Cytotoxic Diterpenoids from the Bark of *Pseudolarix kaempferi* and Their Structure−Activity Relationships

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Four new diterpenoids, 11*S*-deacetylpseudolaric acid A (**2**), deacetylpseudolaric acid A *O*-*β*-D-glucopyranoside (**3**), deacetylpseudolaric acid A 2,3-dihydroxypropyl ester (**4**), and deacetylpseudolaric acid B 2,3-dihydroxypropyl ester (**5**), and nine known diterpenoids were isolated from the bark of *Pseudolarix kaempferi*. Their structures were determined on the basis of chemical and spectroscopic analyses. In addition, their in vitro cytotoxic activities against three human cancer cell lines and their structure−activity relationships were evaluated.

The root and trunk bark of *Pseudolarix kaempferi* Gord. (Pinaceae), known as "Tu-Jing-Pi" in traditional Chinese medicine, are used for the treatment of dermal infections caused by fungi. A series of diterpenoids isolated from the bark are considered to be responsible for the antifungal and antifertility activities.[1−3] These characteristic components also have significant in vitro cytotoxic properties[4] and potent in vivo antitumor effects.[5] Their cytotoxicities are mediated by inhibition of angiogenesis,[6] induction of cell apoptosis,[7] and microtubule destabilization.[5]

In our continuous research on the bioactive natural products derived from traditional Chinese medicines, a systematic phytochemical investigation on the bark of *P. kaempferi* resulted in the isolation of 13 diterpenoids (**1**−**13**), including four new compounds (**2**−**5**). One of these new compounds, 11*S*-deacetylpseudolaric acid A (**2**), is the first diterpenoid isolated from this plant with a different configuration at C-11. Compounds **6**, **7**, **12**, and **13** show significant in vitro cytotoxic activities against human leukemia HL-60 cells and display IC_{50} values of 1.51, 0.59, 4.09, and 2.56 μM, respectively. Compounds **10** and **11** exhibit moderate inhibition against HL-60 cells. The majority of the active compounds, which include **6**, **7**, and **10**−**13**, show weak inhibition against human hepatoma Bel-7402 cells and human gastric cancer BGC-823 cells.

Results and Discussion

The EtOH extract of *P. kaempferi* was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH, respectively. The EtOAc fraction was subjected to silica gel and ODS open column chromatography. It was then purified using preparative HPLC and recrystallization to obtain deacetylpseudolaric acid A (**1**),[8] 11*S*-deacetylpseudolaric acid A (**2**), deacetylpseudolaric acid A *O*-*β*-D-glucopyranoside (**3**), deacetylpseudolaric acid A 2,3-dihydroxypropyl ester (**4**), deacetylpseudolaric acid B 2,3-dihydroxypropyl ester (**5**), pseudolaric acid A (**6**),[8] pseudolaric acid B (**7**),[1] pseudolaric acid C_1 (**8**),[1] pseudolaric acid C_2 (**9**),[1] pseudolaric acid F (**10**),[9] pseudolaric acid G (**11**),[9] pseudolaric acid A *O*-*β*-D-glucopyranoside (**12**),[10] and pseudolaric acid B *O*-*β*-D-glucopyranoside (**13**).[1]

Compound **1** was obtained as a white, amorphous solid. Its molecular formula, $C_{20}H_{26}O_5$, was determined by high-resolution electrospray ionization mass spectroscopy (HRESIMS) ($[M + Na]^+$, *m*/*z* 369.1698). The ^1H, ^{13}C NMR (DEPT), and HMQC data revealed the presence of three methyl, five methylene, five methine, and seven quaternary carbons, including one disubstituted and two trisubstituted double bonds, one carboxyl, one ester carbonyl, and two oxygenated quaternary carbons. These data indicate that compound **1** has considerable structure similarity to pseudolaric acid A (**6**).[8] Comparison of the NMR data of **1** with those of **6** revealed that **1** has the same basic skeleton as **6**, expect for the absence of the C-4-acetyl group. Therefore, **1** was elucidated as the known deacetylpseudolaric acid A. Based on the interpretation of 1D and 2D NMR spectroscopic data, the full spectroscopic assignment of **1** (Table 1) is reported here for the first time.

