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Pallambins A and B, Unprecedented Hexacyclic 19-*nor*-Secolabdane Diterpenoids from the Chinese Liverwort *Pallavicinia ambigua*

Li-Ning Wang,[†] Jiao-Zhen Zhang,[†] Xia Li,[†] Xiao-Ning Wang,[†] Chun-Feng Xie,[‡] Jin-Chuan Zhou,[†] and Hong-Xiang Lou*,[†]

Department of Natural Products Chemistry, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, P. R. China, and College of Pharmacy, Nankai University, Tianjin 300071, P. R. China

louhongxiang@sdu.edu.cn

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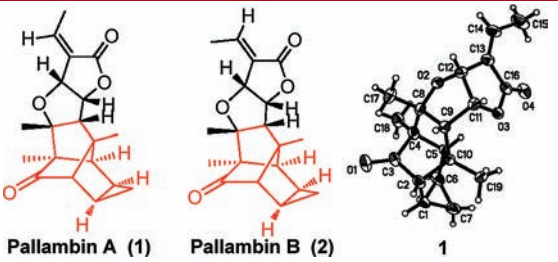
ABSTRACT



Pallambins A (1) and B (2), two novel 19-*nor*-7,8-secolabdane diterpenoids with unprecedented tetracyclo[4.4.0^{3,5}.0^{2,8}]decane skeletons, along with a pair of structurally related isomers, pallambins C (3) and D (4), were isolated from the Chinese liverwort *Pallavicinia ambigua*. Their structures with absolute configurations were determined by means of NMR, X-ray diffraction, and CD analyses. Their preliminary cytotoxicity to human cancer cells was also tested.

Liverworts are the most primitive group of terrestrial plants, which are widely distributed throughout the world.[1] They produce rich terpenoids and aromatic compounds, many of which exhibit a variety of fascinating structures and interesting biological activities.[2] Previous chemical investigations on liverworts of the genus *Pallavicinia* afforded many kinds of di- and sesquiterpenoids, mainly including the labdane and clerodane diterpenoids, as well as sesquiterpeniods.[3] In the current study, pallambin A (**1**) and pallambin B (**2**), two novel 19-*nor*-7,8-secolabdane diterpenoids with unprecedented tetracyclodecane skeletons, along with a pair of structurally related *Z*(*E*)-isomers, pallambin C (**3**) and pallambin D (**4**), were isolated from the epilithic liverwort *Pallavicinia ambigua* (Mitt.) Steph. collected from Zunyi, Guizhou province, P. R. China. Herein, we report the isolation, structural elucidation, and preliminary biological assay of compounds **1**–**4**. In addition, a plausible biosynthetic pathway of **1**–**4** is also proposed.

The liverwort was authenticated by Prof. Yuan-Xin Xiong (College of Life Sciences, Guizhou University, P. R. China). A voucher specimen (no. TX-01-201007-PA)

[†] Shandong University.
[‡] Nankai University.

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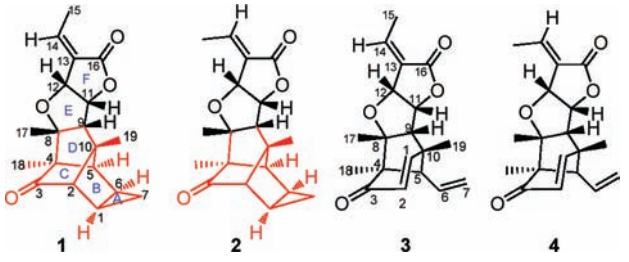
has been deposited in the Department of Natural Products Chemistry, School of Pharmaceutical Sciences, Shandong University, P. R. China. The air-dried powder of the plant material (180 g) was percolated with 95% EtOH at room temperature (3 × 7 days). The obtained crude extract (7.5 g) was chromatographed over an MCI gel column (CHP20P, 70−150 μm, MeOH/H_2O, 0:1 to 1:0) to give thirteen fractions (Fr. 1−13). Fraction 6 (0.8 g) was separated on an RP-18 silica gel column (MeOH/H_2O, 1:9 to 9:1) to give eight subfractions (Fr. 6a−6h). Fraction 6h (70 mg) was further purified by HPLC [Agilent 1100 isopump, Agilent 1100 VWD detector (210 nm), and Phenomenex Luna 5 μm C18(2) column (250 mm × 4.60 mm), MeOH/H_2O 50:50, 0.8 mL/min] to yield **1** (1.8 mg, 31 min), **2** (1.2 mg, 27 min), **3** (15.0 mg, 29 min), and **4** (25.0 mg, 33 min).



