Ainsliaolide D: a new sesquiterpene lactone from *Ainsliaea pertyoides*

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Ainsliaolide D, a new guaiane-type sesquiterpene lactone, was isolated from the roots of *Ainsliaea pertyoides* Fr. var. *albotomentosa* Beauverd. Its structure was determined based on spectral data.

**Keywords:** *Ainsliaea pertyoides*; sesquiterpene lactone; ainsliaolide D; guaiane type

1. Introduction

*Ainsliaea pertyoides* Fr. var. *albotomentosa* Beauverd (Compositae) distributed in south-west China is an important folk medicinal herb widely used to treat colds, asthma, rheumatoid arthritis, paralysis pain, bruises, bleeding, enteritis, diarrhoea, sore throat and urinary system embolism (State Administration of Traditional Chinese Medicine 1999). To date, the systematic and comprehensive research on the chemical composition of *A. pertyoides* has not been reported. This work investigated the roots of *A. pertyoides*, and 11 compounds were isolated. On the basis of spectral data they were identified as baucrenal acetate (1) (Lin 2006), friedelan (2) (Zhang & Koike 1999), friedelinol (3) (Zhang & Koike 1999), isointermedeol (4) (Sun et al. 1999), hexadecanoic acid (5) (State Administration of Traditional Chinese Medicine 1999), stigmasterol (6) (State Administration of Traditional Chinese Medicine 1999), acetyl ursolic acid (7) (Sun & Chen 2004), ainsliaolide D (8) (Bohlmann & Chen 1982), betulinic acid (9) (Liu & Zhang 2006), stigmasterol-β-D-glucoside (10) (Liu & Zhang 2006), and geniposide (11) (Jensen et al. 1996). Compound 8 is a new guaiane-type sesquiterpene lactone.

2. Results and discussion

The structure of compound 8 was elucidated as shown in Figures 1 (as Structure a) and Figure S2, and trivially named ainsliaolide D. The $^1$H NMR and $^{13}$C NMR spectra (Table S1) were completely assigned by HSQC (Figure S10), HMBC and NOESY experiments. The ESI-MS (Figure S4) spectrum exhibited a quasi-molecular ion [M + Na]$^+$ at m/z 353. The molecular formula C$_{20}$H$_{26}$O$_{4}$ was deduced from the ion peak at m/z 353.1710 ([M + Na]$^+$, calculated 353.1728) in HR-ESI-MS. The IR (Figure S5) spectrum suggested the presence of a γ-lactone group (1775.79 cm$^{-1}$) and an ester carbonyl group (1712.86 cm$^{-1}$). The $^1$H NMR (Figure S6) spectrum was quite similar to that of Structure b (Figure 1, as b) (Bohlmann & Chen 1982), except for the ester moiety. Two methyl signals (δ 1.91, 3H, brs, H-4′ and 2.19, 3H, brs, H-5′) and an alkene proton signal (δ 5.72, 1H, brs, H-2′) indicated the presence of a senecioyloxy
group. In the $^{13}$C NMR spectrum (Figure S7), 20 signals were observed, including 3 methyl signals ($\delta$ 11.3, $\delta$ 20.2 and $\delta$ 27.4) and 2 carbonyl signals ($\delta$ 179.6 and $\delta$ 166.2). The HMBC (Figure S8) correlation between H-3 ($\delta$ 5.57, ddt, $J = 8.0, 6.0, 2.0$Hz) and C-1 ($\delta$ 166.2) suggested that the senecioyloxy group was located at C-3. The NOESY (Figure S9) cross-peaks between H-1 and H-3 and between H-1 and H-5 indicated the $\alpha$-orientation of H-3 and H-5. The $\alpha$-orientation of H-7 and H-11 was deduced from the NOESY cross-peaks between H-5 and H-7 and between H-7 and H-11. No NOESY cross peaks were observed between H-6 and H-5 and between H-6 and H-7, suggesting the $\beta$- of H-6.

3. Experimental

3.1. General experimental procedure

Melting points were determined on an XRC-1 micro-melting point apparatus (uncorrected, Sichuan University Scientific Instrument, China). NMR spectra were recorded on a Bruker Avance-600 spectrometer (UIC Inc., USA), and the chemical shifts ($\delta$) are given in ppm. ESI-MS and HR-ESI-MS were carried out on a Finnigen LCQ-DECA mass spectrometer (Finnigen Inc., USA) and a Bruker Daltonics Apex II mass spectrometer (Bruker Daltonics Inc., USA), respectively. IR spectra were recorded on a PerkinElmer Spectra One IR spectrometer (P.E Inc., USA). Column chromatography was carried out with silica gel (200–300 mesh, Qingdao Haiyang Chemical, Inc., Qingdao, China) and polyamide (200 mesh, Linjiang Chemical, Inc., Jiangsu, China).

3.2. Plant material and appraisal

The whole plants of *A. pertyoides* was purchased from the Chengdu Hehuachi market for Traditional Chinese Medicinal Materials in February 2009. A voucher specimen has been deposited in the corresponding author’s laboratory in Chengdu University, China. This specimen was appraised as *A. pertyoides* by Prof. Yan Zhuyun of Chengdu University of Traditional Chinese Medicine.

