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CHEMICAL CONSTITUENTS OF *Aconitum barbatum* VAR. *puberulum*

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Aconitum barbatum var. *puberulum* is widely used in Chinese folk medicine for the treatment of rheumatosis, rheumatoid arthritis, and some other inflammations [1]. In our phytochemical investigation of traditional Chinese medicines, an aporphine alkaloid corydine (**1**) [2], a tricarboxylic unsaturated acid, aconitic acid (**3**) [3], and two diterpenoid alkaloids, tuguaconitine (**2**) [4] and luciculine (**4**) [5], were isolated and identified. These compounds were isolated from this plant for the first time. The ^{13}C NMR of compound **1** was reported for the first time, and the ^{13}C NMR of compounds **2** and **4** were reassigned.

Plant Material. *Aconitum barbatum* var. *puberulum* was collected in Jinzhong Country (Shanxi Province, China) in September 2005 and was identified by Dr. Hu-Yuan Feng (School of Life Science, Lanzhou University). A voucher specimen (No. 20050901AB) is deposited in the Institute of Organic Chemistry, Lanzhou University, China.

Extraction and Isolation. The air-dried and powdered herbs (3.9 kg) were percolated with 90% aq. MeOH (7 days × 3) at room temperature. After evaporation of MeOH under reduced pressure, the defatted aqueous extract was then extracted with EtOAc at two pH levels: pH 4–5 and 9–10. The fraction with pH 9–10, 39.0 g, was subjected to column chromatography on silicon gel eluting with $CHCl_3$–CH_3OH (99:1–5: 1) to afford 5 fractions (1–5). Fraction 1 (12.5 g) was chromatographed on a silica gel column using petroleum ether–EtOAc (15:1–8:1) gradient to give **1** (40 mg). Fraction 2 (5.2 g) was recrystallized from petroleum ether–Me_2CO (4:1) to give **2** (60 mg). Fraction 5 (5.3 g) was further submitted to silica gel CC eluting with petroleum ether–EtOAc–MeOH (4:4:1, 3:3:1, 2:2:1, 1:1:1) to give **4** (23 mg) and **3** (12 mg).

Corydine (1): white crystal, mp 147–149°C; EI-MS: *m/z* 341 $[M^+]$; $C_{20}H_{23}NO_4$; ^{1}H NMR (400 MHz, $CDCl_3$, J/Hz): 6.67 (1H, s, H-3), 2.67 (1H, m, H-4a), 3.18 (1H, m, H-4b), 2.37 (1H, m, H-7a), 3.08 (1H, m, H-7b), 7.05 (1H, d, J = 7.2, H-8), 6.85 (1H, d, J = 7.2, H-9), 3.71 (3H, s, 2-OCH_3), 3.87 (3H, s, 10-OCH_3), 3.87 (3H, s, 11-OCH_3), 8.68 (1H, s, 1-OH); ^{13}C NMR (100 MHz, $CDCl_3$): 142.2 (C-1, s), 149.0 (C-2, s), 111.2 (C-3, d), 123.7 (C-3a, s), 28.8 (C-4, t), 52.6 (C-5, t), 62.6 (C-6a, s), 35.3 (C-7, s), 130.5 (C-7a, s), 124.2 (C-8, d), 110.8 (C-9, d), 151.7 (C-10, s), 143.7 (C-11, s), 126.3 (C-11a, s), 119.1 (C-11b, s), 127.8 (C-11c, s).

Tuguaconitine (2): colorless needles, mp 197–199°C; EI-MS (*m/z*, I_{rel}, %): 437 $[M]^+$ (83), 422 $(M–CH_3)^+$ (100), 406 $(M–CH_2CH_3)^+$ (40); $C_{23}H_{35}NO_7$; ^{1}H NMR (400 MHz, $CDCl_3$): 3.36, 3.41, 3.42 (each 3H, s), 3.21 (1H, d, J = 8.4, H-1), 2.17 (1H, m, H-2a), 1.10 (1H, m, H-2b), 3.06 (2H, m, H-3), 1.35 (1H, s, H-5), 4.34 (1H, s, H-6), 2.84 (1H, dd, J = 8.4, 5.6, H-9), 1.92 (1H, m, H-10), 2.05 (1H, dd, J = 14.0, 7.2, H-12a), 1.54 (1H, dd, J = 14.0, 7.2, H-12b), 2.37 (1H, m, H-13), 3.58 (1H, dd, J = 8.4, 4.0, H-14), 1.72 (1H, t, J = 14.6, 8.2, H-15a), 2.58 (1H, t, J = 7.2, H-15b), 2.85 (1H, s, H-16), 3.89 (1H, s, H-17), 2.55 (1H, d, J = 7.2, H-19a), 3.40 (1H, s, H-19b), 3.00 (2H, m, H-C$\underline{H}_2$$CH_3$), 1.03 (3H, t, J = 7.2, H-CH_2C$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 78.9 (C-1, d), 31.8 (C-2, t), 58.8 (C-3, d), 58.7 (C-4, s), 43.2 (C-5, d), 90.6 (C-6, d), 89.6 (C-7, s), 78.7 (C-8, s), 48.7 (C-9, d), 37.9 (C-10, d), 54.1 (C-11, s), 30.9 (C-12, t), 42.6 (C-13, d), 84.5 (C-14, d), 33.6 (C-15, t), 83.1 (C-16, d), 67.3 (C-17, d), 54.6 (C-19, t), 50.2 (C-20, t), 14.1 (C-21, q), 57.9 (6-OCH_3, q), 56.5 (14-OCH_3, q), 59.0 (16-OCH_3, q).

Aconitic acid (3): white amorphous powder, $C_6H_6O_6$; EI-MS *m/z*: 174.2 $[M]^+$; elemantal analysis: anal. C 41.37%, H 3.42%. Calcd for $C_6H_6O_6$, C 41.39%, H 3.47%. ^{1}H NMR (400 MHz, CD_3COCD_3, J/Hz): 6.92 (1H, d, J = 2.5),

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3.93 (2H, d, J = 3.5), 10.0–10.3 (br.s, 4H); ^{13}C NMR (100 MHz, CD_3COCD_3): 128.98 (d), 140.83 (s), 166.24 (s), 167.0 (s), 170.87 (s), 32.50 (t).

Luciculine (4): white villiform crystal, mp 111–113°C; $C_{22}H_{33}NO_3$; EI-MS: *m/z* 359 $[M]^+$; ^{1}H NMR (400 MHz, δ, $CDCl_3$, J/Hz): 0.76 (3H, s, H-18), 0.93 (3H, t, J = 7.2, H-$CH_2C\underline{H}_3$) and 2.3–2.4 (2H, m, H-$C\underline{H}_2CH_3$); 5.12 and 5.15 (each 1H, br.s, H-17); ^{13}C NMR (100 MHz, $CDCl_3$): 69.7 (C-1, d), 30.7 (C-2, t), 31.3 (C-3, t), 34.1 (C-4, s), 48.2 (C-5, d), 23.4 (C-6, t), 43.9 (C-7, d), 50.1 (C-8, s), 36.5 (C-9, d), 52.9 (C-10, s), 28.6 (C-11, t), 75.9 (C-12, d), 47.1 (C-13, d), 36.1 (C-14, t), 77.6 (C-15, d), 159.1 (C-16, s), 108.6 (C-17, t), 26.3 (C-18, q), 58.2 (C-19, t), 65.7 (C-20, d), 51.3 (C-21, t), 13.0 (C-22, q).

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