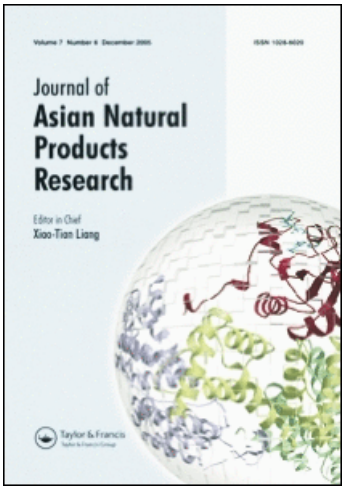

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Two eudesmane sesquiterpenes from *Laggera pterodonta*

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Two new eudesmane sesquiterpenes, 2α-hydroxy pterodontic acid (**1**) and pterodolide [3α-(2-methyl-2,3-epoxy)-butyric-4α-acetoxy-8β-ethoxy eudesma-7(11)-en-8α,12-olide (**2**), along with five known compounds (**3–7**) were isolated from the aerial parts of *Laggera pterodonta*. Their structures were elucidated on the basis of spectroscopic methods. The immunosuppressive activity of the isolated compounds was investigated.

Keywords: *Laggera pterodonta*; Sesquiterpenes; 2α-Hydroxy pterodontic acid; Pterodolide; Immunosuppressive activity

1. Introduction

Laggera pterodonta (DC.) Benth is a kind of medicinal plant growing in Yunnan province, and has been used as folk medicine from ancient times. Pharmacological research indicated that the extract of *L. pterodonta* has antileukaemia, anti-bacterial, anti-inflammatory and anti-malarial activities [1–3]. In our search for pharmacologically active compounds from medicinal plants, the petroleum ether extract of *L. pterodonta* showed significant inhibitory effects on lymphocyte transformation (34.7%, 50 μg/ml). This paper deals with the isolation and structure elucidation of two new and five known compounds (**1–7**) from the aerial parts of *L. pterodonta*. Their immunosuppressive activities were evaluated.

2. Results and discussion

Compound **1** was obtained as an amorphous powder. A dehydrate molecular ion peak at *m/z* 232.1439 $[M-H_2O]^+$ observed in the HREI-MS, indicated the molecular formula of

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$C_{15}H_{22}O_3$ for **1**. Its IR spectrum showed the presence of hydroxyl groups (3398 cm^{-1}) and carbonyl group (1695 cm^{-1}). The ^1H NMR spectrum of **1** revealed the presence of two methyl groups (δ_H 1.26, d, $J = 7.6$ Hz and 1.25, s), one oxygenated methine proton (δ_H 4.51, m), three olefinic protons (δ_H 6.54, d, $J = 1.6$ Hz; 5.68, br s, and δ_H 5.50, s). Its ^{13}C NMR spectrum showed 15 carbons, including two methyls, one oxygenated methine (δ_C 63.3), four methylenes, and one double bond (δ_C 147.4, s; 125.2, d), as well as the α, β-unsaturated carboxylic acid (δ_C 170.1, s, 147.9, s, 123.0, t). The ^{13}C NMR spectrum of **1** was similar to those of 3β-hydroxy pterodontic acid (**4**), except for C–1 to C–4 [4].

