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Note

A new diterpenoid and active stilbenes from *Pinus armandii* heartwood

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8(17),13-Labdadien-16,14-olid-18-oic acid (**1**), a new diterpenoid, has been isolated from the heartwood of *Pinus armandii* Francher, along with seven known diterpenoids (**2–8**) and four known stilbenes (**9–12**). Their structures have been elucidated by spectral evidence. (*E*)-3-Hydroxy-5-methoxystilbene (**9**) showed significant inhibition against white-rot fungi.

Keywords: *Pinus armandii*; 8(17), 13-Labdadien-16, 14-olid-18-oic acid; Diterpenoid; Stilbene; White-rot fungi

1. Introduction

Pinus armandii Francher is an important economic conifer indigenous to the southwest and central regions of China [1]. Chemical investigation on this tree has been reported previously [2–4]. This plant has been used widely for timber and lumber products in north China because its wood is durable and resistant to decay and insects. Thus, we were interested in studying further its bioactive constituents. Here, we report eight diterpenoids, including a new one (**1**) established as 8(17), 13-labdadien-16, 14-olid-18-oic acid, and four known stilbenes from the heartwood of *P. armandii*. On the basis of spectroscopic analysis, the eleven known compounds (**2–12**) (figure. 1) were determined to be pinusolide (**2**) [5], demethylpinusolide (**3**) [6], 15-hydroxy-8(17), 13-labdadien-16, 15-olid-18-oic acid (**4**) [6], 16-hydroxy-8(17), 13-labdadien-15, 16-olid-18-oic acid (**5**) [6,7], 15-hydroxydehydroabietic acid (**6**) [4,8], 4-hydroperoxy-19-nor-8, 11, 13-abietatriene (**7**) [9], isopimara-7-en-18-oic acid (**8**) [10], (*E*)-3-hydroxy-5-methoxystilbene (**9**) [11,12], (*E*)-3,5-dimethoxystilbene (**10**) [11,12], (*Z*)-3,5-dimethoxystilbene (**11**) [11,12] and 3-hydroxy-5-methoxybibenzyl (**12**) [11,12].

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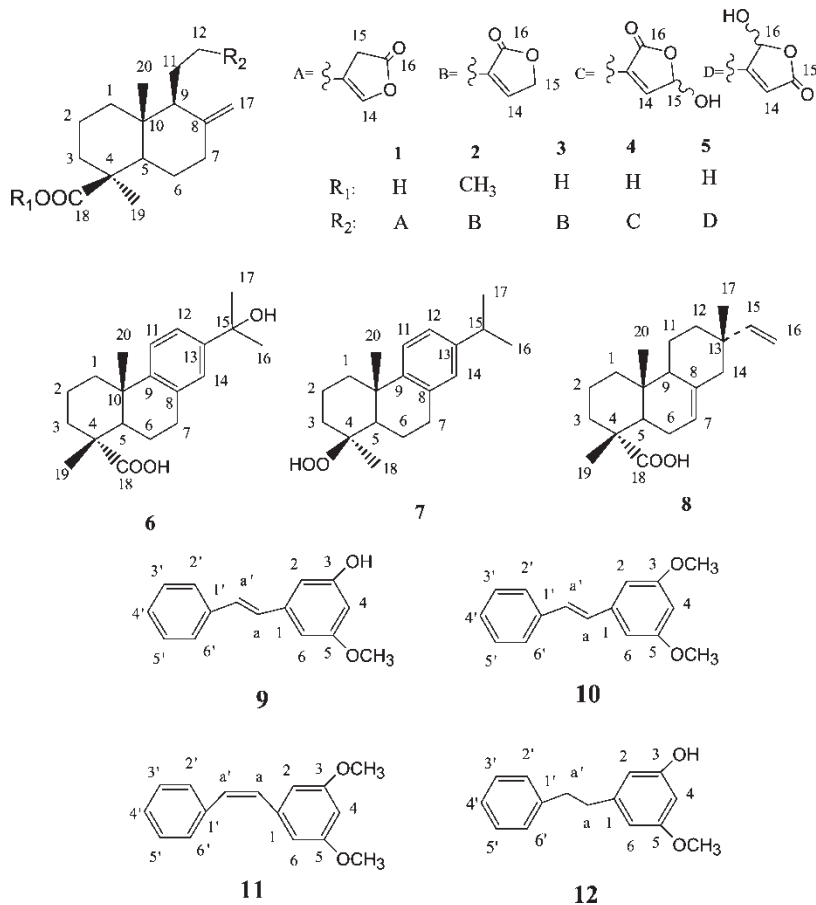
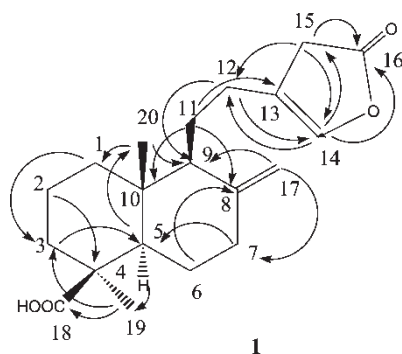


