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Pseudolaridimers A and B, Hetero-Cycloartane–Labdane Diels–Alder Adducts from the Cone of *Pseudolarix amabilis*

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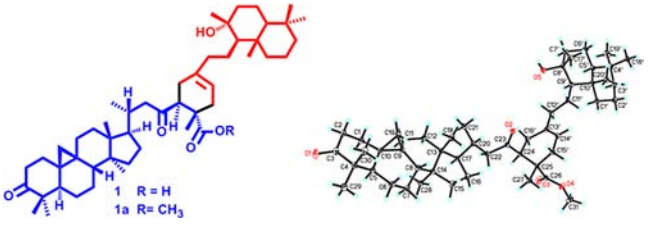
ABSTRACT



Pseudolaridimers A (1) and B (2), two unprecedented heterodimers formed via a [4 + 2] Diels–Alder cycloaddition between a cycloartane triterpenoid unit and a labdane diterpenoid unit, were isolated from the cones of *Pseudolarix amabilis*. Their structures were established by extensive analysis of HRESIMS and NMR spectra. The absolute configuration of 1 was determined by single crystal X-ray diffraction (Cu K_α) of its methyl esterified derivative. Pseudolaridimer A (1) showed strong cytotoxicity against HCT116, ZR-75-30, and HL-60 human tumor cell lines, with IC_{50} values 9.62, 7.84, 8.29 μg/mL, respectively. Pseudolaridimer B (2) only exhibited potent inhibition against the HL-60 cell line with an IC_{50} value of 7.50 μg/mL.

Pseudolarix amabilis (Pinaceae), a monotypic genus plant, is a tall deciduous conifer exclusively distributed in eastern China.[1] In traditional Chinese medicine, the root bark of this plant, known as "Tu-Jing-Pi", have been long used to treat skin disease caused by microbial infection.[2] Previous investigations on the root bark, trunk bark, twigs, and seeds of *P. amabilis* have led to the isolation of a number of structurally novel diterpenoids,[3] triterpenoid lactones,[4] lignans,[5] and flavones[6] from this plant. Some of these compounds, in particular pseudolaric acids and peroxide triterpenoid lactones, have demonstrated potent antifungal,[7] antifertility,[8] and cytotoxic activities.[9,10] These results greatly inspired our interest to search for other structurally unique bioactive compounds from other

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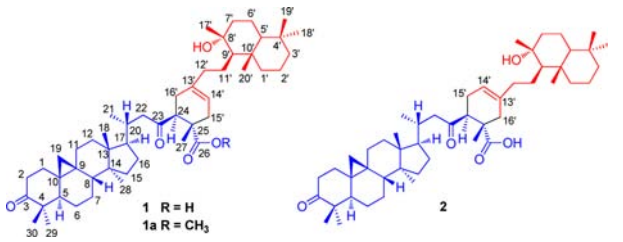

Figure 1. Structures of pseudolaridimers A (**1**) and B (**2**).

parts of the titled plant. Herein, we describe the isolation and structural elucidation of two unique hetero-cycloartane–labdane dimers pseudolaridimers A (**1**) and B (**2**) (Figure 1) from the cones of *P. amabilis*, which include an unusual carbon skeleton by a Diels–Alder [4 + 2] cycloaddition of a cycloartane triterpenoid unit and a labdane diterpenoid unit, together with their cytotoxic assay.

Pseudolaridimer A (**1**),[11] a white amorphous powder, was assigned a molecular formula $C_{50}H_{78}O_5$ as deduced from positive-ion HRESIMS at *m*/*z* 781.5745 $[M + Na]^+$ (calcd. 781.5747), requiring 12 degrees of unsaturation. The IR spectrum of **1** indicated the presence of two characteristic absorptions at 3438 (OH) and 1706 (carbonyl) cm^{-1}. In the ^1H NMR spectrum of **1**, 10 methyl signals, including 9 singlet methyls and 1 doublet methyl, were observed occurring at δ_H 0.83 (3H, s), 0.89 (9H, 3 × CH_3, each s), 1.01 (3H, s), 1.06 (3H, s), 1.16 (3H, s), 1.39 (3H, s), 1.76 (3H, s), and 1.04 (3H, d, J = 6.4 Hz) (Table 1). The proton resonance at δ_H 5.61 (1H, br s) suggested the presence of one olefinic group. The ^{13}C NMR spectrum gave 50 carbon resonances, which were sorted into 10 methyls, 19 methylenes, 8 methines, and 13 quaternary carbons by analysis of ^{13}C and DEPT NMR spectra (Table 1). Moreover, several important functionalities, including two ketone cabonyls (δ_C 215.3, 212.9), one carboxyl (δ_C 180.8), one olefinic group (δ_C 138.1, 118.8), and one oxygenated quaternary carbon (δ_C 73.4), were also identified. Considering 50 carbon atoms and 12 degrees of unsaturation in the structure of **1**, in combination with the number of methyls, it could be speculated that compound **1** may be a dimer of one triterpenoid moiety (Part A) fused with a diterpenoid moiety (Part B).