Compound **2** was obtained as a colorless, amorphous solid. Its molecular formula was also determined as $C_{20}H_{26}O_5$ by HRESIMS ($[M + Na]^+$, *m*/*z* 369.1663). Compound **2** is therefore isomeric with compound **1**. Analysis of the various NMR data confirmed that **2** has the same basic skeleton as and similar spectroscopic data to **1** (Table 1). The major differences between **1** and **2** were the resonances of H-13 and H-14. These two resonances of **1** were well resolved, while the corresponding resonances of **2** were overlapped. Therefore, the ^1H NMR of **1** and **2** were measured using $CDCl_3$ and CD_3OD as solvents, successively. The spectra revealed that in these two solvents the H-13 and H-14 resonances were well resolved and had nearly the same coupling constants (Table 2). In addition, in $CDCl_3$ and CD_3OD, the difference between δ_{H-14} and δ_{H-13} ($\delta_{H-14} - \delta_{H-13}$) of **2** was consistently smaller than that of **1**, at approximately 0.2−0.3 ppm, due to the fact that H-13 of **2** was at lower field compared with that of **1**. This difference was also found in DMSO-d_6. The $\delta_{H-14} - \delta_{H-13}$ value of **1** in DMSO-d_6 was only 0.12 ppm, and that of **2** should also be smaller than 0.12 ppm, indicating that the overlap of the H-13 and H-14 resonances of **2** can be attributed to their close chemical shift values in DMSO-d_6. Therefore, it was concluded that the structures of the conjugated dienoic acid side chain of **1** and **2** must be the same.

The NOE correlations of H-3 and 4-OH to vicinal protons in **1** and **2** were identical, suggesting that they have the same configuration at C-3 and C-4. Furthermore, the similarities of their NMR spectroscopic data and the rigidity of the ring system indicated that the configuration at C-10 is also identical. Circular dichroism (CD) spectra showed that **1** has a negative Cotton effect (CE) at 257 nm, while **2** has a positive CE at 264 nm. On the basis of these results, **1** has nearly the same CD spectrum as pseudolaric acid A (**6**), whereas the spectrum of **2** is opposite that of **6**.[11] The CEs of **1**, **2**, and **6** at about 260 nm are all mainly due to the strong $\pi \rightarrow \pi^*$

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Table 1. NMR Data for Compounds **1**, **2**, and **3** in DMSO-d_6

