since one oxygen atom must be shared between the γ-lactone and the ketal, both of these functional groups must then be located in this side chain. The ether bond would then connect the side chain terminus to the terpene skeleton at a position close to the side chain origin. The ^{1}H-^{1}H COSY spectrum showed coupling between the ether proton (δ 4.09) and signals at δ 1.23, 1.76, and 1.47; the ether proton was assigned to H-16 and the other signals to H-15α, H-15β, and H-17, respectively. Biogenetic considerations would also connect one of the ketal oxygens to C-16; this confirms the ether bond at C-16 and establishes a spiro-ring moiety at C-23 of the side chain.

^{1}H-^{1}H COSY and HETCOR spectra were used for assignment of all proton resonances as listed in Table 1, and a long-range HETCOR was used for assignment of all carbon resonances as shown in Table 2. Some of the assignments are as follows. The methylene protons at C-22 occur at δ 1.39 (dd, J=12, 14 Hz) and δ 1.87 (dd, J=4, 14 Hz); the former was assigned axial and the latter equatorial by comparison of the coupling constants. The oxygen of the ketal causes the downfield shift of the α proton at C-24 (δ 2.36) relative to the β proton (δ 1.72). H-25 was assigned clearly at δ 2.92

TABLE 1. ^{1}H-nmr Chemical Shifts of Compounds **1–5**.[a]

Proton	Compound				
	1	**2**	**3**	**5**	**4**
H-1α	1.79, dt, J=8, 14	1.80, m			1.79, m
H-1β	1.50, td (br), J=6.5, 14	1.51, td (br), J=4, 14			1.52, m
H-2α	2.28, td (br), J=6.5, 14	2.28, td (br), J=4, 14			
			2.65, m	2.68, m	2.68, m
H-2β	2.69, dt, J=8, 14	2.69, dt, J=6.5, 14			
H-5	1.68, dd, J=7.5, 12.5	1.68, dd, J=7, 13			2.05, dd, J=5, 12.5
H-6α	0.90, dt, J=2, 12.5	0.90, dt, J=2, 13	0.64, q (br), J=5.5, 14		0.68, q (br), J=12.5
H-6β	1.54, m	1.54, m	1.84, dd, J=5.5, 14		1.79, m
H-7	1.33, m	1.36, m			1.10, m 1.28, m
H-8	1.53, dd, J=6, 12.5	1.63, dd J=4.5, 13			1.42, m
H-11α	1.14, m	1.21, m			1.12, m
H-11β	2.08 m	2.05, ddd, J=6, 10.5, 15	2.10, m		2.10, m
H-12	1.55, m	1.65, m	1.65, m		1.69, m
H-15α	1.23, dd, J=5.5, 12.5	1.42, dd, J=7.5, 13	1.25, m		1.27, dd, J=5.5, 12
H-15β	1.76, dd, J=10, 12.5	1.96, dd, J=7.5, 13	1.76, dd, J=5.5, 13.5	1.76, dd, J=5.5, 13.5	1.78, dd, J=10, 12
H-16	4.09, td, J=5.5, 10	4.43, q, J=7.5	4.07, td, J=5.5, 10	4.07, m	4.09, dt, J=5.5, 10.5
H-17	1.47, t, J=10	1.70, m	1.45, t, J=10	1.45, t, J=10	1.48, t, J=10.5
Me-18	1.06, s	0.89, s	1.01, s	1.00, s	1.05, s
H_a-19	0.57, d, J=4	0.58, d, J=4	0.52, d, J=4.5	0.52, d, J=4	0.59, d, J=4.5
H_b-19	0.83, d, J=4	0.83, d, J=4	0.70, d, J=4.5	0.70, d, J=4	0.70, d, J=4.5
H-20	2.08, m	1.75, m	2.09, m	2.11, m	2.07, m
Me-21	0.88, d, J=6.5	0.99, d, J=5.5	0.87, d, J=6.5	0.87, d, J=6.5	0.89, d, J=6.5
H-22α	1.39, dd, J=12, 14	1.84, m	1.39, d, J=13	1.39, d, J=15	1.89, dd, J=4, 14
H-22β	1.87, dd, J=4, 14		1.88, dd, J=4, 13	1.88, dd, J=4,15	1.42, m
H-24α	2.36, dd, J=8.5, 13	6.83, d, J=1	2.38, dd, J=8.5, 13	2.39, dd, J=8.5, 13	2.39, dd, J=8.5, 13
H-24β	1.72, dd, J=4, 13		1.76, dd, J=3, 13	1.76, dd, J=3, 13	1.72, m
H-25	2.92, m	—	2.91, m	2.92, m	2.91, m
Me-27	1.23, d, J=7	1.87, d, J=1	1.22, d, J=7	1.23, d, J=6.5	1.23, d, J=7
Me-28	1.03, s	1.03, s	1.20, s	1.20, s	1.38, s
Me-29	1.08, s	1.08, s	1.19, s	1.19, s	1.44, s
Me-30	1.08, s	1.13, s	1.08, s	1.08, s	1.09, s
OMe			3.62, s		