More detailed information about the structure of **1** came from interpretation of its $^1H-^1H$ COSY and HMBC spectra. Four structural fragments, corresponding to H_2-22/H-20(H_3-21)/H-17/H_2-16/H_2-15, H_2-11/H_2-12, H-5/H_2-6/H_2-7/H-8, and H_2-1/H_2-2, were identified by analysis

Table 1. ^1H and ^{13}C NMR Spectral Data for Pseudolaridimers A (**1**) and B (**2**)

	1[a]		**2**[b]	
no.	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)	δ_C
1	1.43 overlap	33.8	1.55 overlap	33.4
	1.78 overlap		1.86 overlap	
2	2.37 overlap	37.9	2.30 brd (13.6)	37.4
	2.72 overlap		2.71 dt (6.4, 14.0)	
3		215.3		216.7
4		50.6		50.2
5	1.65 overlap	48.8	1.71 dd (4.0, 12.0)	48.4
6	0.89 overlap	21.9	0.96 overlap	21.5
	1.44 m		1.56 overlap	
7	1.06 overlap	26.4	1.14 overlap	25.8
	1.30 overlap		1.36 overlap	
8	1.47 overlap	48.2	1.58 overlap	47.8
9		21.3		21.0
10		26.4		25.9
11	1.09 overlap	27.0	1.17 overlap	26.6
	1.95 ddd (2.0, 8.4, 15.6)		2.04 overlap	
12	1.61 overlap	33.3	1.65 overlap	32.7
13		46.0		45.4
14		49.4		48.9
15	1.30 overlap	36.1	1.31 overlap	35.5
16	1.28 overlap	28.9	1.31 overlap	28.3
	1.91 m		1.88 overlap	
17	1.64 overlap	52.8	1.59 overlap	52.2
18	0.89 s	19.4	0.91 s	19.3
19	0.44 d (4.0)	29.9	0.58 d (4.0)	29.5
	0.60 d (4.0)		0.79 overlap	
20	2.30 overlap	33.2	2.03 overlap	32.6
21	1.04 d (6.4)	20.2	0.85 d (6.4)	19.5
22	2.46 dd (10.0, 16.8)	50.2	2.20 dd (10.0, 16.4)	49.2
	2.86 d (17.2)		2.54 dd (2.0, 16.4)	
23		212.9		212.1
24	3.56 dd (4.8, 12)	52.6	3.05 dd (2.4, 11.2)	50.1
25		44.0		43.0
26		180.8		183.0
27	1.76 s	17.5	1.26 s	16.6
28	1.01 s	18.7	1.04 s	18.1
29	1.06 s	21.2	1.10 s	20.7
30	1.16 s	23.0	1.04 s	22.2
1'	1.10 overlap	40.5	0.94 overlap	39.8
	1.80 overlap		1.64 overlap	
2'	1.45 overlap	19.3	1.45 overlap	18.4
	1.65 overlap		1.58 overlap	
3'	1.17 m	42.6	1.14 overlap	42.0
	1.37 overlap		1.38 overlap	
4'		33.8		33.2
5'	1.03 overlap	56.8	0.93 overlap	56.1
6'	1.09 dd (3.6, 12.8)	21.3	1.25 overlap	20.5
	1.62 m		1.65 overlap	
7'	1.76 overlap	45.4	1.38 overlap	44.5
	2.05 dt (2.8, 12.4)		1.87 overlap	
8'		73.4		74.4
9'	1.43 overlap	62.4	1.03 overlap	61.3
10'		39.7		39.2
11'	1.59 overlap	24.7	1.35 overlap	23.7
	2.10 ddd (2.0, 8.4, 15.6)		1.51 overlap	
12'	2.33 overlap	41.8	2.00 overlap	40.9
	2.65 overlap		2.09 overlap	
13'		138.1		137.0
14'	5.61 br s	118.8	5.42 br s	117.7
15'	2.41 overlap	38.8	2.01 overlap	25.4
	2.82 d (16.8)		2.43 overlap	
16'	2.37 overlap	29.6	2.03 overlap	40.4
	2.72 d (17.2)		2.38 overlap	
17'	1.39 s	25.2	1.15 s	23.9
18'	0.83 s	22.1	0.79 s	21.5
19'	0.89 s	33.9	0.87 s	33.4
20'	0.89 s	15.8	0.80 s	15.5

[a] Determined at 400 MHz for ^1H and 100 MHz for ^{13}C in pyridine-d_5. [b] Determined at 400 MHz for ^1H and 100 MHz for ^{13}C in $CDCl_3$.