	1			**2**			**3**		
position	δ_C, mult.	δ_H (J in Hz)	HMBC[a]	δ_C, mult.	δ_H (J in Hz)	HMBC[a]	δ_C, mult.	δ_H (J in Hz)	HMBC[a]
1	34.3, CH_2	1.59−1.71[b]; 1.91−2.00[b]	2, 3, 4, 10	35.0, CH_2	1.42, m; 1.53−1.56[b]		34.3, CH_2	1.60−1.71[b]; 1.92−1.98[b]	2, 4, 10
2	26.4, CH_2	1.59−1.71[b]; 2.68, t (13.5)	1, 4, 10	26.3, CH_2	1.53−1.56[b]; 2.61, t (13.5)	4	26.4, CH_2	1.60−1.71[b]; 2.68, t (13.5)	4
3	53.0, CH	2.17, d (6.5)	1, 2, 4, 10, 12	53.7, CH	2.42, d (6.5)	4, 5, 10	52.9, CH	2.19, d (6.0)	4, 5, 10, 12
4	78.7, qC			78.6, qC			78.6, qC		
5	33.2, CH_2	1.43−1.50[b]; 1.59−1.71[b]	6	33.9, CH_2	1.63−1.71, m		33.2, CH_2	1.46−1.48[b]; 1.60−1.71[b]	4
6	24.3, CH_2	1.43−1.50[b]; 1.91−2.00[b]	5, 7	21.6, CH_2	1.80−1.85[b]; 2.07−2.11[b]	7	24.2, CH_2	1.46−1.48[b]; 1.92−1.98[b]	7
7	138.4, qC			138.3, qC			138.2, qC		
8	123.5, CH	5.37, br, s	9, 10	123.6, CH	5.36, br, s		123.4, CH	5.39, br, s	
9	26.7, CH_2	2.11, dd (8.5, 14.5); 2.31, d (14.5)	4, 7, 8, 10, 20	26.3, CH_2	2.10, dd (8.5, 14.5); 2.30, d (14.5)	8, 10	26.6, CH_2	2.11, dd (8.5, 14.5); 2.31, d (14.5)	4, 7, 8, 10
10	54.5, qC			54.8, qC			54.5, qC		
11	82.8, qC			82.6, qC			82.8, qC		
12	27.8, CH_3	1.47, s	3, 11, 13	29.1, CH_3	1.35, s	3, 11, 13	27.7, CH_3	1.50, s	3, 11, 13
13	146.0, CH	6.19, d (15.0)	11, 12, 14, 15	148.0, CH	6.41−6.42[b]	11, 15	147.4, CH	6.30, d (15.5)	11, 14, 15
14	120.0, CH	6.31, dd (11.5, 15.0)	11, 13, 15, 16	122.5, CH	6.41−6.42[b]	11, 15	119.6, CH	6.35, dd (10.5, 15.5)	15
15	136.5, CH	7.11, d (11.5)	13, 14, 16, 17	136.8, CH	7.08, d (11.5)		138.4, CH	7.26, d (10.5)	17, 18
16	127.4, qC			127.3, qC			125.9, qC		
17	12.5, CH_3	1.81, s	14, 15, 16, 18	12.5, CH_3	1.85, s	15, 16, 18	12.3, CH_3	1.86, s	15, 16, 18
18	168.9, qC			169.0, qC			166.0, qC		
19	26.5, CH_3	1.64, s	7, 8	26.3, CH_3	1.60, s	7, 8	26.4, CH_3	1.64, s	
20	174.2, qC			173.6, qC			174.1, qC		
4-O*H*		4.94, s	3, 4, 10		4.85, s	10		[c]	
18-COO*H*		12.28, br, s			12.26, br, s				
1′							94.6, CH	5.40, d (8.0)	18
2′							72.5, CH	3.17−3.22[b]	
3′							77.7, CH	3.17−3.22[b]	2′, 4′
4′							69.5, CH	3.11, m	3′, 5′
5′							76.3, CH	3.17−3.22[b]	
6′							60.5, CH_2	3.43, m; 3.63, d (10.5)	

[a] HMBC correlations are from proton(s) stated to the indicated carbon. [b] Overlapped signals. [c] nd = not detected.

Table 2. Selected NMR Data for Compounds **1** and **2** in $CDCl_3$ and CD_3OD

	$CDCl_3$ [δ_H (J in Hz)]		CD_3OD [δ_H (J in Hz)]	
	1	**2**	**1**	**2**
H-13	5.94, d (14.7)	6.24, d (15.5)	6.07, d (15.0)	6.27, d (15.3)
H-14	6.58, dd (11.4, 15.0)	6.53, dd (11.5, 15.5)	6.53, dd (11.4, 15.0)	6.54, dd (11.1, 15.6)
H-15	7.28, d (10.2)	7.23, d (11.5)	7.21, d (11.7)	7.15, d (10.8)

transition of the conjugated dienoic acid moiety attached to the chiral carbon C-11.[11,12] Thus, the change in the chirality of C-11 may lead to opposite Cotton effects. The C-11 configuration of **2** could therefore be assigned as *S* relative to the 11*R* configuration of **6** and **1**. Consequently, **1** was defined as 11*R*-deacetylpseudolaric acid A, and **2** as 11*S*-deacetylpseudolaric acid A.

In the 1980s, the structures of compounds **6** and **7** were identified by X-ray diffraction and CD experiments.[8,11,13] Subsequently, all the diterpenoids isolated from *P. kaempferi* were established to possess the same absolute configuration as **6** and **7**. 11*S*-Deacetylpseudolaric acid A (**2**) described here is the first compound isolated from *P. kaempferi* with a different absolute configuration at C-11. The amount of **2** obtained upon isolation did not permit crystallization. Thus, we could obtain only a CD spectrum for this compound and could not conduct any X-ray diffraction experiment.