3.3. Extraction and isolation

The whole plants of *A. pertyoides* (5.0 kg, shade-dried) were extracted under reflux (3 to 4 h each time). The ethanol solution was concentrated under reduced pressure (1/10 × 1 to 1/20 × 1 atm). A total of 353 g of extract was subsequently obtained. The extract was dispersed with 1.5 L MeOH–H$_2$O (90:10), sonic-oscillated extracted with petroleum ether (b.p. 60–90°C) for four times. A total of 85 g petroleum fraction was obtained. After extraction with petroleum ether,
distilled water was added to the MeOH–H2O (90:10) suspension, sonic-oscillated extracted with ethyl acetate (EtOAc) for four times. A EtOAc fraction (64 g) was obtained.

The petroleum ether extract (70 g) was dissolved in a 30:1 petroleum ether–ethyl acetate mixture and subjected to silica gel column (Φ 8 cm × L 80 cm) eluted with a 30:1 to 2:1 petroleum ether–ETOAc mixture. A total of 0.6 L was collected each time, and 100 fractions were collected. The eluates were combined based on TLC monitoring. Compound 1 (100 mg) was obtained by concentrating the 14th fraction and recrystallising from petroleum ether–EtOAc. Compound 2 (210 mg) was obtained by combining the 20th to 26th fractions and recrystallising from CHCl3. The leuco-spiculate crystal compound 3 (180 mg) was obtained by combining the 45th and 46th fractions and recrystallising from petroleum ether–EtOAc. Compound 4 (320 mg) was obtained by combining the 57th to 60th fractions. A white gummy solid 5 (57 mg) was obtained by the combination of the 61st to 65th fractions. The leuco-lumpish crystal compound 6 (135 mg) was obtained by the concentration of the 70th fraction and recrystallisation from methanol. Compound 7 (32 mg) was obtained by the combination of the 76th to 80th fractions and purified over silica gel column eluted with a petroleum ether–acetone mixture (15:1) as solvent. The hexagonal leuco-lumpish crystal compound 8 (132 mg) was obtained by purification of the combined 87th to 92nd fractions over silica gel column, eluted with petroleum ether–acetone (15:1) and recrystallised with a petroleum ether–acetone mixture.

The EtOAc extract (50 g) was divided into 80 fractions over silica gel column (200–300 mesh, Φ 8 cm × L 80 cm, wet loaded column with petroleum ether–acetone mixed solvent 10:1) gradiently eluted with a petroleum ether–acetone mixture (40:1 to 1:1). A white solid compound 9 (87 mg) was obtained by the combination of the 19th to 22nd fractions eluted out with petroleum ether–EtOAc (10:1). A white solid compound 10 (350 mg) was obtained by recrystallisation of the 45th to 50th combined fractions from CHCl3–MeOH. A White spiculate crystal compound 11 (2.7 g) was obtained by recrystallisation of the 67th to 73rd fractions from acetone.

3.4 Structural identification of compound 8

Compound 8 was obtained as a colourless crystal, m.p. 99–101°C, [α]D20 = −136 (c 0.11, CH3COCH3). IR (KBr) cm⁻¹: 1775.8 cm⁻¹ (γ−C==O). EI-MS m/z: [M + Na]+/m/z 353 [C20H26O4]. ¹H NMR (CDCl3) δ: 2.48 (1H,α-H-2), 1.79 (1H,β-H-2), 5.57 (1H,ddt(8,6,2),H-3), 2.85 (1H,m,H-5), 4.11 (1H,t(10),H-6), 2.42 (1H,m,H-7), 1.92 (1H,m,α-H-8), 1.42 (1H,m,β-H-8), 2.01 (1H,m,α-H-9), 2.52 (1H,m,β-H-9), 2.69 (1H,p(8),H-11), 1.16 (3H,d(8),H-13), 4.91 (1H,brs,H-14a), 4.92 (1H,brs,H-14b), 5.28 (1H,brt(2),H-15a), 5.41 (1H,brt(2),H-15b), 1.91 (3H,brs, H-4'), 2.19 (3H,brs,H-5'), 5.72 (1H,brs,H-2'). ¹³C NMR (CDCl3) δ: 43.8 (C-1), 36.34 (C-2), 73.7 (C-3), 148.75 (C-4), 50.0 (C-5), 83.8 (C-6), 45.6 (C-7), 28.7 (C-8), 36.29 (C-9), 148.88 (C-10), 39.2 (C-11), 179.6 (C-12), 11.3 (C-13), 113.23 (C-14), 113.06 (C-15), 166.2 (C-1'), 151.9 (C-2'), 164.9 (C-2'), 179.6 (C-3'), 27.4 (C-4'), 20.2 (C-5').

4. Conclusions

From the petroleum ether extract of the whole plants of A. Pertyoides, a new sesquiterpene lactone was isolated and purified. It is named amsliaolide D.

Supplementary material

The structures of all isolated compounds are available online as supplementary material, alongside Figures S1–S10 and Table S1.
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