[a]Measured in $CDCl_3$; J in Hz; δ=ppm. Multiplicity of signals: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad signal.

TABLE 2. ^{13}C-nmr Data for Compounds **1–4**.[a]

Carbon	Compound			
	1	**2**	**3**	**4**
C-1	33.3 t	33.3 t	33.1 t	29.9 t
C-2	37.4 t	37.3 t	31.2 t	35.0 t
C-3	216.2 s	216.2 s	174.8 s	175.4 s
C-4	50.2 s	50.1 s	75.8 s	87.2 s
C-5	48.6 d	48.2 d	48.2 d	50.0 d
C-6	21.3 t	21.2 t	25.6 t	25.7 t
C-7	26.2 t	26.0 t	26.1 t	25.7 t
C-8	47.8 d	47.4 d	45.4 d	48.4 s
C-9	20.6 s	20.8 s	21.8 s	22.6 s
C-10	26.6 s	26.3 s	27.6 s	28.2 s
C-11	26.8 t	26.3 t	26.8 t	27.6 t
C-12	30.9 t	32.7 t	31.9 t	31.0 t
C-13	44.1 s	44.6 s	43.8 s	43.6 s
C-14	47.1 s	46.2 s	47.5 s	47.2 s
C-15	40.8 t	43.4 t	41.1 t	41.0 t
C-16	77.4 d	75.5 d	77.2 d	77.4 d
C-17	54.8 d	55.6 d	54.8 d	54.8 d
C-18	19.3 q	19.5 q	19.4 q	19.5 q
C-19	30.9 t	30.0 t	29.6 t	30.7 t
C-20	30.0 d	25.4 d	29.8 d	30.0 d
C-21	19.2 q	20.2 q	19.6 q	19.3 q
C-22	44.2 t	37.8 t	44.0 t	44.3 t
C-23	107.3 s	107.2 s	107.2 s	107.2 s
C-24	42.7 t	146.3 d	42.6 t	42.7 t
C-25	34.1 d	130.7 s	34.0 d	34.2 d
C-26	179.6 s	172.3 s	179.4 s	179.7 s
C-27	14.9 q	10.4 q	14.8 q	15.0 q
C-28	22.1 q	22.2 q	29.9 q	31.1 q
C-29	20.8 q	20.7 q	25.8 q	23.0 q
C-30	23.1 q	20.4 q	23.1 q	23.2 q
OMe	—	—	51.3 q	—

[a]Solvent: $CDCl_3$.

with the ^{1}H-^{1}H COSY spectrum showing cross peaks between this signal and the δ 1.23 doublet (Me-27), as well as with H_2-24. These assignments were confirmed by the long-range HETCOR experiment. Thus, the Me-27 protons (δ 1.23) showed two-bond correlation to the carbon signal at δ 34.1 (C-25) and three-bond correlation to the carbonyl at δ 179.6 (C-26), while H_2-24 correlated with the signals at δ 179.7 (C-26, 3-bond), 107.3 (C-23, 2-bond), 34.1 (C-25, 2-bond), and 14.9 (C-27, 3-bond). The Me-21 protons (δ 0.88) coupled to signals at δ 30.0 (C-20, 2-bond), 44.2 (C-22, 3-bond), and 54.8 (C-17, 3-bond). Figure 1 summarizes the observed long-range correlations.