Pallambin A (**1**)[4] was isolated as a colorless crystal (in MeOH). Its molecular formula was determined to be $C_{19}H_{22}O_4$ by HRESIMS requiring 9 degrees of unsaturation. The ^{13}C NMR spectrum revealed one double bond, an ester carbonyl, and a ketone carbonyl, suggesting compound **1** was a six-ring structure in order to achieve its degree of unsaturation. The coupled ^1H and ^{13}C NMR signals at δ_H 2.27 (1H, d, J = 7.1), 4.95 (1H, dd, J = 7.1, 3.5), 4.75 (1H, d, J = 3.5), 6.69 (1H, J = q, 7.3), 2.27 (3H, d, J = 7.3) and at δ_C 168.3, 144.8, 126.0, 89.8, 84.7, 80.1, 60.9, 44.6, 14.2 of rings E and F with ethylidene bear a close resemblance with those of neopallavicinin,[3b] suggesting that **1** was a modified 7,8-secolabdane framework with a Δ^{13} double bond. The gross structure of **1** was constructed by the detailed analysis of 1D and 2D NMR data, especially the HMBC and $^1H-^1H$ COSY spectrum. The fusion of rings D−F were readily established by comparison with those of several known 7,8-secolabdane diterpenoids, such as noepallavicinin (Supporting Information, SI).[5] Furthermore, the ring A cyclopropyl was determined to be assembled through C-1/C-2 and C-5/C-6 on the basis of the $^1H-^1H$ COSY corelations of H-5/H-6, H-1/H-2. Along with the key HMBC correlations of H-9/C-2, H-19/C-2, and H-5/C-2 for C-2/C-10 linkage, ring B was deduced accordingly. The above assigned functional groups and ring system (a double bond, an ester, a ketone, and rings A, B, D, E, F) accounted for 8 out of the 9 degrees of unsaturation. The remaining 1 degree of unsaturation required the presence of an additional ring C in **1**. The linkages of fragments to ring C were again comfirmed by the HMBC correlations of H-18/C-3 for C-3/C-4 linkage. Ring C was furnished via C-2/C-3 to form a highly compact structure, with an unprecedented tetracyclo[4.4.$0^{3,5}$.$0^{2,8}$]decane skeleton, although there was no HMBC correlation of H-2/C-3 observed between the two "ends" C-2 and C-3 (Figure 1).

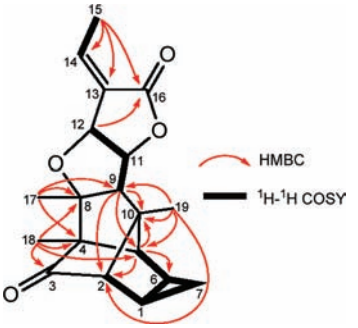


Figure 1. Key HMBC and $^1H-^1H$ COSY correlations of **1**.

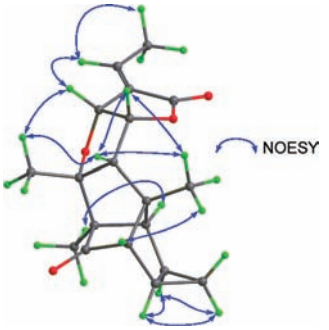


Figure 2. Key NOESY correlations of **1**.

The NOESY correlations (Figure 2) determined the relative configuration of **1**. Moreover, this assignment was aslo confirmed by a single-crystal X-ray diffraction study (Figure 3).

Pallambin B (**2**)[6] was obtained as a colorless oil. Its molecular fomula $C_{19}H_{22}O_4$ could be established by the HRESIMS, which showed an $[M+Na]^+$ ion peak at m/z 337.1410 (calcd 337.1410). The close resemblance between the NMR spectra of **1** and those of **2** indicated that **2** was another labdane diterpenoid analogue of **1**. The proton and carbon assignments (Table 1) were achieved by

(4) Pallambin A (**1**): Colorless crystals (MeOH); $[\alpha]^{20}_D$ −260.9 (c 0.092, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.94) nm; CD (MeOH) 195 ($\Delta\varepsilon$ +2.61), 214 ($\Delta\varepsilon$ −0.90), 249 ($\Delta\varepsilon$ +0.42), 297 ($\Delta\varepsilon$ −6.26) nm; ^1H and ^{13}C NMR, see Table 1; positive ESIMS m/z (relative intensity) 332.6 $[M+H_2O]^+$ (100), 315.4 $[M+H]^+$ (78), 337.6 $[M+Na]^+$ (20); positive HRESIMS m/z 337.1412 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_4Na$, 337.1410).