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(11) Pseudolaridimer A (**1**): $C_{50}H_{78}O_5$; white amorphous powder; $[\alpha]^{20}_D$ +21.3 (*c* 0.3, EtOH); CD (*c* 0.69 mmol/L, MeOH, 20 °C) nm ($\Delta\varepsilon$) 290 (−4.6), 218 (+0.82); IR (KBr) ν_{max} 1706, 3000, 3438 cm^{-1} ; for ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive) *m*/*z* 781.8 $[M + Na]^+$; HRESIMS *m*/*z* 781.5745 $[M + Na]^+$ (calcd for $C_{50}H_{78}O_5Na$, 781.5747].

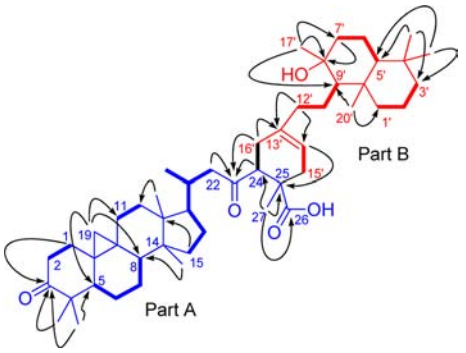


Figure 2. Key ^{1}H−^{1}H COSY (bold bond) and HMBC (arrows) correlations for pseudolaridimer A (**1**).

of their ^{1}H−^{1}H COSY correlations (Figure 2). Two characteristic proton resonances at δ_H 0.44 (*d*, J = 4.0 Hz) and 0.60 (*d*, J = 4.0 Hz) attributed to a methylene (δ_C 29.9) by an HSQC experiment were correlated with C-5 (δ_C 48.8) and C-8 (δ_C 48.2) in the HMBC spectrum, indicative of a cyclopropane ring. The above information coupled with 5 singlet methyls and a doublet methyl confirmed that the triterpenoid moiety of **1** is a cycloartane-type derivative. The HMBC spectrum of **1** exhibited the key correlations (Figure 2) from H_2-22 (δ_H 2.46, 2.86) and H-24 (δ_H 3.56) to a ketone carbonyl at δ_C 212.9, and from methyl proton CH_3-27 (δ_H 1.76) to C-24 (δ_C 52.6), C-25 (δ_C 44.0), and carboxyl (δ_C 180.7), assigning the ketone carbonyl at δ_C 212.9 and carboxyl to C-23 and C-26, respectively. Moreover, the ketone carbonyl at δ_C 215.3 was attributed to C-3 based on its HMBC correlation with two methyls CH_3-29 at δ_H 1.06 and CH_3-30 at δ_H 1.16.

By deducting the triterpenoid moiety, the remaining 20 carbon resonances, including 4 methyls, 9 methylenes, 3 methines, and 4 quaternary carbons, constructed a diterpenoid unit. The ^{1}H−^{1}H COSY spectrum exhibited the correlations of H_2-1′/H_2-2′/H_2-3′, of H-5′/H_2-6′/H_2-7′, of H-9′/H_2-11′/H_2-12′, and of H-14′/H_2-15′ (Figure 2). Key HMBC correlations (Figure 2) of CH_3-18′ (δ_H 0.83, s) and CH_3-19′ (δ_H 0.89, s) with C-3′ (δ_C 42.6) and C-5′ (δ_C 56.8) and of CH_3-17′ (δ_H 1.39, s) with C-7′ (δ_C 45.4) and C-9′ (δ_C 62.4) further supported the presence of a labdane-type diterpenoid skeleton. A hydroxyl was attached to C-8′ (δ_C 73.4) due to the HMBC correlations from H_2-7′ (δ_H 1.76, 2.05) and H_3-17′ (δ_H 1.39) to the oxygenated quaternary carbon at δ_C 73.4, while the HMBC correlations of H_2-12′ with two olefinic carbons at δ_C 138.1 and 118.8, and of H_2-16′ (δ_H 2.37, 2.72) with olefinic carbon at δ_C 138.1, assigned the two olefinic carbons to C-13′ and C-14′. On the basis of the above data, the diterpenoid moiety of **1** was characterized as a labdane-type diterpenoid derivative.

