CHEMICAL CONSTITUENTS OF *Pulsatilla dahurica*

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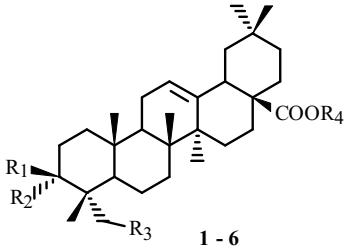
The roots of *Pulsatilla dahurica* (Fisch. ex DC.) Spreng (Ranunculaceae) are used as a traditional Chinese medicine for the treatment of amoebic disease, diarrhea, vaginal trichromoniasis, and bacterial infections [1]. Previous pharmacological investigations had suggested that triterpene glycosides are the main bioactive components of this plant genus [2–4].

The roots of *P. dahurica* were collected in Shangzhi county, Heilongjiang province of P. R. China in August of 2003. The air-dried powdered roots (4 kg) were extracted with 70% EtOH three times (each 3h, 12 L) under reflux condition. The 70% EtOH extract was evaporated under reduce pressure at 60°C. The residue (615 g) was subsequently suspended in water and partitioned with ethyl acetate and *n*-butanol.

The ethyl acetate extract (30 g) was subjected to silica gel column chromatography using $CHCl_3$–CH_3OH (19:1→4:1) as eluents to yield 1 (50 mg) and 2 (8 mg). The *n*-butanol extract (410 g) was chromatographed on a D101 resin column using EtOH–H_2O (1:9→9:1) as eluents to yield 12 fractions (Fr. I – Fr. XII). Fraction V (2.8 g), containing a mixture of compounds **3** and **4**, was further separated on an ODS column using CH_3OH–H_2O (35:65→60:40) as eluents to yield **3** (8 mg) and **4** (27 mg). Fraction II (3.1 g) was chromatographed on an ODS column using CH_3OH–H_2O (3:7→1:1) as eluents to afford **5** (32 mg) and **6** (12 mg). These compounds were identified by comparing their physical and spectral data with literature values.

Hederagonic Acid (1), $C_{30}H_{46}O_4$, colorless needles, mp 202–205°C; UV (CH_3OH, λ_{max}, nm): 211, 220, 307; IR (KBr, ν_{max}, cm^{-1}): 3450 (OH), 2920 (CH), 1730 (C=O), 1690 (C=O); ESI-MS *m/z*: 471 $[M+H]^+$ [5]. ^1H NMR (400 MHz, C_5D_5N, δ, J/Hz): 0.91 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.01 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.20 (3H, s, CH_3), 5.53 (1H, t, J = 3.2, H-12), 3.32 (H, dd, H-18), 3.70 (1H, d, J = 10.3, H-23a), 4.06 (1H, d, J = 10.3, H-23b). ^{13}C NMR (100 MHz, C_5D_5N, δ): 37.8 (C-1), 36.8 (C-2), 216.8 (C-3), 52.6 (C-4), 47.3 (C-5), 19.9 (C-6), 32.3 (C-7), 39.6 (C-8), 46.7 (C-9), 36.6 (C-10), 23.8 (C-11), 122.4 (C-12), 144.7 (C-13), 42.3 (C-14), 28.2 (C-15), 23.8 (C-16), 46.3 (C-17), 42.1 (C-18), 46.3 (C-19), 30.9 (C-20), 34.2 (C-21), 33.1 (C-22), 68.2 (C-23), 17.8 (C-24), 15.3 (C-25), 17.2 (C-26), 25.8 (C-27), 180.0 (C-28), 33.2 (C-29), 23.7 (C-30).



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1: $R_1 + R_2$ = O, R_3 = OH, R_4 = H
2: R_1 = Rha(1→2)Ara-O, $R_2 = R_4$ = H, R_3 = OH
3: R_1 = OH, $R_2 = R_3$ = H, R_4 = Rha(1→4)Glc(1→6)Glc
4: R_1 = Ara-O, R_2 = H, $R_3 = R_4$ = Rha(1→4)Glc(1→6)Glc
5: R_1 = Ara-O, R_2 = H, R_3 = OH, R_4 = Rha(1→4)Glc(1→6)Glc
6: R_1 = Rha(1→2)Ara-O, $R_2 = R_3 = R_4$ = Rha(1→4)Glc(1→6)Glc

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Tauroside E (2), $C_{41}H_{66}O_{12}$, amorphous powder, mp 258–260°C; UV (CH_3OH, λ_{max}, nm): 205, 313; IR (KBr, ν_{max}, cm^{-1}): 3400 (OH), 2920 (CH), 1700 (C=O); ESI-MS *m/z*: 773 $[M+Na]^+$; NMR data were identical to the literature values [6].

Acid hydrolysis of **2** afforded hederagenin, L-rhammose, and L-arabinose.

Hederacaucaside B (3), $C_{48}H_{78}O_{17}$, amorphous powder, mp 196–198°C; UV (CH_3OH, λ_{max}, nm): 204, 315; IR (KBr, ν_{max}, cm^{-1}): 3450 (OH), 2930 (CH), 1710 (C=O); ESI-MS *m/z*: 949 $[M+Na]^+$; NMR data were identical to the literature values [7].

Acid hydrolysis of **3** afforded oleanolic acid, L-rhammose, and D-glucose.

Ciwujianoside C3 (4), $C_{53}H_{86}O_{21}$, amorphous powder, mp 220–222°C; UV (CH_3OH, λ_{max}, nm): 205, 311; IR (KBr, ν_{max}, cm^{-1}): 3450 (OH), 2920 (CH), 1680 (C=O); ESI-MS *m/z*: 1081 $[M+Na]^+$; NMR data were identical to the literature values [8].

Acid hydrolysis of **4** afforded oleanolic acid, L-rhammose, L-arabinose, and D-glucose.

Hederasaponin D (5), $C_{53}H_{86}O_{22}$, amorphous powder, mp 182–185°C; UV (CH_3OH, λ_{max}, nm): 205, 313; IR (KBr, ν_{max}, cm^{-1}): 3420 (OH), 2930 (CH), 1690 (C=O); ESI-MS *m/z*: 1097 $[M+Na]^+$; NMR data were identical to the literature values [9].

Acid hydrolysis of **5** afforded hederagenin, L-rhammose, L-arabinose, and D-glucose.

Hederacoside C (6), $C_{59}H_{96}O_{26}$, amorphous powder, mp 225–228°C; UV (CH_3OH, λ_{max}, nm): 205, 311; IR (KBr, ν_{max}, cm^{-1}): 3400 (OH), 2920 (CH), 1690 (C=O); ESI-MS *m/z*: 1243 $[M+Na]^+$; NMR data were identical to the literature values [10].

Acid hydrolysis of **6** afforded hederagenin, L-rhammose, L-arabinose, and D-glucose.

All the above compounds were isolated from the title plant for the first time. The NMR data of compound **1** were assigned for the first time by extensive NMR methods.

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