The stereochemistry in rings E and F was determined by 2D ^{1}H nOe (NOESY) and 1D nOe difference experiments (Table 3). Irradiation of the multiplet at δ 4.09 (H-16) markedly enhanced both the signal at δ 2.08 (H-20) and the doublet at δ 1.06 (Me-18); H-16 thus has a β orientation (*R* configuration at C-16). However, irradiation of the H-24 protons at δ 1.72 and 2.36 did not enhance the H-16 signal, placing C-24 and C-16 far apart and assigning the configuration at C-23 as *S*. Also, irradiation of H-24 (δ 2.36) enhanced the H-25 methine, indicating an α orientation of H-25 (*R* configuration at C-25). Therefore the structure of **1** was elucidated as 25*R*-3-oxo-16*R*,23-epoxycycloartan-26,23*S*-olide.

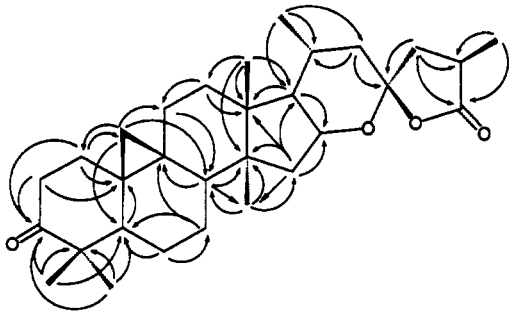


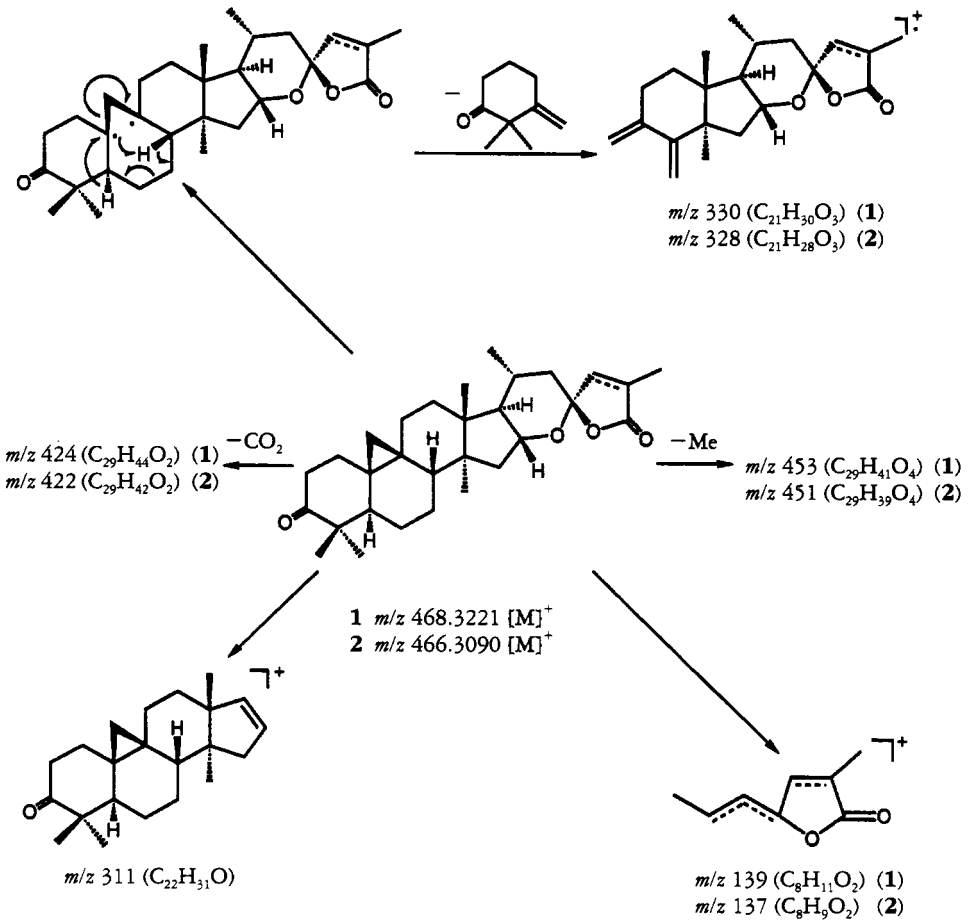
FIGURE 1. The long-range HETCOR responses of **1**.