Finally, the triterpenoid moiety of **1** was fused with the diterpenoid moiety *via* two C−C bonds (between C-16′ and C-24, C-15′, and C-25) as part of an additional cyclohexene unit due to the key ^{1}H−^{1}H COSY correlation of H_2-16′/H-24 and two HMBC correlations of H_2-16′ with C-23 (δ_C 212.9) and H-14′ (δ_H 5.61, br s) with C-25 (δ_C 44.0), respectively. Consequently, the planar structure of **1** was established as shown in Figure 1.

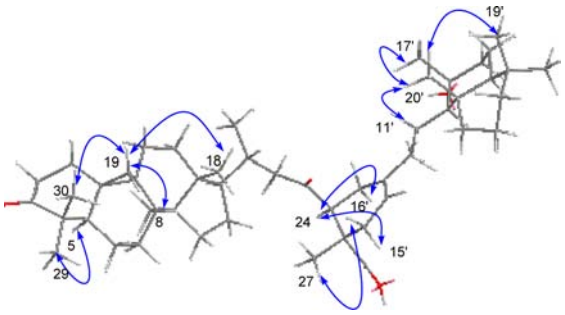


Figure 3. Key ROESY (blue arrows) correlations for **1**.

In the ROESY spectrum of **1**, the ROESY correlations (Figure 3) of CH_3-29 (δ_H 1.06) with H-5 (δ_H 1.65) and of H_2-19 with H-8 (δ_H 1.47), CH_3-18 (δ_H 0.89), and CH_3-30 (δ_H 1.16) established the relative configuration of the triterpenoid moiety as those of usual cycloartanes. Moreover, the relative configuration of the diterpenoid moiety was determined to be identical with those of usual labdanes on the basis of the key ROESY correlations (Figure 3) from CH_3-20′ (δ_H 0.89) to CH_3-17′ (δ_H 1.39) and H_2-11′ (δ_H 1.59, 2.10) and from CH_3-17′ to CH_3-19′. However, the stereochemistry of the cyclohexene moiety, including H-24 (δ_H 3.56) and CH_3-27 (δ_H 1.76), could not be determined due to the lack of sufficient ROESY correlations and structural flexibility. To solve this issue, the 26-carboxyl of **1** underwent methyl esterification using diazomethane, yielding a methylesterified product **1a** (see Figure 1). Comparison of the CD spectra between **1** and **1a** displayed very similar CD curves (see Supporting Information (SI)), suggesting that **1a** possesses the same stereoconfiguration as **1**. A suitable crystal of **1a** was obtained from an acetone/cyclohexane solution. The absolute configuration of **1** was finally determined as 5*R*, 8*R*, 9*S*, 10*R*, 13*R*, 14*S*, 17*R*, 20*R*, 24*S*, 25*S*, 5′*S*, 8′*R*, 9′*R*, and 10′*S*, by the single crystal X-ray diffraction of **1a** (Figure 4),[12] and named pseudolaridimer A.

Pseudolaridimer B (**2**)[13] was obtained as a white amorphous powder. The molecular formula of **2** was deduced to be identical with that of **1** from positive-ion HRESIMS at m/z 781.5750 $[M + Na]^+$ (calcd. 781.5747). The ^{1}H and ^{13}C NMR data (Table 1) of **2** were very close to those of **1**, except that C-15′ in **2** was shifted upfield from δ_C 29.6 (C-16′ in **1**) to 25.4, while C-16′ was shifted downfield from δ_C 38.8 (C-15′ in **1**) to 40.4. Additionally, key HMBC correlations of H_2-12′ (δ_H 2.00, 2.09) and CH_3-27 (δ_H 1.26)

(12) CCDC 875931 contains the crystallographic data for compound **1a**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centra via www.ccdc.cam.ac.uk/data_request/cif.

(13) Pseudolaridimer B (**2**): white amorphous powder; $[\alpha]^{20}_D$ +11.3 (*c* 0.3, $CHCl_3$); CD (*c* 0.65 mmol/L, MeOH, 20 °C) nm ($\Delta\varepsilon$) 294 (−2.1), 207 (−3.9); IR (KBr) ν_{max} 1708, 3000, 3440 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Table 1; ESIMS (positive) m/z 781.7 $[M + Na]^+$; HRESIMS m/z 781.5750$[M + Na]^+$ (calcd for $C_{50}H_{78}O_5Na$, 781.5747].