Compound **3** was also isolated as a colorless, amorphous solid. Its molecular formula was determined to be $C_{26}H_{36}O_{10}$ by HRESIMS ([M + Na]$^+$, m/z 531.2220). The ^{1}H and ^{13}C NMR and CD data revealed that compound **3** has the same skeleton and absolute configuration as compound **1**. The anomeric proton [δ_H 5.40 (1H, d, J = 8.0 Hz)] displayed an HMBC correlation to δ_C 166.0, suggesting glycosylation at C-18. Enzymatic hydrolysis of **3** furnished D-glucose. This finding was confirmed by HPLC analysis of its 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative, which corresponded to the derivative of standard sugar. The coupling constant for H-1 and H-2 of the glucosyl moiety was 8.0 Hz. The anomeric configuration of glucose was therefore determined to be β. Thus, the structure of compound **3** was elucidated as deacetylpseudolaric acid A *O*-β-D-glucopyranoside.

Compound **4** was isolated as a colorless, amorphous solid with a molecular formula of $C_{23}H_{32}O_7$ as determined by HRESIMS ([M + Na]$^+$, m/z 443.2051). ^{1}H and ^{13}C NMR (Table 3) and CD data indicated that compound **4** also possesses the same skeleton and absolute configuration as **1**, but contains another oxygenated methine (δ_C 69.3) and two more oxygenated methylenes (δ_C 65.9, 62.6). Analyses of the 1D and 2D NMR data of **4** indicated the presence of a 2,3-dihydroxypropyl group.[9] This observation was supported by ^{1}H−^{1}H COSY correlations between H_2-1′ (δ 4.13, 3.99), H-2′ (δ 3.69), and H_2-3′ (δ 3.38) and by their HMQC, HMBC correlations to C-1′ (δ 65.9), C-2′ (δ 69.3), and C-3′ (δ 62.6). Furthermore, the HMBC correlations from H_2-1′ (δ 4.13, 3.99) to the C-18 carbonyl carbon (δ 167.3) corroborated that the 2,3-dihydroxypropyl group was linked to C-18 in the side chain, forming an ester of deacetylpseudolaric acid A. On the basis of all these findings, **4** was elucidated as deacetylpseudolaric acid A 2,3-dihydroxypropyl ester.

Table 3. NMR Data for Compounds **4** and **5** in DMSO-d_6

	4			**5**		
position	δ_C, mult.	δ_H (J in Hz)	HMBC[a]	δ_C, mult.	δ_H (J in Hz)	HMBC[a]
1	34.3, CH_2	1.61−1.73[b]; 1.93−2.04[b]	4, 10, 11	34.4, CH_2	1.52−1.70[b]; 2.02−2.09[b]	
2	26.4, CH_2	1.61−1.73[b]; 2.70, t (13.4)		24.3, CH_2	1.52−1.70[b]; 2.02−2.09[b]	
3	52.9, CH	2.20, d (6.3)	2, 4, 5, 10	53.1, CH	2.27, d (6.0)	4, 5, 10, 11
4	78.7, qC			78.5, qC		
5	33.2, CH_2	1.45−1.53[b]; 1.61−1.73[b]	4	33.2, CH_2	1.52−1.70[b]	
6	24.3, CH_2	1.45−1.53[b]; 1.93−2.04[b]	4, 5, 7	19.7, CH_2	2.55−2.62[b]	4, 7, 8
7	138.4, qC			133.9, qC		
8	123.5, CH	5.39, d (6.5)	9	142.7, CH	7.02, dd (8.2, 3.5)	10, 19
9	26.7, CH_2	2.12, dd (8.4, 14.6); 2.33, d (14.6)	4, 7, 8, 10	27.1, CH_2	2.44, dd (8.7, 14.6); 2.51−2.52, m	4, 7, 8, 10
10	54.5, qC			54.6, qC		
11	82.8, qC			83.5, qC		
12	27.7, CH_3	1.50, s	3, 11, 13	27.8, CH_3	1.48, s	11, 13
13	146.7, CH	6.26, d (15.1)	11, 14, 15	146.3, CH	6.26, d (15.1)	11, 14, 15
14	119.7, CH	6.35, dd (11.0, 15.1)	11, 13, 15	119.9, CH	6.34, dd (10.8, 15.1)	11, 15
15	137.0, CH	7.21, d (11.0)	13, 16, 17	137.0, CH	7.20, d (10.5)	13, 17, 18
16	126.6, qC			126.9, qC		
17	12.5, CH_3	1.87, s	15, 16, 18	12.6, CH_3	1.87, s	15, 16, 18
18	167.3, qC			167.4, qC		
19	26.5, CH_3	1.66, s	7, 8	167.6, qC		
20	174.2, qC			174.0, qC		
−OCH_3				51.8, CH_3	3.64, s	19
1′	65.9, CH_2	3.99, dd (6.3, 11.1); 4.13, dd (4.1, 11.1)	18, 2′	66.0, CH_2	3.99, dd (6.2, 11.1); 4.13, dd (4.0, 11.1)	18, 2′, 3′
2′	69.3, CH	3.69, q (5.1)		69.4, CH	3.70, q (5.1)	
3′	62.6, CH_2	3.38, m[b]	1′	62.7, CH_2	3.39, m[b]	1′, 2′
4-O*H*		4.97, s	10		nd[c]	
2′-O*H*		4.92, d (5.2)			nd[c]	
3′-O*H*		4.66, d (5.6)			nd[c]	