Pseudolarolide B [**2**], obtained as needles (mp 229–231°), has the molecular formula $C_{30}H_{42}O_4$ as found from its hrms (*m/z* 466.3090) and confirmed by ^{1}H- and ^{13}C-nmr spectra. A yellow-brown color was obtained with a Lieberman-Burchard test. The ir spectrum of **2** closely resembled that of **1** (γ-lactone at 1769 and six-membered ring ketone at 1705 cm^{-1}) except for the presence of double bond absorptions at 3040 and 1668 cm^{-1}. Comparison of the ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) suggested that **2** also has a cycloartane skeleton. The mass spectra of the two compounds were similar except for the loss of two units in most fragments of **2** (Scheme 1). The strong fragment peak at *m/z* 137.0620 ($C_8H_9O_2$) suggested that the double bond is in the side chain rings. The most prominent differences in the ^{1}H-nmr spectra of **1** and **2** were the disappearance of the ABX system for the H_2-24 and H-25 protons (δ 2.36 dd, 1.72 dd, 2.92 m) in the former compound and the appearance of an olefinic proton (δ 6.83) in the latter compound. Also, Me-27 shifted downfield from δ 1.23 in **1** to δ 1.87 in **2**. Differences in the E and F ring carbons in the ^{13}C-nmr spectra were consistent with location of the double bond between C-24 and C-25. Thus, the structure of **2** was designated as 3-oxo-16*R*,23-epoxycycloartan-24-en-26,23*S*-olide.

Pseudolarolide C [**3**], obtained as prisms (mp 205–207°), has the molecular formula $C_{31}H_{48}O_6$ as found from hrms and eims (*m/z* 498.3328, $C_{30}H_{46}O_5$ $[M-H_2O]^+$ and 517 $[M+H]^+$) and confirmed by ^{1}H- and ^{13}C-nmr spectra. The Lieberman-Burchard test gave a yellow color that changed to violet. The ir spectrum showed the presence of hydroxyl (3496), γ-lactone (1757), and ester (1728 cm^{-1}) groups. The ^{1}H-nmr spectrum

TABLE 3. Results of nOe Enhancement Studies on Compound **1**.

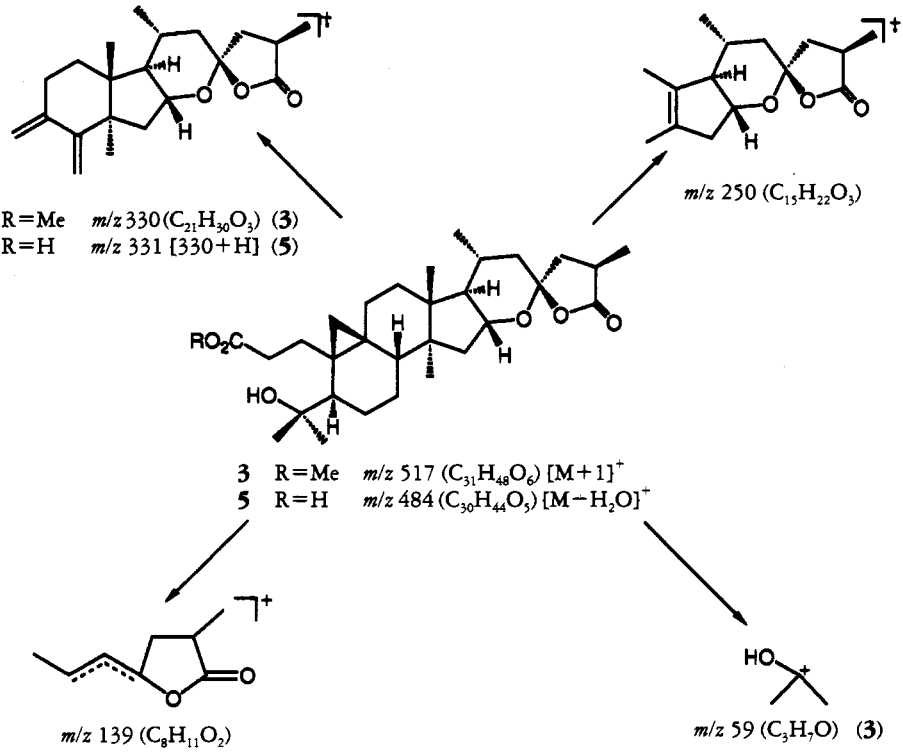
Irradiation	Observation	nOe Enhancement (%)
4.09 (H-16)	2.08 (H-20)	6.0
	1.76 (H-15β)	6.1
	1.06 (Me-18)	increase
2.08 (H-20)	4.09 (H-16)	3.7
	0.88 (Me-21)	increase
0.88 (Me-21)	1.55 (H-12)	5.2
1.39 (H-22α)	1.48 (H-17)	2.7
	1.87 (H-22β)	23
1.87 (H-22β)	2.08 (H-20)	8.4
	1.39 (H-22α)	increase
2.36 (H-24α)	1.72 9H-24β)	26.9
	2.92 (H-25)	12.2
1.72 (H024β)	1.23 (Me-27)	4.6
	2.36 (H-24α)	30.9



SCHEME 1. Mass spectral fragmentation of compounds **1** and **2**.