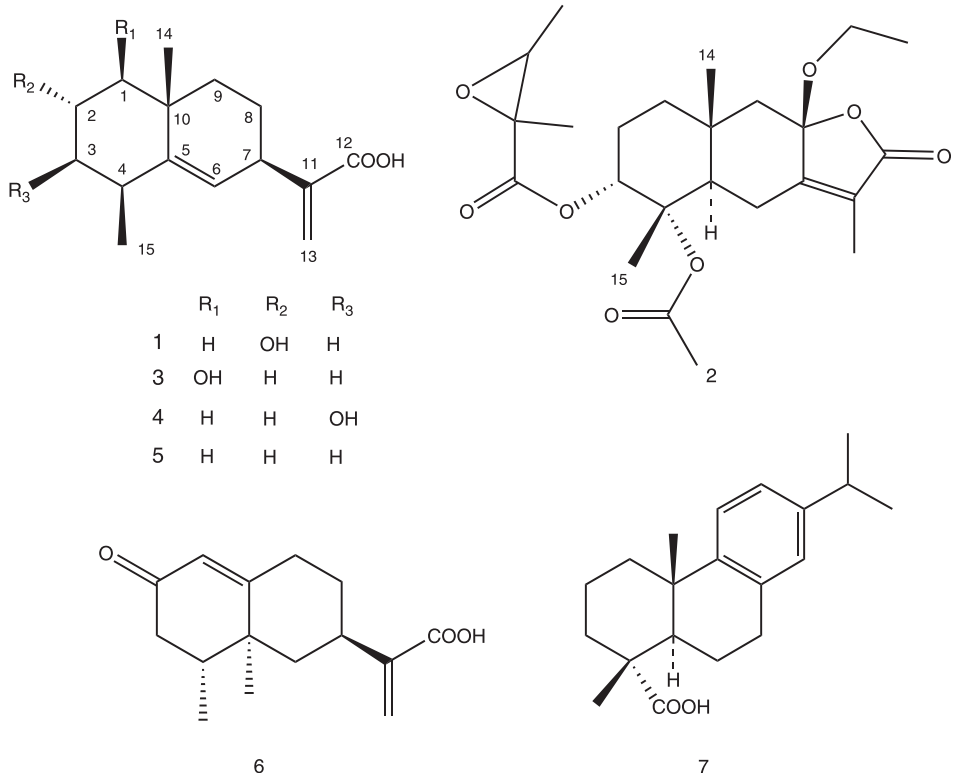
Furthermore, in the HMBC spectrum of **1**, H–1 at δ_H 2.16 and 1.48 correlated with C–2 at δ_C 63.3, H–4 at δ_H 2.68 correlated with C–2 at δ_C 63.3, C–3 at 43.7, C–5 at 147.4, C–6 at 125.2, and C–10 at 35.9, and the methyl proton at δ_H 1.26 (H_3–15) correlated with C–3 at δ_C 43.7, C–4 at 39.9, and C–5 at 147.4, suggesting that the hydroxyl group was located at C–2. On the other hand, in the NOESY spectrum, H–2 at δ_H 4.51 correlated with 14-CH_3 at δ_H 1.26 and 15-CH_3 at 1.25, indicating the hydroxyl group at C–2 has equatorial orientation. Based on above facts, compound **1** was deduced as 2-hydroxy pterodontic acid.

Pterodolide (**2**) had a molecular formula of $C_{24}H_{34}O_8$ as inferred from its HRFT-MS (*m/z* 451.2329 $[M + H]^+$). Its IR spectrum showed an intensive absorption 1755 cm^{-1} for the unsaturated lactone. The ^1H NMR spectrum of **2** revealed the presence of an acetyl group (δ_H 2.00), an ethoxyl group (3.44, 3.30; each 1H, dq, $J = 11.0$, 7.0 Hz; 1.20, 3H, t, $J = 7.0$ Hz), one oxygenated methine (δ_H 5.83; 1H, br t, $J = 2.7$ Hz), as well as three methyl groups (δ_H 1.89, 1.59, 1.20; each 3H, s). From HSQC and HMBC spectra, it also showed the presence of 2-methyl-2,3-epoxy-butyric group (δ_H 3.05; 1H, q, $J = 5.4$ Hz; 1.29, 3H, d, $J = 5.4$ Hz; 1.52, 3H, s). The ^{13}C NMR spectrum of **2** revealed seven methyls, two oxygenated methines, one oxygenated methylene, one ketal carbon (δ_C 105.7), and two carbonyl carbons (δ_C 169.1 and 168.2), in addition to a α, β-unsaturated lactone moiety (δ_C 171.6, 159.3, 124.1).

In the HMBC spectrum, the H–9a at δ_H 2.30 correlated with C–7 at δ_C 159.3, C–8 at 105.7, C–5 at 50.3, and C–10 at 35.2, the H–6a at δ_H 2.89 correlated with C–7 at δ_C 159.3, C–8 at 105.7, and C–10 at 35.2, and 13-CH_3 at δ_H 1.89 correlated with C–7 at δ_C 159.3, C–11 at 124.1, and C–12 at 171.6. Therefore, the 8,12-olide partial structure was proposed, and eight degree unsaturations for **2** also supported the presence of a lactone ring. Furthermore, 15-CH_3 at δ_H 1.59 correlated with C–3 at δ_C 73.3, C–4 at 82.7, and C–5 at 50.3, H–3 at δ_H 5.83 correlated with the carbonyl carbon signal at δ_C 168.2 (2-methyl-2,3-epoxy-butyric group), and the ethoxyl proton at δ_H 3.44 and 3.30 correlated with C–8 at δ_C 105.7. Therefore, ethoxyl and 2-methyl-2,3-epoxy-butyric groups should be located at positions C–8 and C–3, respectively. The acetyl group would be assigned at C–4. From the above information, **2** was also assumed to be a eudesmane sesquiterpene lactone, similar to the structure of 1β,8β-dihydroxy eudesman 3,7(11)-dien-8α,12-olide [5].

