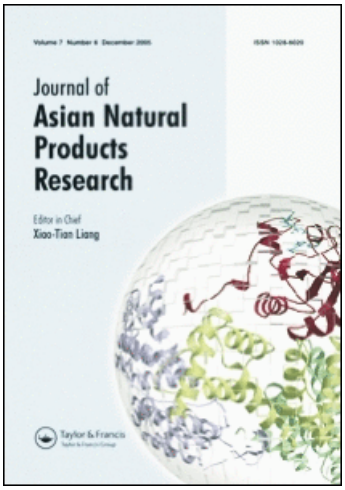

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Labdane diterpenes from the seeds of *Platycladus orientalis*

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Two new labdane diterpenes, 14(*R*),15-dihydroxy-8(17),12(*E*)-labdadien-19-oic acid (**1**) and 16-methyl–12,15-epoxy-8(17),13-labdadien-19-oic acid (**2**), together with four known compounds, were isolated from the seeds of *Platycladus orientalis*. Their structures were established by spectroscopic methods. The stereochemistry of compound **1** was defined by X-ray crystallographic analysis.

Keywords: *Platycladus orientalis*; Labdane diterpene; X-ray crystallographic analysis

1. Introduction

Platycladus orientalis (L.) Franco is also called *Biota orientalis* (L.) Endl. Sesquiterpenoids [1–3], diterpenoids [4–9], flavonoids [10,11] and monolignol derivatives [12] have been isolated from different parts of this plant. The seed of the plant has been used as a traditional Chinese medicine widely in haemostatic, expectorant and anti-cough remedies. In the CNS animal test, *P. orientalis* seed extract and ethyl acetate fraction showed good activity learning impairment in mice [9]. During the course of screening for anti-neurodegenerative agents from natural resources, the ethyl acetate extract of the seeds of *P. orientalis* was found to reverse scopolamine-induced cognitive deficit significantly in mice. In this paper, we describe the isolation and structure elucidation of two new labdane diterpenoid acids, 14(*R*),15-dihydroxy-8(17),12(*E*)-labdadien-19-oic acid (**1**) and 16-methyl-12,15-epoxy-8(17),13-labdadien-19-oic acid (**2**) from the ethyl acetate fraction of the plant. In addition, 15,16-dihydroxy-8(17),13(*E*)-labdadien-19-oic acid (**3**) [13,14], labdane diterpenoid isopinusolide (**4**) [15], sandaracopimaric diterpene sandaracopimara diene-3β,18-diol (**5**) [16] and 3-(4′-formylphenoxy)-4-methoxybenzaldehyde (**6**) [17] were also obtained for the first time from the species. Their structures were elucidated by spectroscopic measurements including ESI-MS, IR, ^{1}H NMR, ^{13}C NMR and 2D NMR spectra (figure 1).

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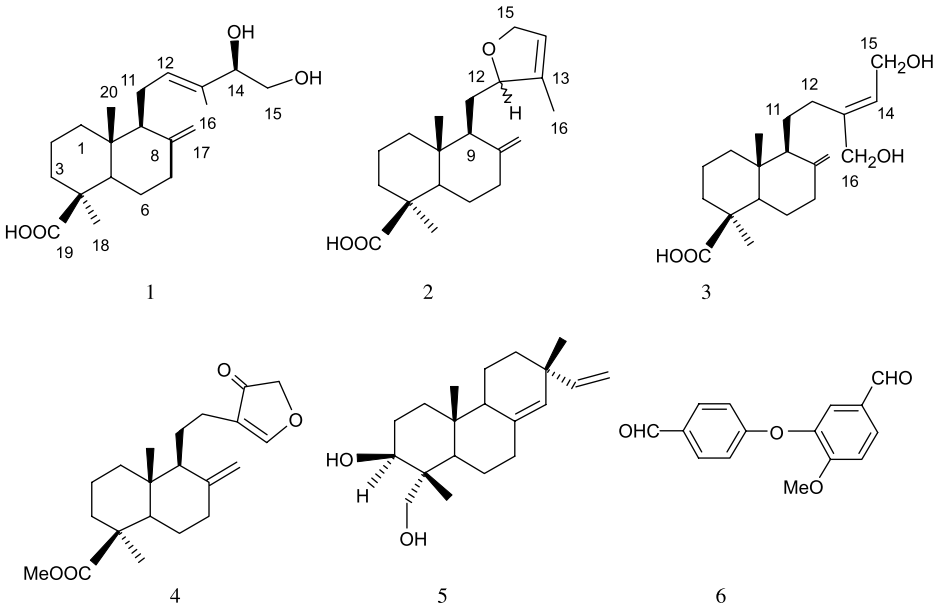

Figure 1. Structures of **1**–**6**.