(Table 1) showed signals for a cyclopropyl methylene (δ 0.52 and 0.70) and six methyls (δ 0.87, 1.01, 1.08, 1.19, 1.20, and 1.22), again establishing **3** as a triterpene with a cycloartane skeleton. A methoxy singlet was found at δ 3.62. The ^{13}C-nmr spectrum (Table 2) showed carbonyl carbons at δ 179.4 and 174.8, a ketal carbon at δ 107.2, and three oxygenated carbons at δ 77.2 (methine), 75.8 (quaternary), and 51.3 (methyl). Compounds **1** and **3** have the same side chain moiety, as shown by similarities in the ^{1}H- and ^{13}C-nmr spectra. The mass spectral fragment at *m/z* 139.0745 ($C_8H_{11}O_2$, Scheme 2) supports this conclusion and is characteristic of the spiro E and F rings in these pseudolarolides. A fragment at *m/z* 59 (C_3H_7O) in the mass spectrum of **3** indicated the presence of an isopropyl group in the parent compound, accounting for the quaternary carbon at δ 75.8. Also, the carbon signal at δ 51.3 and the proton singlet at δ 3.62 were attributed to a methyl ester. Oxidative cleavage of ring A betwen C-3 and C-4 formed a methyl ester at C-3 and an isopropyl moiety at C-4 and assigns **3** the final structure as shown.

Pseudolarolide D [**4**], obtained as colorless needles (mp 222–223°), has the molecular formula $C_{30}H_{44}O_5$ as found from its hrms (*m/z* 484.3238) and confirmed by ^{1}H- and ^{13}C-nmr spectra. Its ir spectrum contains γ-lactone (1772) and lactone (1709 cm^{-1}) absorption bands. A cycloartane skeleton was suggested by the AB doublet at δ 0.58 and 0.70 (cyclopropyl) in the ^{1}H-nmr spectrum, together with the six methyls (δ



SCHEME 2. Mass spectral fragmentation of compounds **3** and **5**.

0.89, 1.05, 1.09, 1.23, 1.38, and 1.44). The ^{1}H- and ^{13}C-nmr spectra of **4** (Tables 1 and 2) were quite similar to those of **1** and **3**, with some differences in the A ring, especially the absence of the ester methyl. Compound **4** has one more oxygen atom than **1** but lacks a CH_4O found in **3**. Together with biogenetic considerations, this indicates an oxidative cleavage of ring A between C-3 and C-4 with formation of a seven-membered lactone. The effect of the carbonyl C-3 in the ring A lactone and the γ- effect of the C-5–C-6 bond cause a low-field shift of Me-24 (δ_C 31.1). The structure of **4** was assigned as shown.

Saponification of **3** with 1% KOH gave two compounds, lactone **4**, confirming its structural assignment, and the free acid **5**. The fragments in the mass spectra of **3** and **5** were identical, except for a fragment at *m/z* 484 [M−H_2O]$^+$ in the spectrum of the free acid. The ^{1}H-nmr spectra of the two compounds differed only in the presence (in **3**) or absence (in **5**) of the ester methyl (Table 1).

Pseudolarolides A [**1**], B [**2**], C [**3**], and D [**4**] were tested for in vitro cytotoxicity in four human cancer cell lines, KB (nasopharyngeal), A-549 (lung), HCT-8 (colon), and TE671 (medulloblastoma), and one murine tumor cell line, P-388 (leukemia) (13). The data are shown in Table 4. Compounds **1**, **3**, and **4** were inactive in all five cell lines. Pseudolarolide B [**2**] was also inactive in the TE671 cell line but showed good cytotoxicity in KB, A-549, HCT-8, and P-388 tumor cells with ED_{50} values of 0.49, 0.67, 0.73, and 0.79 μg/ml, respectively. The unsaturated lactone in ring F may play a role in the antitumor activity of **2**. Compounds **1** and **2** also exhibit antiviral activity, showing in vitro inhibition of HSV-2 plaque formation in Vero cells.