In the NOESY spectrum, the15-CH_3 at δ_H 1.59 correlated with the 14-CH_3 at δ_H 1.20 and H–3 at 5.83, the acetyl methyl at δ_H 2.00 correlated with the H–5 at δ_H 1.76; the methylene signal of ethoxyl group at δ_H 3.44 and 3.30 correlated with the 14-CH_3 signal at δ_H 1.20. Therefore, the structure of **2** was determined as pterodolide (figure 1).

Five known compounds, 1β-hydroxy pterodontic acid (**3**) [4], 3β-hydroxy pterodontic acid (**4**) [4], pterodontic acid (**5**) [4], tessaric acid (**6**) [6], and dehydroabietic acid (**7**) [7], were identified by comparison of their spectroscopic data with those of literature values.



	R_1	R_2	R_3
1	H	OH	H
3	OH	H	H
4	H	H	OH
5	H	H	H

6 7

Figure 1. Structures of compounds **1–7**.

In a search for immunosuppressive activity, we examined the immunoinhibitory effect of these sesquiterpenes on lymphocyte transformation [8,9] (table 2). The values of inhibition percent of compounds **1**, **4** and **5** revealed a significant distinction to the Con A control group ($P < 0.05$, $n = 6$), and showed an inhibitory effect on lymphocyte transformation by comparing with a reference compound (dexamethasone).

3. Experimental

3.1 General experimental procedures

NMR spectra were performed on a Bruker Avance 300 instrument with teramethylsilane as an internal standard. HRFT-MS and EI-MS were obtained on a Bruker apexIII 7.0 Tesla and VG ZAB-HS instrument, respectively. Column chromatography was performed on silica gel (Qingdao Haiyang Chemical Co. Ltd.), Sephadex LH-20 (Amersham Pharmacia Biotech) and Toyopearl HW-40 (Tosoh). HPLC was a Jasco Gulliver Series with PU-1580 (pump), RI-1530 and UV-1575 (detector). Preparative HPLC column was used as follows: ODS (YMC-Pack ODS-A, SH-343-5), GPC (Shodex, Asahipak GS-310, 20G, MeOH). IR spectra were recorded on a FTS3000 Infrared Fourier Transform sepectrometer (Bio-Rad). Optical rotation was measured with a MC 241 digital polarimeter (Perkin Elmer).

Table 1. ^{1}H NMR and ^{13}C NMR spectral data of **1** and **2**.

No.	*1* (C_5D_5N)		*2* ($CDCl_3$)	
	δ_C	δ_H mult. (J=Hz)	δ_C	δ_H mult. (J=Hz)
1	52.3	2.16, 1.48 m	33.6	1.33 m
2	63.3	4.51 m	22.9	1.98 m
3	43.7	2.16, 1.81 m	73.3	5.83 br t (2.7)
4	39.9	2.68 m	82.7	–
5	147.4	–	50.3	1.76 dd (12.0, 2.6)
6	125.2	5.50 s	21.9	2.89 dd (12.8, 2.6), 2.20 dd (12.8, 12.0)
7	39.6	3.74 m	159.3	–
8	26.9	2.17, 1.50 m	105.7	–
9	42.3	1.50, 1.59 m	53.5	2.30, 1.42 d (13.5)
10	35.9	–	35.2	–
11	147.9	–	124.1	–
12	170.1	–	171.6	–
13	123.0	5.68 br s, 6.54 d (1.6)	8.1	1.89 s
14	28.7	1.25 s	19.1	1.20 s
15	24.7	1.26 d (7.6)	17.9	1.59 s

3.2 Plant material

Laggera pterodonta (DC.) Benth was purchased from Kunming, Yunnan province of China in August 2002 and identified by Professor Wen-Yuan Gao. A voucher specimen (D20020818) is deposited at the School of Pharmacy, Tianjin Medical University, China.

3.3 Extraction and isolation

The dried aerial parts (0.85 kg) of *L. pterodonta* were crushed and extracted three times with EtOH (95%, 10 L each) at 60°C for 6 h. The EtOH extracts were concentrated under reduced pressure to give a residue (110 g), which was suspended in H_2O, and then partitioned with petroleum ether, EtOAc and *n*-BuOH, respectively.