The stereochemistry of **1** was determined by a NOESY spectrum and was confirmed by a single crystal X-ray diffraction analysis.

2. Results and discussion

Compound **1** was obtained as colourless needles with $[\alpha]_D^{20} + 43$ (*c* 0.145, MeOH) and its molecular formula was assigned as $C_{20}H_{32}O_4$ by HRESI-MS analysis. The presence of two rings in the structure of **1** was deduced from the molecular formula and the analysis of ^{13}C NMR spectrum with four unsaturated carbons at δ_C 150.1(s), 129.8 (d), 135.6 (s) and 108.4 (t), and a ketonic carbon at 182.0 (s). Analysis of NMR spectral data revealed two geminal vinyl protons at δ_H 4.45 (1H, s) and 4.80 (1H, s) with δ_C 108.4 (t) and two methyl groups at δ_H 1.18 (3H, s), 0.66 (3H, s). From these structural elements, compound **1** appeared to be a diterpenoid belonging to the labdane group. The structure of the labdane skeleton could then be deduced by a careful analysis of the NMR spectral data of **1**. The ^{13}C NMR spectrum of **1** presented two quaternary carbons (δ_C 150.1 and 135.6). According to the labdane skeleton, one quaternary carbon should be located at C-8 position and another should be attributed to C-13. Analysis of HMBC correlations between H-11 at δ_H 2.05–2.30 with C-13 at δ_C 135.6 (s) and H-12 at δ_H 5.40 (t) with C-9 at δ_C 58.4 (d) supported this vinyl group attached to C-9. Further the oxygenated methine (δ_H 3.95 and δ_C 79.6) and ethyl (δ_H 3.45 and δ_C 66.5) moieties were assigned to the C-14 and C-15 positions, respectively, and the oxygenated groups were attached to C-13 according to the HMBC correlations between H-14 at δ_H 3.95 and Me-16 at δ_C 12.8 and C-12 at δ_C 129.8 as well as H-15 at δ_H 3.45 and C-13 at δ_C 135.6.

The stereochemistry of **1** was deduced from the NOESY experiment of **1**. In particular, the cross-peaks implicating Me-18, H-5 indicated that these protons were all

on the same α-face of the molecule while those observed with carboxyl C-19 and Me-20, H-11 established that these substituents were on the other side of the molecule (β-face). Finally, the absolute configuration was defined by single-crystal X-ray diffraction analysis. Thus compound **1** was concluded to be 14(*R*),15-dihydroxy-8(17),12(*E*)-labdadien-19-oic-acid.

Compound **2** was obtained as an amorphous powder with $[\alpha]_D^{20} + 68$ (*c* 0.095, MeOH) and its molecular formula was assigned as $C_{20}H_{30}O_3$ by HREI-MS. The presence of three rings in **2** was deduced from the molecular formula and the analysis of ^{13}C NMR spectrum with four unsaturated carbons at δ_C 149.4 (s), 141.4 (s), 124.8 (d) and 106.6 (t), and a ketonic carbon at 178.7 (s). Similarly, two methyl groups at δ_H 1.20 (Me-18), 0.60 (Me-20), two germinal vinyl protons at δ_H 4.80 (1H, brs s), 4.50 (1H, brs s) (H-17) and a carboxyl group at δ_C 178.7 (C-19) suggested that compound **2** belongs to labdane diterpenoid also. By analysis of NMR spectral data of **2**, we found that **2** had nearly the same functional groups as **1**, including an oxygenated methine (δ_H 3.91, δ_C 74.9), an oxygenated ethyl (δ_H 4.30, δ_C 59.0) and a vinyl group (δ_H 5.50, δ_C 124.8) associated with quaternary carbon at δ_C 141.4 (C-13). However, there is a little difference between **2** and **1** in elemental formula and degree of unsaturation. Compared with compound **1**, **2** has one oxygen less but one ring more than **1**. So it can be deduced that **2** was derived from **1** due to the loss of one molecule of H_2O. This conclusion was further proved by long-range $^1H-^{13}C$ correlations between H-12 at δ_H 3.91 and C-15 at δ_C 59.0 (t) and H-15 at δ_H 4.30 (d), H-14 at 5.50 (t) and C-12 at δ_C 74.0 (d). So a β-methyl-2-olefin-furan ring can be established. In HMBC correlation, H-12 at δ_H 3.91 and C-9 at δ_C 52.5 suggested this fruan ring was annexed to C-11. The stereochemistry of **2** was deduced from the NOESY spectrum the same as for **1**. Thus compound **2** can be defined as 16-methyl-12,15-epoxy-8(17),13-labdadien-19-oic acid.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Fisher-Johns Melting Point Apparatus and are uncorrected. Optical rotations were obtained on a DIP-181 digital polarimeter. IR spectra were recorded on a Nicolet 750 instrument. NMR spectra were measured on a Bruker AM-400 spectrometer using TMS as internal standard. ESI-MS spectra were taken on an LCQ Deca mass spectrometer. The HRESI-MS spectra were obtained on an Apex mass spectrometer. Single crystal X-ray diffraction data were measured and collected on a Bruker Smart Apex CCD diffractometer with graphite monochromated Mo-Kα radiation. The structure was solved by direct methods and expanded using Fourier techniques.