TABLE 4. Cytotoxicity of Pseudolarolides A [**1**], B [**2**], C [**3**], and D [**4**] against Cancer Cell Lines.

Compound	Cell Line EC_{50} (μg/ml)				
	P388	A549	HCT	KB	TE671
1	I[a]	I	I	I	I
2	0.79	0.67	0.73	0.49	I
3	I	I	I	I	I
4	I	I	I	I	I

[a]I: Inactive

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler micro-melting point apparatus and are uncorrected. Ir spectra were recorded as KBr pellets on a Perkin-Elmer 983 spectrophotometer. Cd and uv spectra were meaasured on a JASCO 500-C and a Shimazu UV-260 spectrophotometer, respectively, in absolute EtOH. Ms was determined on a JMSO-D 300S mass spectrometer. ^{1}H- and ^{13}C-nmr spectra were measured on Bruker AM-400 and AC-300, Varian XL-400 and FT-80AM, and JEOL FX-90Q spectrometers with TMS as an internal standard. Si gel H (10–40 μ, Qing Dao or Shanghai) was used for cc under 0.5–2 kg/cm^2. Analytical tlc was performed on Si gel plates (prepared with Si gel G or Si gel H and carboxymethylcellulose) with petroleum ether-Et_2O (1:1). Pseudolarolides were detected by spraying with a 50% H_2SO_4 solution containing 1% anisaldehyde in 95% EtOH, followed by heating.

PLANT MATERIAL.—The seeds of *P. kaempferi* were collected at Changle 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* (1800 g) were pulverized and extracted with Et_2O (1100 ml) for 30 h using a Soxhlet extraction apparatus. After evaporation of the Et_2O, the oily extract was added to 3.5 liters of petroleum ether, resulting in 150 g of precipitate. The precipitate was chromatographed on Si gel under low pressure, employing a petroleum ether-Et_2O gradient (100:0 to 0:100) as eluent. Fractions of petroleum ether-Et_2O (4:1) were combined and further purified by flash chromatography with petroleum ether-Et_2O (6:1) as eluent to afford 60 mg (0.0033% yield) of pseudolarolide A [**1**] and 150 mg (0.0083% yield) of pseudolarolide B [**2**]. The fractions of petroleum ether-Et_2O (3:2) were also combined and subjected to flash chromatography using petroleum ether-Et_2O (3:1) to yield 180 mg (0.01% yield) of pseudolarolide C [**3**] and 40 mg (0.0022% yield) of pseudolarolide D [**4**].

Pseudolarolide A [**1**].—Colorless plates (MeOH): mp 257.0–259.0°; R_f 0.57; cd Δε (nm) 2.05 (200), 0.88 (295) (c=0.47); uv nm (log ε) 203.2 (3.27); ir 3040, 2936, 2880, 1775, 1702, 1457, 1381, 1213, 1080, 979, 896 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* [M]$^+$ 468.3221 ($C_{30}H_{44}O_4$, calcd 468.3239) (100%), 453 (56), 425 (29), 424 (50), 395 (8), 383 (10), 382 (10), 356 (11), 338 (11), 330 (64), 315 (14), 311 (18), 303 (16), 295 (11), 271 (16), 250 (21), 201 (21), 187 (16), 173 (20), 159 (24), 147 (23), 139 (86), 135 (34), 121 (28), 119 (28), 107 (28), 105 (28), 95 (26), 93 (28), 91 (26), 81 (19), 79 (20), 67 (28), 55 (29), 42 (29).

Pseudolarolide B [**2**].—Colorless needles (Me_2CO): mp 229–231°; R_f 0.49; cd Δε (nm) −4.65 (216), 3.75 (245), 0.83 (295), (c=0.565); uv nm (log ε) 208.4 (3.76); ir 3040, 2956, 2936, 2904, 2872, 1769, 1705, 1668, 1453, 1380, 1321, 1309, 1176, 1114, 1030, 972 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* [M]$^+$ 466.3090 ($C_{30}H_{42}O_4$, calcd 466.3083) (23%), 451 (15), 423 (3), 422 (4), 381 (3), 380 (3), 356 (3), 338 (8), 328 (44), 323 (6), 313 (21), 311 (8), 218 (15), 207 (19), 203 (11), 201 (11), 189 (13), 173 (14), 161 (15), 159 (17), 155 (17), 147 (24), 137 (100), 135 (29), 133 (34), 121 (42), 119 (61), 107 (39), 105 (44), 95 (32), 93 (43), 91 (40), 81 (40), 79 (32), 69 (40), 67 (41), 55 (41), 42 (43).