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(6) Pallambin B (**2**): Colorless oil; $[\alpha]^{20}_D$ −320.7 (c 0.106, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.98) nm; CD (MeOH) 194 ($\Delta\varepsilon$ +0.54), 216 ($\Delta\varepsilon$ −3.12), 248 ($\Delta\varepsilon$ +0.70), 297 ($\Delta\varepsilon$ −6.80) nm; ^1H and ^{13}C NMR, see Table 1; positive ESIMS m/z (relative intensity) 332.6 $[M+H_2O]^+$ (100), 315.4 $[M+H]^+$ (93), 337.6 $[M+Na]^+$ (50); positive HRESIMS m/z 337.1410 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_4Na$, 337.1410).

Table 1. ^{1}H and ^{13}C NMR Data of **1**, **2**, **3**, and **4** (in $CDCl_3$)[a]

	1		**2**		**3**		**4**	
position	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C
1	1.43 (m)	14.5 d	1.40 (m)	14.2 d	6.69 (m)	156.9 d	6.70 (dd, 9.6, 2.1)	157.0 d
2	2.49 (br s)	58.0 t	2.50 (br s)	58.0 t	5.89 (d, 9.6)	126.8 d	5.91 (d, 9.6)	126.8 d
3		214.4 s		214.4 s		201.3 s		201.3 s
4		70.0 s		66.8 s		62.6 s		62.7 s
5	2.48 (br s)	54.9 d	2.42 (br s)	54.9 d	2.84 (dd, 9.9, 2.0)	63.8 d	2.78 (dd, 9.9, 2.0)	63.9 d
6	0.87 (m)	15.0 d	0.87 (m)	15.0 d	5.54 (dt, 17.0, 9.9)	132.2 d	5.55 (dt, 16.9, 9.9)	132.2 d
7α	0.56 (dd, 13.8, 7.5)	12.0 t	0.56 (dd, 13.8, 7.5)	11.9 t	5.19 (2H, m)	121.5 t	5.19 (2H, m)	121.5 t
7β	1.40 (m)		1.42 (m)					
8		89.8 s		90.1 s		94.0 s		94.4 s
9	2.27 (d, 7.1)	60.9 d	2.48 (d, 7.1)	60.5 d	2.54 (d, 6.7)	60.6 d	2.58 (d, 7.0)	60.3 d
10		44.6 s		44.6 s		46.8 s		46.7 s
11	4.95 (dd, 7.1, 3.5)	84.7 d	4.98 (dd, 7.1, 3.9)	85.4 d	4.89 (dd, 6.8, 3.3)	82.9 d	4.92 (dd, 7.0, 3.6)	83.6 d
12	4.75 (d, 3.5)	80.1 d	5.00 (d, 3.9)	75.6 d	4.85 (d, 3.3)	82.1 d	5.11 (dd, 3.5, 0.8)	77.6 d
13		126.0 s		127.5 s		125.9 s		127.5 s
14	6.69 (q, 7.3)	144.8 d	7.03 (q, 6.8)	142.0 d	6.69 (1H, m)	144.6 d	7.07 (qd, 7.2, 0.8)	141.7 d
15	2.27 (3H, d, 7.3)	14.2 q	2.05 (3H, d, 6.8)	16.0 q	2.26 (3H, d, 7.3)	14.4 q	2.06 (3H, d, 7.2)	16.0 q
16		168.3 s		170.3 s		168.1 s		169.3 s
17	1.11 (3H, s)	19.4 q	1.13 (3H, s)	19.4 q	1.15 (3H, s)	22.2 q	1.18 (3H, s)	22.3 q
18	1.18 (3H, s)	7.3 q	1.17 (3H, s)	7.8 q	1.04 (3H, s)	12.7 q	1.03 (3H, s)	12.7 q
19	1.34 (3H, s)	21.5 q	1.39 (3H, s)	21.6 q	1.42 (3H, s)	19.5 q	1.44 (3H, s)	19.6 q

[a] Recorded at 600 MHz (^{1}H NMR) or 150 MHz (^{13}C NMR). *J* in Hz. ^{13}C multiplicities were determined by HSQC experiments.