Figure 1. The structures of compounds 1–12.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder with mp 132–134°C and $[\alpha]_D^{23} + 49.7$ (c 0.20, $CHCl_3$). Its high-resolution EIMS shows a molecular ion peak at m/z 332.1985 $[M]^+$, suggesting a molecular formula of $C_{20}H_{28}O_4$. Its IR spectrum shows absorption bands at 3500–2500 cm^{-1} (broad) for a carboxyl, at 1770 and 1694 cm^{-1} (strong) for two carbonyl groups, and at 1645 and 1634 cm^{-1} for two double bonds. ^{13}C NMR and DEPT spectra reveal 20 carbon atoms, including two carbonyl (δ_C 179.6 and 175.0) and four olefinic carbons. Thus, **1** is composed of three rings as well as two carboxyl groups and two double bonds with a degree of unsaturation of $n = 7$. The 1H NMR spectrum shows two methyl signals at δ 0.53, 1.09 (each 3H, s) and two olefinic signals at δ 4.52, 4.79 (each 1H, brs), which accord with the signals at δ 11.7 (C-20), 28.0 (C-19) and 105.4 (C-17) in the ^{13}C NMR spectrum. Comparison with the 1H and ^{13}C NMR spectral data in the literature [5,6] suggests that **1** has a typical labdane skeleton. The 1H and ^{13}C NMR spectral data are similar to those of demethylpinusolide (**3**) [6], except for δ_C 45.9 instead of δ_C 69.8 in **3**, indicating that **1** has the same skeleton as **3**. The signal at δ_C 45.9 is assigned to the C-15 methylene in **1** based on HMQC and HMBC analysis. Cross-peaks in the HMBC spectrum (figure. 2) from H-14 (δ_H 6.77, brs) to C-12 (δ_C 23.7), C-15 (δ_C 45.9) and C-16 (δ_C 175.0), and from H-15 (δ_H 3.82, brs)

Figure 2. The selected HMBC correlations for compound **1**.

to C-12 (δ_C 23.7), C-14 (δ_C 137.6) and C-16 (δ_C 175.0), accord with the assignment of the unconjugated furan ring of **1**—a new ring structure. The stereochemistry of **1** was confirmed by correlations between H-20/H-11, H-19/H-5 and H-5/H-9 in the NOESY experiment. Based on above evidence, **1** was elucidated as 8(17), 13-labdadien-16,14-olid-18-oic acid.

The structures of eleven known compounds (**2–12**) were established by comparison of their spectral data with those reported previously. The polyhydroxystilbenes have a broad spectrum of physiological activities, including antifungal [13]. Compounds **9** and **12** have obvious nematocidal activity [12]. Some bibenzyls (dihydrostilbenes) exhibit bioactivity against some species of brown-rot fungi [14] and white-rot fungi [15]. Previous studies showed that 3-hydroxylated stilbenes have fungicidal activity against the white-rot fungus *Coriolus versicolor* [15], while 4-hydroxystilbenes have no such activity [16]. The results of the bioactivity of stilbenes from *Pinus armandii* showed that **9** and **12** could obviously inhibit the growth of five strains of white-rot fungi, which has laccase activity [17]; **9** could completely inhibit three strains, *Spongipellis spumeus*, *Pycnoporus sanguineus* and *Fomitopsis pinicola* at 0.64 mM, while **12** showed complete inhibition on *Fomitopsis pinicola* at 0.80 mM (table 1). The content of **9** and **12** is as high as 0.5% in the heartwood of *Pinus armandii*, based on our tests, which is consistent with a previous report [2]. The durability of the wood may be due to the presence of active stilbenes, such as **9** and **12**.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a XRC-1 micro-melting point apparatus and are uncorrected. Optical rotations were determined on a Jasco-20 polarimeter. IR spectra were

Table 1. The length of fungal mycelium after 7 days (mm).