Pseudolarolide C [**3**].—Colorless prisms (Me_2CO): mp 205.5–207.5°; R_f 0.42; cd Δε (nm) 1.24 (200) (c=0.405); uv nm (log ε) 203.8 (2.95); ir 3496, 3045, 2964, 2950, 2940, 2880, 1757, 1728, 1454, 1378, 1260, 1218, 1180, 1150, 1080, 978, 965, 925, 895, 875 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2: hrms *m/z* [M−H_2O]$^+$ 498.3328 ($C_{31}H_{46}O_5$, calcd 498.3345); eims *m/z* [M+1]$^+$ 517 (0.5%), 498 (9), 485 (2), 483 (14), 458 (12), 443 (12), 414 (7), 330 (8), 301 (9), 250 (5), 261 (6), 173 (10), 161 (11), 159 (14), 157 (12), 147 (18), 145 (15), 139 (76), 135 (16), 133 (23), 121 (24), 119 (27), 109 (17), 107 (27), 105 (25), 95 (25), 93 (26), 91 (23), 81 (20), 79 (19), 69 (39), 67 (21), 59 (100), 55 (28), 43 (26).

Pseudolarolide D [**4**].—Colorless needles (Me_2CO): mp 222–223°; R_f 0.33; cd $\Delta\epsilon$ (nm) 1.70 (218) (c=0.45); uv nm (log ϵ) 201.2 (2.57); ir 2940, 2885, 1772, 1709, 1457, 1447, 1380, 1290, 1215, 1118, 1080, 982, 894, 875 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* $[M]^+$ 484.3238 ($C_{30}H_{44}O_5$, calcd 484.3189) (34%), 469 (53), 466 (4), 451 (10), 440 (23), 426 (5), 411 (17), 354 (17), 339 (9), 331 (12), 250 (12), 139 (100), 121 (29), 119 (29), 109 (20), 107 (31), 105 (25), 95 (29), 93 (29), 69 (45), 56 (35), 42 (31).

HYDROLYSIS OF **3**.—To a solution of **3** (30 mg) in 6.5 ml EtOH, 6.5 ml of 2% KOH in EtOH was added. After refluxing for 30 min, the reaction mixture was acidified to pH 2 with 5% HCl. The acidic solution was evaporated to remove EtOH, and the residue was extracted with $CHCl_3$. The extract was evaporated to dryness, and the residue was chromatographed on Si gel H under low pressure with petroleum ether-Et_2O (1:1) to afford 15 mg of **5** and 4 mg of a second compound. The R_f value, ms, and ^1H-nmr spectrum of this second compound were identical to those of **4**. The structure of compound **5** was identified as shown.

Compound **5**.—^1H nmr see Table 1; eims *m/z* $[M]^+$ 484 (3%), 469 (4), 440 (2), 425 (1), 411 (2), 354 (2), 339 (2), 331 (3), 313 (2), 303 (2), 289 (3), 287 (4), 273 (4), 250 (6), 219 (5), 201 (5), 173 (14), 159 (18), 147 (22), 145 (21), 139 (100), 135 (25), 133 (29), 131 (19), 121 (38), 120 (19), 119 (40), 109 (28), 107 (48), 105 (43), 95 (43), 93 (46), 91 (46), 81 (36), 79 (34), 69 (64), 67 (38), 55 (44), 43 (37), 42 (40).

BIOASSAY METHOD.—The in vitro cytotoxicity assay was carried out according to a National Cancer Institute protocol (13).

ACKNOWLEDGMENTS

This investigation was supported by grants from the Science Fund of the Chinese Academy of Sciences (Z.L.L.) and from the National Cancer Institute, Grant Nl. CA-17625 (K.H.L.). We thank Dr. Jer-Jang Chang, School of Medicine, and Mr. Mike Fisher, Cancer Research Center, University of North Carolina at Chapel Hill, for the bioassays.

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Received 7 December 1992