

# A New Pregnane from *Munronia delavayi* Franch (Meliaceae)

Xiang-Hai Cai<sup>1,2</sup>, Xiao-Dong Luo<sup>1\*</sup>, Jun Zhou<sup>1</sup> and Xiao-Jiang Hao<sup>1</sup>

(1. State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming 650204, China;

2. Graduate School of the Chinese Academy of Sciences, Beijing 100039, China)

## Abstract

To search for pharmacologically active constituents of folk medicine, a new pregnane, 2 $\alpha$ ,3 $\alpha$ ,15 $\beta$ -trihydroxy-20(S)-tigloyl-pregnane (compound 1), and nine known compounds, geranylgeraniol (compound 2),  $\beta$ -daucosterol (compound 3), 6-hydroxystigmast-4-en-3-one (compound 4), sitoindoside I (compound 5), sitoindoside II (compound 6),  $\beta$ -sitosterol (compound 7), kaempferol (compound 8), quercetin (compound 9), and rutin (compound 10), were isolated from the ethanol extract of whole plants of *Munronia delavayi* Franch using chromatographic methods. The structures of compounds 1–10 were elucidated on the basis of spectral data.

**Key words:** chemical constituents; Meliaceae; *Munronia delavayi*; pregnane; 2 $\alpha$ ,3 $\alpha$ ,15 $\beta$ -trihydroxy-20(S)-tigloyl-pregnane.

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Because the family Meliaceae has attracted considerable interest as a possible new source for unique natural products for integrated pest management, many chemical constituents have been isolated. However, until now, no chemical research has been performed on the genus *Munronia*, except for our work (Qi et al. 2003a, 2003b, 2003c). *Munronia delavayi* Franch is a small semi-bush that has been used for the treatment of many diseases, such as tuberculosis, cough, stomachache, and sores, in Chinese traditional medicine (Yunnan Institute of Botany 1977). During the course of our research on Meliaceae, we explored the constituents of *M. delavayi* (Luo et al. 2002a, 2002b). A new pregnane, namely 2 $\alpha$ ,3 $\alpha$ ,15 $\beta$ -trihydroxy-20(S)-tigloyl-pregnane (compound 1), together with nine known compounds (2–10), was obtained from this species.

## Results and Discussion

The ethanol (EtOH) extract of whole plants of *Munronia*

*delavayi* Franch was partitioned between H<sub>2</sub>O with ethyl acetate (EtOAc). The EtOAc layer was loaded on column chromatography (CC) over silica gel to afford 10 compounds: 2 $\alpha$ ,3 $\alpha$ ,15 $\beta$ -trihydroxy-20(S)-tigloyl-pregnane (1), geranylgeraniol (2; Nagasampagi et al. 1967),  $\beta$ -daucosterol (3; Voutquenne et al. 1999), 6-hydroxystigmast-4-en-3-one (4; Khan and Malik 1989), sitoindoside I (5) and sitoindoside II (6; Luo et al. 2001),  $\beta$ -sitosterol (7), kaempferol (8) and quercetin (9; Lu et al. 2004), and rutin (10; Maddlam and Temai 1976).

Compound 1 was found to possess a molecular formula of C<sub>26</sub>H<sub>42</sub>O<sub>5</sub>, as evidenced by HRESIMS at *m/z* 457.293 1 ([M+Na]<sup>+</sup>). The IR spectrum of compound 1 showed absorption bands for hydroxyl (3 443 cm<sup>-1</sup>), carbonyl groups (1 705 cm<sup>-1</sup>), and double bonds (1 651 cm<sup>-1</sup>). The <sup>1</sup>H-, <sup>13</sup>C-NMR and DEPT spectra displayed signals for 26 carbons, five methyl groups ( $\delta_c$  20.0, 15.3, 14.4, 12.4, 12.0), three of them were tertiary methyl; seven methylenes ( $\delta_c$  41.0, 40.6, 38.4, 34.2, 31.3, 27.5, 20.8); 10 methines ( $\delta_c$  137.0, 72.1, 70.6, 69.2, 69.1, 60.4, 55.5, 54.8, 38.3, 30.7), four of which were oxymethines; four quaternary carbons ( $\delta_c$  167.2, 129.1, 42.1, 37.1); and a tigloyl group ( $\delta_c$  167.2 (s), 129.1 (s), 137.0 (d), 14.4 (q), 12.4 (q)). Comparison of these data with those of azedarachol revealed that the structure of the ring A–D portion of the molecule was identical to that of azedarachol, including the locations of the hydroxyls at C-2 and C-3, as well as a hydrogen atom at C-5, with the exception

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\*Author for correspondence. Tel: +86 (0)871 522 3188; Fax: +86 (0)871 515 0227; E-mail: <xdluo@mail.kib.ac.cn>.

of the side chain attached to C-17 and the location of the third hydroxyl group (Nakatani et al. 1985) (Figure 1).

