# Two Eudesmane Sesquiterpenes from *Laggera Pterodonta*

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## Abstract:

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.

## Article:

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.

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 $C_{15}H_{22}O_3$ 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 ($\delta_H$ 1.26, d, $J$ = 7.6 Hz and 1.25, s), one oxygenated methine proton ($\delta_H$ 4.51, m), three olefinic protons ($\delta_H$ 6.54, d, $J$ = 1.6 Hz; 5.68, br s, and $\delta_H$ 5.50, s). Its $^{13}C$ NMR spectrum showed 15 carbons, including two methyls, one oxygenated methine ($\delta_C$ 63.3), four methylenes, and one double bond ($\delta_C$ 147.4, s; 125.2, d), as well as the α, β-unsaturated carboxylic acid ($\delta_C$ 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 $\delta_H$ 2.16 and 1.48 correlated with C–2 at $\delta_C$ 63.3, H–4 at $\delta_H$ 2.68 correlated with C–2 at $\delta_C$ 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 $\delta_H$ 1.26 ($H_3$–15) correlated with C–3 at $\delta_C$ 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 $\delta_H$ 4.51 correlated with 14-$CH_3$ at $\delta_H$ 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 ($\delta_H$ 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 ($\delta_H$ 5.83; 1H, br t, *J* = 2.7 Hz), as well as three methyl groups ($\delta_H$ 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 ($\delta_H$ 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 ($\delta_C$ 105.7), and two carbonyl carbons ($\delta_C$ 169.1 and 168.2), in addition to a α, β-unsaturated lactone moiety ($\delta_C$ 171.6, 159.3, 124.1).

In the HMBC spectrum, the H–9a at $\delta_H$ 2.30 correlated with C–7 at $\delta_C$ 159.3, C–8 at 105.7, C–5 at 50.3, and C–10 at 35.2, the H–6a at $\delta_H$ 2.89 correlated with C–7 at $\delta_C$ 159.3, C–8 at 105.7, and C–10 at 35.2, and 13-$CH_3$ at $\delta_H$ 1.89 correlated with C–7 at $\delta_C$ 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 $\delta_H$ 1.59 correlated with C–3 at $\delta_C$ 73.3, C–4 at 82.7, and C–5 at 50.3, H–3 at $\delta_H$ 5.83 correlated with the carbonyl carbon signal at $\delta_C$ 168.2 (2-methyl-2,3-epoxy-butyric group), and the ethoxyl proton at $\delta_H$ 3.44 and 3.30 correlated with C–8 at $\delta_C$ 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 $\delta_H$ 1.59 correlated with the 14-$CH_3$ at $\delta_H$ 1.20 and H–3 at 5.83, the acetyl methyl at $\delta_H$ 2.00 correlated with the H–5 at $\delta_H$ 1.76; the methylene signal of ethoxyl group at $\delta_H$ 3.44 and 3.30 correlated with the 14-$CH_3$ signal at $\delta_H$ 1.20. Therefore, the structure of **2** was determined as pterodolide (figure 1).

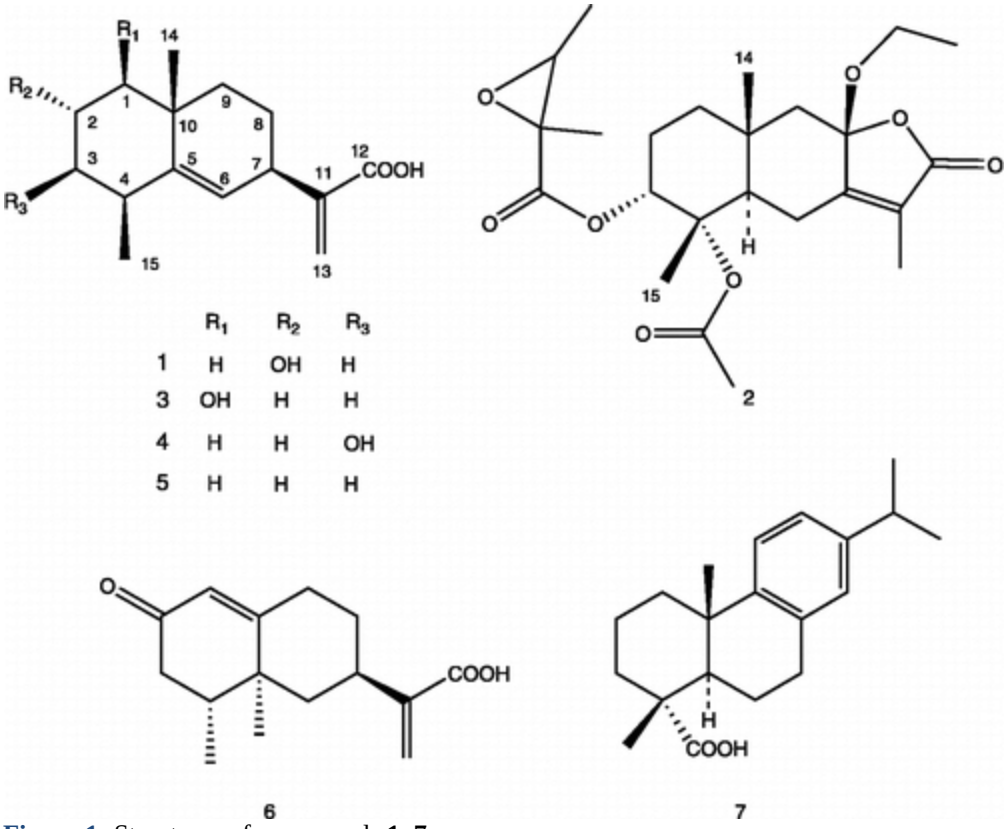


**Figure 1:** Structures of compounds **1–7**.

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.

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

| No. | 1 ($C_5D_5N$) | | 2 ($CDCl_3$) | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ mult. (J=Hz) | $\delta_C$ | $\delta_H$ 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 |

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).

**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).

## EXPERIMENTAL

### *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).

### *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.

### *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 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) $\nu_{max}$ $cm^{-1}$: 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). $^1$H 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) $\nu_{max}$ $cm^{-1}$: 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). $^1$H NMR ($CDCl_3$), see table 1; $\delta_H$ 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; $\delta_C$ 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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