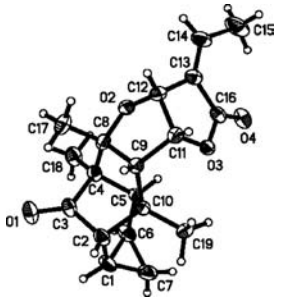

Figure 3. X-ray crystal structure of compound **1**.

HSQC, HMBC, and $^{1}H-^{1}H$ COSY spectra. The stereochemistry was deduced from the NOESY spectrum (SI). The key NOE correlation of H-12/H-15 in **2** which supported *E*-$\Delta^{14,15}$ was unambiguously observed, whereas a cross peak of H-12/H-14 for *Z*-$\Delta^{14,15}$ was clearly shown in the NOESY spectrum of **1**. *Z*-form could also explain the downfield shift of H-14 which was deshielded at δ_H 7.03, as a consequence of the anisotropic influence of the lactone carbonyl function.[7] Accordingly, the structure of **2** was determined as shown.

Compounds **3**[8] and **4**[9] showed identical EIMS and very similar NMR spectra. In addition their NMR data resembled those of **1** and **2**. But **3** and **4** showed one ethenyl group connected to C-5, and both demonstrated the same molecular formula, $C_{19}H_{22}O_4$, by HRESIMS. The structures of compounds **3** and **4** were confirmed by ^{1}H, ^{13}C, COSY, HSQC, and HMBC spectra (SI) experiments. They were assigned as a pair of geometric isomers at C-14, with **3** as an *E*-isomer, showing H-14 at δ_H 6.69 (1H, m), and **4** as a *Z*-isomer, showing H-14 at δ_H 7.07 (qd, 7.2, 0.8). The relative configuration of **3** was established by the key NOESY correlations of H-14/H-12, H-12/H-11, H-12/H-8, H-8/H-9, and H-9/H-11 as depicted. The NOESY NMR spectroscopic data also assigned the relative configuration of **4**, which was further elucidated by X-ray diffraction analysis (SI).

Compound **4** was even isolated from liverwort *P. subciliata* by Asakawa et al.,[3c] providing the X-ray crystallographic result,[3c] but no physicochemical data were reported. Herein, we further report the data and supply **4** with the trivial name pallambin D followed by the other three compounds.

The absolute configurations of **1**–**4** were determined on the basis of circular dichroism (CD) analyses. In the CD spectrum of **1**, the $\pi\rightarrow\pi^*$ transition of the lactone moiety gave rise to a negative Cotton effect at *ca.* 225 nm as that reported for neopallavicinin. This implied that the

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(8) Pallambin C (**3**): Colorless crystals (MeOH); $[\alpha]^{20}_D$ −165.1 (*c* 0.103, MeOH); UV (MeOH) λ_{max} (log ε) 193 (3.99), 214 (4.06) nm; CD (MeOH) 208 ($\Delta\varepsilon$ −2.58), 250 ($\Delta\varepsilon$ +3.96) nm; ^{1}H and ^{13}C NMR, see Table 1; positive ESIMS *m*/*z* (relative intensity) 315.3 $[M+H]^+$ (100), 337.6 $[M+Na]^+$ (15); positive HRESIMS *m*/*z* 337.1411 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_4Na$, 337.1410).

(9) Pallambin D (**4**): Colorless oil; $[\alpha]^{20}_D$ −158.4 (*c* 0.106, MeOH); UV (MeOH) λ_{max} (log ε) 191 (3.96), 217 (3.86) nm; CD (MeOH) 214 ($\Delta\varepsilon$ −4.44), 249 ($\Delta\varepsilon$ +3.51) nm; ^{1}H and ^{13}C NMR, see Table 1; positive ESIMS *m*/*z* (relative intensity) 315.3 $[M+H]^+$ (100), 337.6 $[M+Na]^+$ (33); positive HRESIMS *m*/*z* 337.1416 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_4Na$, 337.1410).