[a] HMBC correlations are from proton(s) stated to the indicated carbon. [b] Overlapped signals. [c] nd = not detected.

Compound **5** was also obtained as a colorless, amorphous solid. Its molecular formula was $C_{24}H_{32}O_9$ as determined by HRESIMS ([M + Na]$^+$, m/z 487.1942). The ^{1}H and ^{13}C NMR (DEPT) and HMQC spectra revealed the presence of one methoxy, two methyl, seven methylene, six methine, and eight quaternary carbons, including one disubstituted and two trisubstituted double bonds, three ester carbonyls, and two oxygenated quaternary carbons (Table 3). Together with its CD spectrum, these data suggest that compound **5** is a derivative of pseudolaric acid C_1 (**8**),[1] viz., deacetylpseudolaric acid B, with an additional oxygenated methine (δ_C 69.4) and two additional oxygenated methylenes (δ_C 66.0, 62.7). Analyses of the NMR data showed that compound **5** also had a 2,3-dihydroxypropyl group attached to C-18, forming an ester of deacetylpseudolaric acid B. The structure of **5** was therefore elucidated as deacetylpseudolaric acid B 2,3-dihydroxypropyl ester.

The structures of the eight known compounds (**6**−**13**) (Chart 1) were determined by the interpretation of spectroscopic data and by comparison with literature data.[1,8−10]

The activities of the isolated diterpenoids against three human tumor cell lines are summarized in Table 4. Compounds **6** and **7** had the strongest cytotoxic activities against HL-60 cells among the tested compounds. Compounds **1** and **8,** which are deacetyl derivatives of **6** and **7**, respectively, exhibited no cytotoxicities, indicating that the presence of the C-4-acetoxy is crucial for the cytotoxic property.

Glycosylations at the C-18 carboxyl of compounds **6** and **7** yield compounds **12** and **13**, respectively. Compounds **12** and **13** have lower cytotoxicities than **6** and **7**. Compound **3** is the deacetylated and 18-glucosylated product of **6**, while **4** and **5** are the deacetylated and 18-esterified products of **6** and **7**, respectively. None of these compounds (**3**−**5**) displayed any cytotoxic activity.

The C-7-carboxyl substitution of **6** leads to **9** and results in the loss of cytotoxic activity. 8-Carbonylation or 8α-hydroxylation and then isomerization of the C-7 double bond of **6** remarkably reduced the cytotoxicities. The corresponding compounds **10** and **11** exhibited only moderate cytotoxicities. The results obtained in this study led to a preliminary cytotoxic structure−activity relationship (SAR) of the diterpenoids, as illustrated in Figure 1.

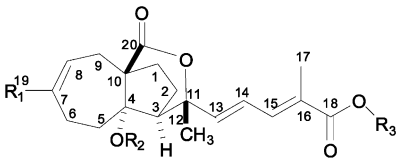
Overall, our study provides new insights into the cytotoxicity of diterpenoids **3**−**5**.[4] This cytotoxic SAR is similar to the antifungal SAR of the diterpenoids offered by Yang et al.[2] The major difference between these two results is that glycosylation at C-18 leads to the loss of antifungal activity with only a slight reduction of cytotoxicity. The explanation of this phenomenon needs further investigation.