In the HMBC spectrum signals of  $\delta_{\text{H}}$  4.27 (t,  $J=6.0$  Hz) correlated with  $\delta_{\text{C}}$  42.1 (s, C-13) and 55.5 (d, C-17) placed the hydroxyl  $\beta$ -orientation at C-15 in combination with correlations between  $\delta_{\text{H}}$  4.27 (H-15) and  $\delta_{\text{H}}$  0.93 (H-14 $\alpha$ , d,  $J=6.0$  Hz) and H-16 $\alpha$  ( $\delta_{\text{H}}$  2.28, m) in the ROESY spectrum (Figures 1 and 2). The presence of the tigloyl group attached on C-20 can also be confirmed by cross-peaks ( $\delta_{\text{H}}$  5.03 (q, H-20) with  $\delta_{\text{C}}$  167.2 (s, C-1'), 20.9 (d, C-21), 38.4 (t, C-16), 42.1 (s, C-13) and 55.5 (d, C-17)) from the HMBC data. The absolute configuration of C-20 was determined S from correlations (H-20 ( $\delta_{\text{H}}$  5.03, m) with H-16 $\beta$  ( $\delta_{\text{H}}$  1.37, m) and H-18 ( $\delta_{\text{H}}$  0.91, s), H-21 ( $\delta_{\text{H}}$  1.17, d,  $J=6.0$  Hz) with H-18) in the ROESY spectrum (Jing et al. 2002). So, compound 1 was established as 2 $\alpha$ ,3 $\alpha$ ,15 $\beta$ -trihydroxy-20 (S)-tigloyl-pregnane. All the signals of  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR were assigned by HSQC, HMBC, and ROESY spectra.

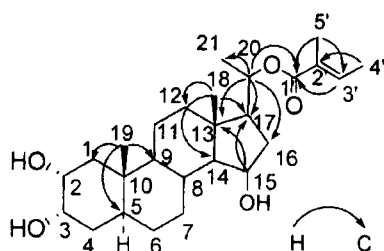


Figure 1. Structure and HMBC correlations of compound 1.

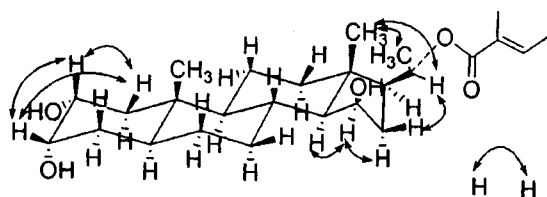


Figure 2. Key ROESY correlations of compound 1.

## Experimental

### General experimental procedures

Optical rotations were measured using a Horiba SEAP-300 spectropolarimeter. The UV spectra were measured with a Shimadzu (Kyoto, Japan) double-beam 210A spectrophotometer in methanol (MeOH) solution. The IR (KBr) spectra were obtained on a Bio-Rad (Hercules, CA, USA) FTS-135 infrared spectrophotometer. One- and two-dimensional NMR spectra were recorded on a Bruker DRX-500 MHz NMR spectrometer with tetramethylsilane (TMS) as the internal standard. The MS spectral data were obtained on a VG Autospec-3000

spectrometer. Silica gel (200–300 mesh) for CC and GF<sub>254</sub> for TLC were obtained from the Qingdao Marine Chemical Factory (Qingdao, China). Spots on chromatograms were detected by spraying with 5% H<sub>2</sub>SO<sub>4</sub>.

### Plant materials

The whole plant of *Munronia delavayi* Franch was collected in Qianjia County, Yunnan Province, China, in May 2004 and was identified by Professor Li-Shan Xie (Kunming Institute of Botany, the Chinese Academy of Sciences).