Species	9			12		
	Contrast	0.16 mM	0.64 mM	Contrast	0.16 mM	0.80 mM
<i>Trameeis versicolor</i>	18–23	11	4–5	37	21	7.5–10
<i>Cerrena unicolor</i>	10–12	5.5	0–0.5	35	21.5	13.5
<i>Spongipellis spumeus</i>	5–6	0.5	0	22	11	7.5–8.5
<i>Pycnoporus sanguineus</i>	10	1–2	0	30	12	8–9
<i>Fomitopsis pinicola</i>	3–4	1	0	14	3	0

obtained on KBr pellets using a Bio-Rad FTS-135 spectrometer. UV spectra were recorded on a UV 210A spectrometer. MS spectra were carried out on a VG Auto Spec-3000 spectrometer. The 1D and 2D NMR spectra were obtained on Bruker AM-400 and DRX-500 spectrometers. TLC was carried on silica-gel G pre-coated plates. Separation and purification were performed by column chromatography on silica gel (200–300 mesh).

3.2 Bioassay

The inhibition of fungi by stilbenes **9** and **12** was tested by using five strains of white-rot fungi, *Trametes versicolor*, *Cerrena unicolor*, *Spongipellis spumeus*, *Pycnoporus sanguineus* and *Fomitopsis pinicola*. Fungal growth was determined by measuring the length of mycelium after 7 days. These fungi were isolated from specimens aseptically and kept on PDA slants at 4°C. In the control group, fungal cultures were grown in MA medium containing malt extract (2%) and agar (2%), and the incubation was carried out at 26°C. Inhibition of **9** and **12** on fungal growth was measured at 0.16, 0.64 and 0.80 mM.

3.3 Plant material

Heartwoods of *P. armandii* were collected in April 2001 from Kunming, Yunnan Province of China, and identified by Mr. Zhang Xue-Xing of the Sciences Academy of Yunnan Forestry. A voucher specimen has been deposited in the Kunming Institute of Botany, Chinese Academy of Sciences.

3.4 Extraction and isolation

Dried heartwoods of *Pinus armandii* (1 kg) were extracted (3 ×) with 95% EtOH under reflux. After removal of the solvent by evaporation, the EtOH extracts gave a residue (78 g), of which 30 g was subjected to column chromatography on silica gel, eluting with light petroleum–acetone (4:1) to yield 5 fractions. Compound **1** (30 mg) was obtained from fraction 5 by repeated chromatography on silica gel H. Similarly, fraction 1 yielded **2** (32 mg), **10** (6 mg) and **11** (7 mg). Compounds **7** (8 mg) and **8** (8.5 g) were isolated from fraction 2. Fraction 3 produced **3** (7 mg), **9** (35 mg), **12** (60 mg), and fraction 4 yielded **4** (15 mg), **5** (10 mg) and **6** (25 mg).

3.4.1 8(17),13-Labdadien-16, 14-olid-18-oic acid (1). C₂₀H₂₈O₄, white amorphous powder; mp 132–134°C; $[\alpha]_D^{23} + 49.75$ (c 0.201, CHCl₃); UV (CHCl₃) λ_{\max} (nm): 203; IR ν_{\max} (cm⁻¹): 3428, 3080, 2937, 2848, 1770, 1694, 1645, 1634, 1465, 1449, 1386, 1253, 1234, 1175, 1046, 971, 894, 820, 798, 618, 587; HR-EIMS m/z 332.1985 (calcd. for C₂₀H₂₈O₄ 332.1987); EIMS m/z (%): 332 [M]⁺ (14), 314 (5), 286 (14), 270 (48), 235 (30), 221 (31), 189 (27), 177 (37), 166 (45), 133 (26), 121 (49), 110 (53), 97 (100), 81 (58), 69 (30); ¹H NMR (500 MHz, CDCl₃) δ (ppm): 6.77 (1H, br.s, H-14), 4.79 (1H, s, H-17 α), 4.52 (1H, s, H-17 β), 3.82 (2H, br.s, H-15), 2.32 (1H, t, $J = 2.8$ Hz, H-12 α), 2.30 (1H, m, H-7 α), 2.03 (2H, d, $J = 13.1$ Hz, H-3), 1.95 (1H, t, $J = 7.6$ Hz, H-12 β), 1.89 (1H, m, H-6 α), 1.84 (1H, d, $J = 4.2$ Hz, H-7 β), 1.81 (1H, t, $J = 2.9$ Hz, H-1 α), 1.76 (1H, m, H-2 α), 1.74 (1H, d, $J = 4.0$ Hz, H-6 β), 1.69 (1H, m, H-11 α), 1.59 (1H, d, $J = 11.1$ Hz, H-11 β), 1.56 (1H, d, $J = 11.1$ Hz, H-9), 1.37 (1H, m, H-2 β), 1.26 (1H, dd, $J = 3.1, 11.9$ Hz, H-5), 1.09 (3H, s, H-19), 0.96 (1H, t, $J = 4.0$ Hz, H-1 β), 0.53 (3H, s, H-20); for ¹³C NMR spectral data see table 2.