Experimental Section

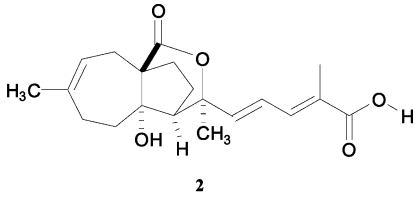
General Experimental Procedures. Optical rotations were performed with a Perkin-Elmer 243B polarimeter in MeOH at 25 °C. UV data were recorded on a TU-1901 UV−vis spectrophotometer. CD spectra were measured with a JASCO J-810 spectropolarimeter in MeOH flushed with N_2. IR spectra were determined with a Nicolet Avatar FT-IR spectrophotometer. NMR spectra were obtained on Bruker DRX-500 and ARX-400 spectrometers, and the chemical shifts are given in δ (ppm) values with reference to TMS. The coupling constants (J values) are reported in Hz. ESIMS and HRESIMS were carried out on ABI Qstar and Jeol AccuTof CS mass spectrometers, respectively. Analytical HPLC was conducted using an Agilent 1100 liquid chromatograph system, which was set up with a quaternary pump, a diode array detector, and YMC-Park ODS-A (4.6 mm i.d. × 150 mm, 5 μm) and Inertsil ODS-3 (4.6 mm i.d. × 250 mm, 5 μm) columns. Preparative HPLC was performed using a YMC-Park ODS-A column (10 mm i.d. × 250 mm, 5 μm). The HPLC was set up with a TSP P100 pump connected to a TSP UV 100 detector. Silica gel (200−300 mesh, Qingdao Marine Chemical Co. Ltd.) and ODS (Fuji Silysia Chemical Ltd.) were used in open column chromatography fractionations. All solvents used for isolation were of analytical grade. Standard sugars and reagents for derivatization were purchased from Sigma.

Plant Material. The bark of *P. kaempferi* was collected from Zhejiang Province, China, and identified by one of the authors (H.G.). A voucher specimen (040309TJP01) was deposited in the Division of Pharmacognostical Biotechnology, School of Pharmaceutical Sciences, Peking University, China.

Chart 1. Structures of **1**−**13**



	R_1	R_2	R_3
1	Me	H	H
3	Me	H	Glc
4	Me	H	$CH_2CH(OH)CH_2OH$
5	COOMe	H	$CH_2CH(OH)CH_2OH$
6	Me	Ac	H
7	COOMe	Ac	H
8	COOMe	H	H
9	COOH	Ac	H
12	Me	Ac	Glc
13	COOMe	Ac	Glc



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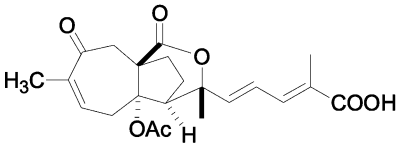

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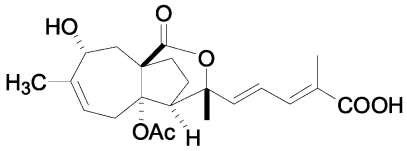

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Table 4. Cytotoxic Activities of Compounds **1** and **3**−**13**[a] against Three Human Cancer Cell Lines ($n = 3$)

	IC_{50} (μM)		
compound	HL-60	Bel-7402	BGC-823
fluorouracil	0.16	23.21	31.16
1	—[b]	—	—
3	—	—	—
4	—	—	—
5	—	—	—
6	1.51	NE[c]	NE
7	0.59	NE	NE
8	—	—	—
9	—	—	NE
10	55.44	NE	—
11	10.97	NE	NE
12	4.09	NE	NE
13	2.56	NE	NE

[a] Compound **2** was not available in sufficient amounts. [b] — = no cytotoxic activity. [c] NE = $IC_{50} > 100$ μM.