3.2 Plant material

The seeds of *Platycladus orientalis* were collected from Wenshan County in November 2001 in Yunnan province, China, and authenticated by Professor Shen Jin-Gui of the Shanghai Institute of Materia Medica. A voucher specimen has been deposited at the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3 Extraction and isolation

The seeds of *Platycladus orientalis* (8 kg) were extracted with petroleum ether (PE bp 60–90°C), ethyl acetate and ethyl alcohol, successively, after grinding. The ethyl acetate fraction (70 g) was separated by silica gel column chromatography using gradient elution with petroleum ether/acetone (v:v = 20:1, 15:1, 10:1, 6:1, 3:1, 1:1) and then MeOH. Ten fractions were collected after checked by TLC. The eighth fraction (10.52 g) was subjected to a silica gel column chromatography again using gradient elution with $CHCl_3$/acetone/H_2O. Three compounds, **1** (20 mg), **3** (83 mg) and **4** (110 mg), were obtained by repeated chromatography. Compound **1** was recrystallised in PE/acetone mixture. Compound **2** (85 mg) was obtained from the ninth fraction (6.68 g) by repeated chromatography. The fifth fraction (2.1 g) and the sixth fraction (4.42 g) were separated and purified by silica gel to furnish known compounds **5** (73 mg) and **6** (26 mg) separately.

3.3.1 14(*R*),15-Dihydroxy-8(17),12(*E*)–labdadien-19-oic acid (1). Colourless needle (20 mg); mp 198°C; $[\alpha]_D^{20} + 43$ (*c* 0.145, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3340 (OH), 2943 (CH), 1687 (carboxyl C=O), 1645, 1440, 1236, 1178, 1105, 887; For ^1H NMR and ^{13}C NMR spectra, see table 1; ESI-MS (positive ion) *m/z* (%): 359.2 ($[M + Na]^+$, 100); ESI-MS (negative ion) *m/z* (%): 335 ($[M - H]^-$, 100); HRESI-MS *m/z* 359.2202 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2198).

*Crystal data for **1** (figure 2):* Compound **1** crystallised in the monoclinic space group P(2)2(1)2(1) with molecules of composition $C_{20}H_{32}O_4$, ($Z = 4$), MW = 336.46, accurate cell constants of $a = 10.3010$ (10)Å, $b = 13.2301$ (13)Å, $c = 14.1148$ (14)Å, $\beta = 90°$, $D_c = 1.162$ mg/m^3, $V = 1923.6(3)$Å^3. All reflections were collected on the Bruker Smart Apex CCD, with graphite monochromator, MoKα radiation ($\lambda = 0.71073$ Å), maximum

Table 1. ^1H and ^{13}C NMR spectral data for compounds 1 and 2 (δ in ppm, *J* in Hz).