Table 2. ^{13}C NMR data for **1–8** in CDCl_3 (a: 125 MHz; b: 100 MHz).

Carbon	<i>1a</i>	<i>2b</i>	<i>3b</i>	<i>4b</i>	<i>5b</i>	<i>6b</i>	<i>7a</i>	<i>8b</i>
1	38.5	40.3	38.8	39.1	39.1	37.8	38.4	38.8
2	19.2	19.9	19.5	19.8	19.8	18.5	18.4	17.9
3	37.4	38.2	37.6	37.8	38.0	36.7	34.7	36.8
4	43.2	44.3	43.8	44.1	43.9	47.3	84.1	46.3
5	55.5	56.3	55.9	56.2	56.0	44.5	50.5	44.9
6	25.5	26.2	25.7	26.0	26.6	21.7	18.3	25.1
7	37.9	38.7	38.3	38.5	38.5	30.1	30.2	121.0
8	147.1	147.5	147.0	147.2	147.3	134.7	134.5	135.6
9	55.0	55.7	55.3	55.7	55.6	145.9	146.4	52.0
10	39.6	40.3	40.1	40.5	40.4	36.9	37.2	35.0
11	21.2	21.8	21.5	21.8	20.9	124.1	124.2	20.0
12	23.7	24.6	24.3	24.4	25.9	121.9	123.8	36.0
13	139.0	134.9	134.5	138.7	170.9	147.8	145.6	36.9
14	137.6	143.8	143.5	143.1	116.3	124.9	126.8	46.0
15	45.9	70.1	69.8	96.7	172.0	72.4	33.4	150.2
16	175.0	174.3	174.0	171.8	99.1	31.5	24.0	109.3
17	105.4	106.7	106.5	106.8	106.4	31.5	24.0	21.5
18	179.6	177.7	182.5	183.4	180.3	184.5	24.5	185.7
19	28.0	28.8	28.7	28.9	28.9	16.2	–	17.1
20	11.7	12.6	12.4	12.7	12.5	25.0	25.4	15.3
CH_3O	–	51.1	–	–	–	–	–	–

3.4.2 Isopimara-7-en-18-oic acid (8). $\text{C}_{20}\text{H}_{30}\text{O}_2$, viscous oil; EIMS m/z 302 $[\text{M}]^+$ (100); ^1H NMR (400 MHz, CDCl_3) δ (ppm): 5.81 (1H, dd, $J = 10.5, 17.3$ Hz, H-15), 5.31 (1H, m, H-7), 4.93 (2H, dd, $J = 10.5, 17.3$ Hz, H16), 1.25 (3H, s, H-19), 0.89 (3H, s, H-17), 0.84 (3H, s, H-20); for ^{13}C NMR spectral data see table 2.

3.4.3 (*E*)-3,5-Dimethoxystilbene (10). $\text{C}_{16}\text{H}_{16}\text{O}_2$, amorphous solid; MS and ^1H NMR are consistent with those in the literature [10,11]; ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 161.3 (C-3,5), 139.7 (C-1), 137.5 (C-1'), 129.5 (C-a), 129.0 (C-2', 6'), 128.0 (C-3', 5'), 126.9 (C-4', a'), 104.9 (C-2, 6), 100.3 (C-4), 55.7 (OCH₃).

3.4.4 (*Z*)-3,5-Dimethoxystilbene (11). $\text{C}_{16}\text{H}_{16}\text{O}_2$, amorphous solid; MS and ^1H NMR are consistent with those in the literature [10,11]; ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 160.6 (C-3, 5), 139.2 (C-1), 137.4 (C-1'), 130.8 (C-2', 6'), 130.3 (C-3', 5'), 129.0 (C-a), 128.3 (C-4'), 127.3 (C-a'), 106.8 (C-2, 6), 100.0 (C-4), 55.3(OCH₃).

3.4.5 3-Hydroxy-5-methoxybibenzyl (12). $\text{C}_{15}\text{H}_{16}\text{O}_2$, amorphous solid; MS and ^1H NMR consistent with those in the literature [10,11]; ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 160.4 (C-3), 156.4 (C-5), 144.4 (C-1), 141.4 (C-1'), 128.2 (C-2', 6'), 128.1 (C-4'), 125.7 (C-3', 5'), 108.0 (C-2), 106.5 (C-6), 100.0 (C-4), 55.0 (OCH₃), 37.7 (C-a), 37.2 (C-a').

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