The petroleum ether layer was concentrated to afford a residue (44 g), which was subjected to column chromatography with silica gel, and was eluted with increased polarity petroleum ether/EtOAc (8:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 100% EtOAc) to yield nine fractions. Fraction 4 (3.9 g) was chromatographed on silica gel column ($CHCl_3$/*n*-hexane, 9:1) to give four fractions (frs. 4.1–4.5). Fraction 4.4 (463 mg) was chromatographed on Toyopearl HW-40 ($CHCl_3$/MeOH, 2:1) to give **5** (295 mg). Fraction 7 (4.7 g) was chromatographed on a silica gel column ($CHCl_3$/MeOH, 99:1, 98:2, 95:5, 9:1) to give five fractions (fr. 7.1–7.5). Fraction 7.4 (1.5 g) was chromatographed on a Toyopearl HW-40 ($CHCl_3$/MeOH, 2:1) to give four fractions (fr. 7.4.1–7.4.4). Fraction 7.4.1 (505 mg) was separated by HPLC (ODS, MeOH/H_2O 8:2, and then GPC, MeOH) to give **2** (9 mg). Fraction 3 (2.5 g) was chromatographed on silica gel column ($CHCl_3$/*n*-hexane, 9:1) to give eight fractions (fr. 3.1–3.8). Fraction 3.8 (850 mg) was chromatographed on a Toyopearl HW-40 ($CHCl_3$/MeOH, 2:1) to give three fractions (fr. 3.8.1–3.8.3). Fraction 3.8.3 (135 mg) was separated by HPLC (GPC, MeOH) to give **7** (21 mg).

Fraction 8 (1.5 g) was chromatographed on Sephadex LH-20 (MeOH) to give four fractions (fr. 8.1–8.4). Fraction 8.2 (298 mg) was purified by HPLC (ODS, MeOH/H_2O, 8:2, and then 7:3) to give **1** (19 mg), **3** (7 mg), and **4** (12 mg). Fraction 8.3 (0.7 g) was

Table 2. Inhibitory effects of compounds **1**, **3–5**, and **7**.

Compounds	*Inhibition (%)*		
	80 μg/ml	*20 μg/ml*	*5 μg/ml*
1	26.8	18.4	6.6
3	−13.6	−10.3	6.2
4	13.1	4.8	1.8
5	11.1	10.3	5.7
7	−3.5	−0.4	2.9

Inhibition ratio of dexamethasone = 60.7% (50 μg/ml).

chromatographed on a Toyopearl HW-40 ($CHCl_3$/MeOH, 2:1) and then separated by HPLC (ODS, MeOH/H_2O, 8:2) to give **6** (5 mg).

2α-Hydroxy pterodontic acid (**1**) was isolated as an amorphous powder, $[\alpha]_D^{25}$ −2.6 (*c* 1.5, MeOH). IR (KBr) ν_{max} cm^{-1}: 3398, 2929, 1695, 1623, 1456, 1375, 1253, 1149, 1043, 1022, 950, 908, 862. EI-MS: *m/z* $[M-H_2O]^+$232 (41), 217 (23), 191 (38), 171 (23), 145 (74), 119 (42), 105 (56), 91 (79), 84 (77), 77 (53), 41 (100). HREI-MS *m/z* 232.1439 $[M-H_2O]^+$ (calcd for $C_{15}H_{20}O_2$, 232.1463). ^1H NMR and ^{13}C NMR (C_5D_5N), see table 1.

Pterodolide (**2**) was isolated as an amorphous powder, $[\alpha]_D^{25}$ + 10.0 (*c* 0.2, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 2956, 2925, 2853, 1755, 1730, 1703, 1454, 1373, 1262, 1248, 1146, 1089, 1013, 907, 764. EIMS: *m/z* 450 $[M]^+$(4), 293 (8), 246 (99), 229 (40), 217 (21), 201 (49), 173 (28), 154 (12), 131 (10), 116 (22), 105 (14), 91 (15), 43 (100). HRFTMS *m/z* 451.2329 $[M+H]^+$ (calcd for $C_{24}H_{35}O_8$, 451.2326). ^1H NMR ($CDCl_3$), see table 1; δ_H 3.05 (1H, q, $J = 5.4$ Hz), 1.29 (3H, d, $J = 5.4$ Hz), 1.52 (3H, s) (2-methyl-2,3-epoxy-butyrate); 2.00 (3H, s) (acetoxyl group); 3.44, 3.30 (each 1H, dq, $J = 11.0$, 7.0 Hz), 1.20 (3H, t, $J = 7.0$ Hz) (ethoxyl group). ^{13}C NMR ($CDCl_3$), see table 1; δ_C 168.2 (s), 59.9 (s), 59.6 (d), 19.3 (q), 13.9 (q) (2-methyl-2,3-epoxy-butyric group); 169.1 (s), 22.1 (q) (acetoxyl group); 58.7 (t), 15.2 (q) (ethoxyl group).

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