Position	***1** (in MeOH-d)*		***2** (in acetone-d)*	
	δ_C	δ_H	δ_C	δ_H
1	39.9 t	2.13a, m 1.06b, m	39.7 t	1.16a, m 1.80b, m
2	27.9 t	1.87a, m 1.97b, m	20.7 t	1.47a, m 1.90b, m
3	21.7 t	1.87a, m 1.50b, m	38.9 t	1.06a, m 2.12b, m
4	45.7 s	-	44.5 s	-
5	58.0 d	1.37 dd ($J = 12.0, 2.0$)	56.8 d	1.41 dd ($J = 3.0, 11.0$)
6	41.1 t	1.18a, m 1.89b, m	27.0 t	1.98a, m 1.88b, m
7	40.2 t	1.92a, m 2.38b, m	39.4 t	1.93a, m 2.38b, m
8	150.1 s	-	149.9 s	-
9	58.4 d	1.75 brd ($J = 10.5$)	52.5 d	2.13 m
10	41.9 s	-	40.6 s	-
11	24.2 t	2.05a, m 2.30b, m	31.3 t	1.62a, m 1.47b, m
12	129.8 d	5.40 t ($J = 6.3$)	74.9 d	3.91 brd ($J = 9.6$)
13	135.6 s	-	141.4 s	-
14	79.6 d	3.95 t ($J = 6.1$)	124.8 d	5.50 t ($J = 6.3$)
15	66.5 t	3.45 t ($J = 6.1$)	59.0 t	4.30 d ($J = 6.3$)
16	12.8 q	1.55 s	12.0 q	1.61 s
17	108.4 t	4.45a, brs 4.80b, brs	106.6 t	4.80a, brs 4.50b, brs
18	30.1 q	1.18 s	29.2 q	1.20 s
19	182.0 s	-	178.7 s	-
20	13.9 q	0.66 s	13.3 q	0.60 s

Data were assigned by ^1H–^1H COSY, HMQC, HMBC spectra (^1H, 400 MHz, ^{13}C, 100 MHz).
Multiplicity was established by DEPT 135°.

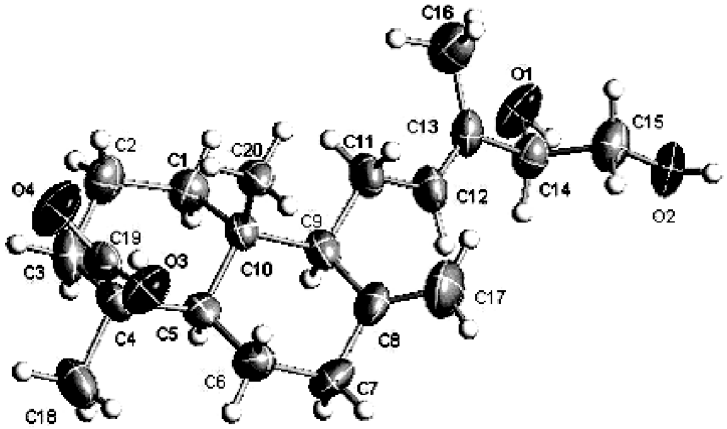

Figure 2. X-ray crystal structure of compound **1**.

2θ value of 25.0°, independent reflections: 9653, observed number of reflections: 3369 [$|F| \geq 8\delta|F|^2$]. The structure was resolved by direct method SHELXS-86 and expanded using difference Fourier techniques, refined by full-matrix least-squares calculation. Hydrogen atoms were fixed at calculated positions. The final indices were $R_f = 0.0640$, $w_R = 0.1410$.

3.3.2 16-Methyl-12,15-epoxy-8(17),13-labdadien-19-oic acid (2). White powder (85 mg); mp 92°C; $[\alpha]_D^{20} + 68$ (*c* 0.095, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3426 (OH), 2935 (CH), 1695 (lactone C=O), 1643, 1444, 1174, 1035, 565; For ^1H NMR and ^{13}C NMR spectra, see table 1; EI-MS *m/z* (%): 318 [M^+] (10); HREI-MS *m/z* 318.2193 $[M]^+$ (calcd for $C_{20}H_{30}O_3$, 318.2195).

3.3.3 15,16-Dihydroxy-8(17),13(*E*)–labdadien-19-oic acid (3). Yellow oil (83 mg); $[\alpha]_D^{20} + 47$ (*c* 0.16, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3423 (OH), 2939 (CH), 2848, 1699 (lactone C=O), 1643, 1467, 1446, 1261, 1243, 1004, 754; ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 182.6 (s, C-19), 147.9 (s, C-8), 144.3 (s, C-13), 44.2 (s, C-4), 40.6 (s, C-10), 126.2 (d, C-14), 56.4 (d, C-5), 55.8 (d, C-9), 106.6 (t, C-17), 58.6 (t, C-15), 60.9 (t, C-16), 34.8 (t, C-12), 22.5 (t, C-11), 38.8 (t, C-11), 26.2 (t, C-6), 38.1 (t, C-3), 20.2 (t, C-2), 39.2 (t, C-1), 29.0 (q, C-18), 12.9 (q, C-20). EI-MS *m/z* (%): 336 [M^+] (12); HREI-MS: *m/z* 336.2185 (calcd for $C_{20}H_{32}O_4$, 336.2195).

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