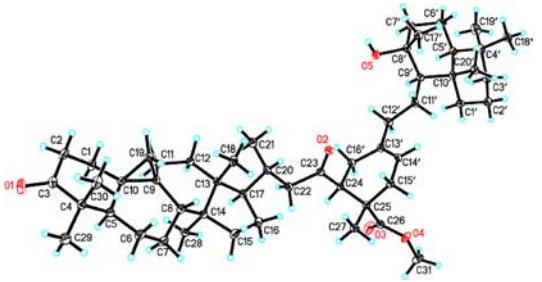
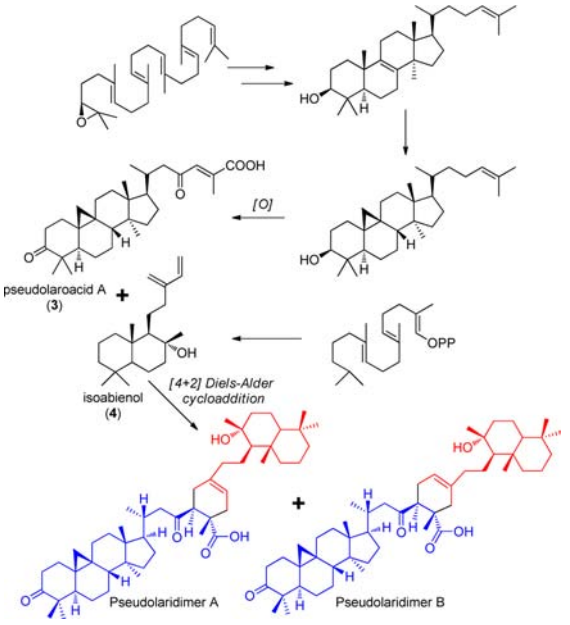


Figure 4. Single X-ray diffraction of the methyl ester of pseudolaridimer A (**1a**).

with C-16′ (δ_C 40.4), and of the olefinic proton (δ_H 5.42) with C-24 (δ_C 50.1), were observed. These results disclosed that the triterpenoid and diterpenoid moieties of **2** were connected by two C−C bonds C-15′−C-24 and C-16′−C-25, instead of the C-16′−C-24 and C-15′−C-25 bonds of **1**. The absolute configuration of **2** was determined to be identical with that of **1** based on the quite similar CD curves (see SI) of these two molecules.

Although many terpenoid dimers have been reported,[14] triterpenoid−diterpenoid dimers are still very rare. Pseudolaridimers A and B are the first two cycloartane−labdane dimers and represent an unusual carbon skeleton. The Diels−Alder reaction is synthetically very useful because it can form a new six-membered carbon ring under mild conditions. A number of structurally complex natural products were often explained to be biogenetically synthesized by an enzymatic Diels−Alder cycloaddition reaction[14] and attracted many research groups to explore and identify enzymatic Diels−Alder synthesis of natural products. Recently, the isolation and identification of some natural Diels−Alderases, such as solanapyrone synthase,[15] lovastatin nonaketide synthase,[16] and macrophomated synthase,[17] have increasingly proved that the presence of Diels−Alderases in plants is possible, and it may play an important role in the biological synthesis of natural products. In our investigation, a cycloartane-type triterpenoid acid named pseudolaroacid A (**3**) (for data see SI) and several labdane diterpenoids also had been isolated from the cones of *P. amabilis*. A plausible biogenetic pathway for pseudolaridimers A and B was thus proposed in Scheme 1. The key step of this biogenetic pathway is a Diels−Alder reaction between a triterpenoid precursor pseudolaroacid A (**3**)[18] and a diterpenoid precursor isoabienol (**4**).[19] Therefore, the possible Diels−Alderases in the biosynthesis of

Scheme 1. Proposed Biogenetic Pathway for Pseudolaridimers A (**1**) and B (**2**)



pseudolaridimers A and B will be an interesting research topic.

Compounds **1** and **2** were evaluated for cytotoxicity against five human cancer cell lines, A549 (lung carcinoma), HCT116 (colorectal carcinoma), ZR-75-30 (breast carcinoma), HL-60 (promyelocytic leukemia cells), and HepG2 (liver hepatocellular carcinoma), using the MTT method. Compound **1** showed significant cytotoxicity against HCT116, ZR-75-30, and HL-60 cell lines, with the IC_{50} value range 7.84−9.62 μg/mL. Compound **2** only exhibited potent inhibition against the HL-60 cell line with an IC_{50} value of 7.50 μg/mL.

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Supporting Information Available. Detailed isolation procedure, NMR spectra, and single crystal X-ray diffraction data. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.