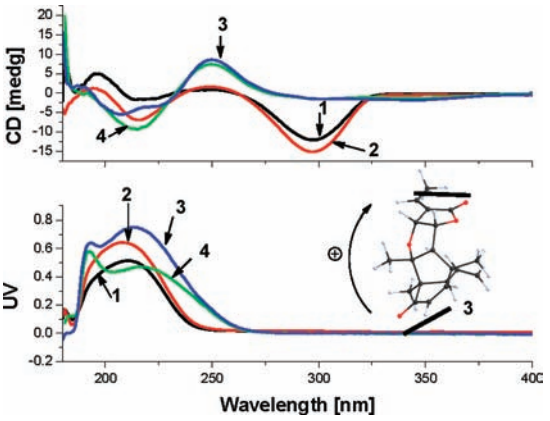


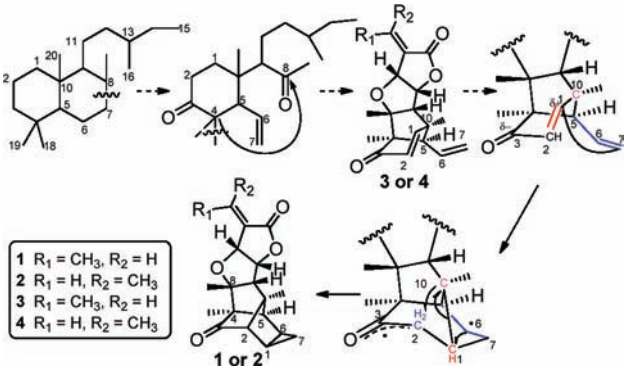
Figure 4. ECD spectra of **1**−**4**.

endoform lactone rings in **1** had the same fusion geometry with that of neopallavicinin.[3d] Furthermore, the positive Cotton effect with a maximum near 250 nm ($n\rightarrow\pi^*$) supported a *cis*-fused γ-lactone for compound **1**.[3d] Thus the absolute configurations of the seven chiral centers in **1** were determined as 1*R*, 2*S*, 4*R*, 5*R*, 6*S*, 8*R*, 9*S*, 10*S*, 11*R*, 12*R*. The absolute configuration of **2** was determined as shown by the similar Cotton effects to **1** in the CD spectrum (Figure 4). The absolute configurations of **3** and **4** were determined by applying the CD exciton chirality method.[10] The CD spectrum of **3** (Figure 4) exhibited the first positive Cotton effect at 250 nm ($\Delta\varepsilon$ +3.96) and the negative one at 208 nm ($\Delta\varepsilon$ −2.58) corresponding to an exciton coupling between the α,β-unsaturated ketone and the α,β-unsaturated γ-lactone, indicating that the transition dipole moments of the two chromophores were oriented in a clockwise manner (Figure 4). Their absolute configurations were thus defined as depicted.

Pallambin A (**1**) and pallambin B (**2**) were unprecedented hexacyclic 19-*nor*-secolabdane diterpenoids with an exotetracyclo[4.4.$0^{3,5}$.$0^{2,8}$]decane framework, formed by an unprecedented C-2/C-10 linkage. Compounds **1**−**4** were derived from the same labdane precursor. First, compounds **3** and **4** were formed by C_7−C_8 bond cleavage and C_8−C_4 bond reconstruction and then tranformed into **1** and **2** as intermediates by intramolecular free-radical cascade additions. A plausible biogenetic pathway for **1**−**4** was proposed as shown in Scheme 1.

Compounds **1**−**4** were tested for cytotoxic effects on the Hela, Hep G2, U87, and A172 cell lines using the MTT assay.[11] All compounds were inactive (IC_{50} > 10 μM) against the cell lines tested. But **1**−**4** exhibited the ability to reverse the adriamycin-induced resistance of K562/A02 cells at a concertration of 10 μM, with reversal fold values of 4.3, 1.9, 2.0, 1.9, respectively.

Scheme 1. Plausible Biosynthetic Pathway of **1**−**4**



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Supporting Information Available. Experimental procedures; detailed HMBC correlations (in tables and figures), detailed NOESY correlations (in figures), 1D and 2D NMR, ESIMS, HRESIMS, UV, and CD spectra of pallambins A−D (**1**−**4**); CIF data for crystal structures of **1** and **4**; the preliminary cytotoxicity results of **1**−**4**. 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.