Extraction and Isolation. The bark of *P. kaempferi* (15 kg) was refluxed with 95% EtOH (45 L, 2 h, × 2). The resulting EtOH extract was concentrated (2 kg), suspended in H_2O, and partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc fraction (154.7 g) was then subjected to silica gel column chromatography. Fractions A−G were obtained through gradient elution with $CHCl_3$−MeOH (30:1 to 2:1). Fraction B was then applied to silica gel using a gradient solvent system of petroleum ether−EtOAc (3:1 to 1:1) and ODS open column chromatography (MeOH−H_2O, 6:4). The eluate was subsequently crystallized in MeOH, yielding compounds **6** (0.6023 g) and **7** (5.0615 g). Fraction D was also applied to silica gel ($CHCl_3$−MeOH, 20:1). The major resulting eluate was crystallized in MeOH, yielding compound **8** (1.7654 g). The remaining fractions of D were combined and subjected to ODS open column chromatography using a gradient elution (MeOH−H_2O, 4:6 to 6:4). Fractions were further purified by HPLC (MeOH−0.05% aqueous TFA, 55:45), thus yielding compounds **1** (108 mg), **2** (1.5 mg), **9** (120 mg), **10** (1.6 mg), and **11** (3.5 mg). Fraction E was isolated with silica gel column chromatography (petroleum ether−EtOAc, 1:8) and HPLC (MeOH−0.05% aqueous TFA, 52:48), resulting in compounds **4** (15.4 mg) and **5** (44.8 mg). Fraction F was subjected to silica gel column chromatography eluted with EtOAc−Me_2CO (10:1 to 2:1) and subsequently ODS open column chromatography (MeOH−H_2O, 2:8 to 6:4). The eluate was subjected to HPLC (MeOH−0.05% aqueous TFA, 50:50), yielding compounds **12** (20 mg) and **13** (150 mg). Fraction G was applied to silica gel column chromatography and eluted with $CHCl_3$−MeOH (10:1 to 2:1). The eluate was further separated using ODS column chromatography (MeOH−H_2O, 2:8). The main fractions were then purified by HPLC (MeOH−0.05% aqueous TFA, 12:88) to furnish compound **3** (5.1 mg).

Deacetylpseudolaric acid A (1): white, amorphous solid; $[\alpha]^{25}_D$ −58.0 (*c* 0.50, MeOH); CD (MeOH) $[\theta]_{257.1}$ -32.9×10^3; UV (MeOH) λ_{max} (log ϵ) 259.0 (4.30) nm; IR (KBr) ν_{max} 3489, 2924, 1718 cm^{-1}; ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data, see Table 1; HRESIMS $[M + Na]^+$ *m*/*z* 369.1698 (calcd for $C_{20}H_{26}O_5Na$, 369.1678).

11*S*-Deacetylpseudolaric acid A (2): colorless, amorphous solid; $[\alpha]^{25}_D$ +32.0 (*c* 0.06, MeOH); CD (MeOH) $[\theta]_{263.6}$ $+17.0 \times 10^3$; UV (MeOH) λ_{max} (log ϵ) 262.0 (4.29) nm; IR (KBr) ν_{max} 3424, 2923, 1737 cm^{-1}; ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data, see Table 1; HRESIMS $[M + Na]^+$ *m*/*z* 369.1663 (calcd for $C_{20}H_{26}O_5Na$, 369.1678).

Deacetylpseudolaric acid A *O*-β-D-glucopyranoside (3): colorless, amorphous solid; $[\alpha]^{25}_D$ −56.0 (*c* 0.25, MeOH); CD (MeOH) $[\theta]_{267.1}$ -5.88×10^3; UV (MeOH) λ_{max} (log ϵ) 263.5 (4.44) nm; IR (KBr) ν_{max} 3411, 2926, 1679 cm^{-1}; ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data, see Table 1; HRESIMS $[M + Na]^+$ *m*/*z* 531.2220 (calcd for $C_{26}H_{36}O_{10}Na$, 531.2206).

Enzymatic Hydrolysis and Derivatization of 3. A solution of compound **3** (2.0 mg) in 0.1 M acetate buffer (pH 4.0, 0.5 mL) was hydrolyzed with naringinase (Sigma, 4.0 mg) by stirring at 40 °C for 24 h. Using a Sep-Pak C_{18} cartridge (Waters), the reaction mixture was eluted with H_2O and MeOH, successively. The H_2O eluate was then concentrated, and the residue was dissolved in 1 mL of H_2O. Subsequently, L-(−)-α-methylbenzylamine (4 mg) and $NaBH_3CN$ (6 mg) in EtOH (1 mL) were added. After stirring this solution for 4 h at 40 °C, 0.2 mL of glacial HOAc was added, and the mixture was dried. The resulting solid was then acetylated with Ac_2O (0.3 mL) in pyridine (0.3 mL) at room temperature for 24 h. To remove pyridine from this reaction mixture, H_2O was added and evaporated off five times. The mixture was then filtered through a Sep-Pak C_{18} cartridge and successively eluted with 10 mL each of MeCN−H_2O (1:4) and MeCN−H_2O (1:1). The MeCN−H_2O (1:1) eluate was concentrated and analyzed by HPLC. The 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative was characterized by co-HPLC analysis with the same derivative of standard sugar prepared under the same conditions. HPLC