### Extraction and isolation

Air-dried whole plant (11.5 kg) was crushed and extracted with EtOH (20 L  $\times$  4) at room temperature (48 h  $\times$  3) to yield an EtOH extract. After removal of the EtOH under reduced pressure, the viscous concentration was partitioned between H<sub>2</sub>O and EtOAc. The EtOAc (210 g) fraction was chromatographed on a prepacked Si gel (2.0 kg) column, using a mixture of CHCl<sub>3</sub>-Me<sub>2</sub>CO (from CHCl<sub>3</sub> to CHCl<sub>3</sub>-Me<sub>2</sub>CO (1 : 1)), to give eight fractions (I–VIII) according to differences in composition monitored by TLC (Si gel GF<sub>254</sub>). Fraction III (23.0 g) was subjected to CC over silica gel (300 g), eluted with petroleum ether-Me<sub>2</sub>CO (7 : 1), to give compounds 7 (18 mg), 2 (55 mg), 4 (36 mg), and 1 (16 mg). Fraction IV (31.0 g) was chromatographed over silica gel (500 g), eluted by petroleum ether-Me<sub>2</sub>CO (5 : 1), to give two fractions (A and B). Fractions A (1.8 g) and B (2.5 g) were further purified by RP<sub>18</sub> gel (80 g) CC, eluted with MeOH-H<sub>2</sub>O (from 8 : 2 to 9 : 1), to obtain compounds 8 (25 mg) and 9 (22 mg), respectively. Fraction V (25.0 g) was purified by repeated CC on silica gel (280 g) using petroleum ether-EtOAc and petroleum ether-CHCl<sub>3</sub> as eluents to afford compounds 5 (120 mg) and 6 (34 mg). Compound 3 was crystallized from Fraction VI and compound 10 was from the H<sub>2</sub>O layer.

### Identification

#### 2 $\alpha$ ,3 $\alpha$ ,15 $\beta$ -Trihydroxy-20 (S)-tigloyl-pregnane (compound 1)

C<sub>26</sub>H<sub>42</sub>O<sub>5</sub>, white powder,  $[\alpha]_{\text{D}}^{20.5} -10.5^\circ$  (MeOH, c 0.430); UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 215 (3.98); IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 3 443, 2 929, 2 852, 1 705, 1 651, 1 446, 1 380, 1 258;  $^1\text{H}$ -NMR (CDCl<sub>3</sub>)  $\delta$ : 6.83 (1H, q,  $J=7.0$  Hz, H-3'), 5.03 (1H, m, H-20), 4.27 (1H, m, H-15 $\alpha$ ), 3.97 (1H, brs, H-3 $\beta$ ), 3.76 (1H, brs, H-2 $\beta$ ), 2.28 (1H, m, H-16 $\alpha$ ), 1.37 (1H, m, H-16 $\beta$ ), 1.90 and 1.10 (each 1H, m, H-7), 1.82 (3H, s, H-5'), 1.79 (1H, d,  $J=7.0$  Hz, H-4'), 1.75 (1H, m, H-1 $\beta$ ), 1.16 (1H, m, H-1 $\alpha$ ), 1.68 and 1.25 (each 1H, m, H-12), 1.62 (1H, m, H-17 $\alpha$ ), 1.60 (1H, m, H-4 $\beta$ ), 1.52 (1H, m, H-4 $\alpha$ ), 1.53 (1H, m, H-5 $\alpha$ ), 1.51 and 1.25 (each 1H, m, H-11), 1.34 (1H, m, H-6 $\beta$ ), 1.20 (1H, m, H-6 $\alpha$ ), 1.23 (1H, m, H-8), 1.17 (3H, d,  $J=6.0$  Hz, H-21), 0.93 (1H, d,  $J=6.0$  Hz, H-14 $\alpha$ ), 0.91 (3H, s, H-18), 0.85 (1H, m, H-9 $\alpha$ ),

0.82 (3H, s, H-19);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 167.2 (s, C-1'), 137.0 (d, C-3'), 129.1 (s, C-2'), 72.1 (d, C-20), 70.6 (d, C-15), 69.2 (s, C-3), 69.1 (d, C-2), 60.4 (d, C-14), 55.5 (d, C-17), 54.8 (d, C-9), 42.1 (s, C-13), 41.0 (t, C-12), 40.6 (t, C-1), 38.4 (t, C-16), 38.3 (d, C-5), 37.1 (s, C-10), 34.2 (t, C-4), 31.3 (t, C-7), 30.7 (d, C-8), 27.5 (t, C-6), 20.8 (t, C-11), 20.0 (q, C-21), 15.3 (q, C-18), 14.4 (q, C-4'), 12.4 (q, C-5'), 12.0 (q, C-19); EIMS  $m/z$ : 434  $[\text{M}]^+$  (2), 416 (5), 398 (3), 334 (12), 316 (85), 83 (100), 55 (65); HRESIMS  $m/z$   $[\text{M}+\text{Na}]^+$  457.293 1 (calcd. for  $\text{C}_{26}\text{H}_{42}\text{O}_5\text{Na}$ , 457.292 9).

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