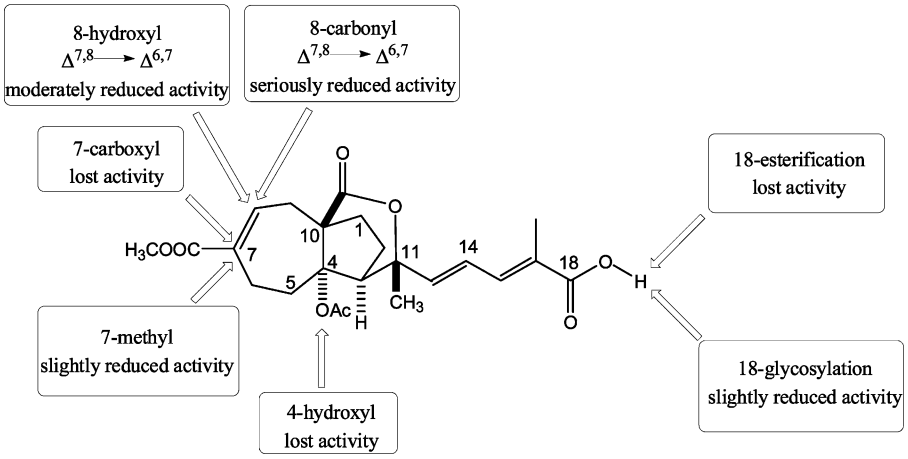

Figure 1. Structure−cytotoxic activity relationships of isolated diterpenoids. Activity was measured as growth inhibition on HL-60 cells. C-4-acetoxy is essential for the cytotoxicity.

conditions were as follows: Inertsil ODS-3 column (4.6 mm i.d. × 250 mm, 5 μm); solvent, MeCN−H_2O (2:3); flow rate, 0.8 mL/min; column temperature, 40 °C; detection, UV 230 nm.[14a−c] The derivative of D-glucose was detected at a t_R of 27.30 min.

Deacetylpseudolaric acid A 2,3-dihydroxypropyl ester (4): colorless, amorphous solid; $[\alpha]^{25}_D$ −59.7 (*c* 1.88, MeOH); CD (MeOH) $[\theta]_{261.0}$ −12.3 × 10^3; UV (MeOH) λ_{max} (log ϵ) 261.5 (4.34) nm; IR (KBr) ν_{max} 3354, 2931, 1704 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data, see Table 3; HRESIMS $[M + Na]^+$ *m*/*z* 443.2051 (calcd for $C_{23}H_{32}O_7Na$, 443.2046).

Deacetylpseudolaric acid B 2,3-dihydroxypropyl ester (5): colorless, amorphous solid; $[\alpha]^{25}_D$ −47.2 (*c* 5.0, MeOH); CD (MeOH) $[\theta]_{259.0}$ −14.4 × 10^3; UV (MeOH) λ_{max} (log ϵ) 261.5 (4.16) nm; IR (KBr) ν_{max} 3339, 2952, 1704 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data, see Table 3; HRESIMS $[M + Na]^+$ *m*/*z* 487.1942 (calcd for $C_{24}H_{32}O_9Na$, 487.1944).

Bioassays. Human tumor cell lines HL-60, Bel-7402, and BGC-823 were maintained in RPMI 1640 medium (Gibco/BRL) supplemented with 10% (v/v) fetal bovine serum, 100 IU/mL penicillin, and 100 IU/mL streptomycin. Cells were cultured in 96-well microtiter plates for the assay. Appropriate dilutions (10^{-2} to 10^2 μM) of the test compounds were added to the cultures. After a 48 h incubation in humidified air containing 5% CO_2 at 37 °C, growth inhibitions of the cancer cells were evaluated by the MTT method.[15] The activity is shown as IC_{50} value, which is the concentration (μM) of tested compound that results in 50% inhibition of cell growth. Results are expressed as the mean value of triplicate data points. Fluorouracil was used as a positive control.

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