CHEMICAL CONSTITUENTS OF *Incarvillea mairei* VAR. *grandiflora*

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Incarvillea mairei var. *grandiflora*, a variety of *Incarvillea maireia* (Bignoniaceae), is distributed mainly in Yunnan, Sichuan, and Qinghai provinces. It often grows in mountains with an altitude of about 2500–3650 meters. Its roots and leaves can be used as folk medicine for the treatment of anemia [1].

In our systematic investigation on the chemical constituents of the whole plant of *I. mairei* var. *grandiflora*, 14 compounds were isolated from this plant for the first time.

The whole plants of *I. mairei* var. *grandiflora* were collected by the Yunnan Gesang Flowering Plants Co. Ltd. in late October 2006, and were authenticated by Prof. Li-shan Xie of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 2006101020) is deposited in the School of Pharmacy, Second Military Medical University, Shanghai, P. R. China.

The whole plants of *I. mairei* var. *grandiflora* (8.5 kg) were extracted with methanol at room temperature. The methanol extract was partitioned with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. Each fraction was purified by column chromatography with silica gel, Rp-18, and Sephadex LH-20 to yield compounds **1–14**.

The compounds were investigated by spectroscopic methods, including NMR and mass spectrometry. All compounds were identified as rengyolone (**1**) [2], cleroindicin B (**2**) [2], cleroindicin C (**3**) [2], polygalacerebroside (**4**) [3], schizandrin (**5**) [4], 1-*O*-caffeoylglycerol (**6**) [5], 4-hydroxycinnamic acid (**7**) [6], isoacteoside (**8**) [7], eutigoside A (**9**) [8], caffeic acid ethyl ester (**10**) [5], *β*-amyrin (**11**) [9], quercetin (**12**) [10], quercetin-7-*O*-rhamnoside (**13**) [10], and stigmasterol (**14**) [11].

Rengyolone (Halleridone) (1). Colorless oil, $[\alpha]_D^{20}$ –2.56° (*c* 0.03, MeOH), ESI-MS *m/z:* 153.0 $[M–H]^–$, 177.1 $[M+Na]^+$. PMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.98 (1H, m, H-2a), 3.76 (1H, m, H-2b), 2.24 (1H, m, H-3a), 2.10 (1H, m, H-3b), 6.69 (1H, dd, J = 10.2, 1.5, H-4), 5.87 (1H, d, J = 10.2, H-5), 2.68 (1H, dd, J = 16.8, 4.5, H-6), 2.48 (1H, dd, J = 16.8, 4.2, H-7), 4.12 (1H, dd, J = 4.5, 4.2, H-7a); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 65.9 (C-2), 39.1 (C-3), 74.7 (C-3a), 148.9 (C-4), 127.9 (C-5), 197.7 (C-6), 39.7 (C-7), 80.9 (C-7a).

Cleroindicin B (2). Colorless oil, EI-MS *m/z:* 158 $[M]^+$, 140 $[M–H_2O]^+$, 122 $[M–2H_2O]^+$, 112 $[140–C_2H_4]^+$. PMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.16 (2H, dt, J = 13.2, 4.8, H_e-2, 6), 1.84 (2H, dt, J = 13.2, 6.2, H_a-2, 6), 2.95 (2H, dt, J = 13.6, 6.2, H_a-3, 5), 2.32 (2H, dt, J = 13.6, 4.8, H_e-3, 5), 2.05 (2H, t, J = 6.6, H-7), 4.18 (2H, t, J = 6.6, H-8); ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 69.8 (C-1), 37.6 (C-2, 6), 37.8 (C-3, 5), 211.5 (C-4), 44.4 (C-7), 58.8 (C-8).

Cleroindicin C (3). Colorless oil, ESI-MS *m/z:* 157.0 $[M+H]^+$; PMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.91 (2H, m, H-2), 2.02 (1H, m, H-3a), 2.07 (1H, m, H-3b), 2.13 (1H, m, H-5a), 2.22 (1H, m, H-5b), 2.32 (1H, ddd, J = 15.8, 10.8, 3.6, H-6a), 2.63 (1H, ddd, J = 11.6, 6.2, 3.4, H-6e), 2.76 (1H, dd, J = 15.7, 4.2, H-8), 2.97 (1H, dd, J = 15.7, 4.2, H-8), 4.25 (1H, t, J = 4.2, H-9); ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 65.7 (C-2), 39.9 (C-3), 76.9 (C-3a), 32.9 (C-4), 34.8 (C-5), 211.3 (C-6), 42.1 (C-7), 83.1 (C-7a).

Polygalacerebroside (4). White powder, ESI-MS: *m/z* 732.5632 $[M+H]^+$, HR-ESI-MS *m/z:* 732.5632 $[M+H]^+$ (calcd for $C_{40}H_{78}O_{10}N$, 732.5626). PMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.81 (1H, dd, J = 11.0, 7.0, H-1a), 3.67 (1H, m, H-1b), 4.11 (1H, m, H-2), 3.38 (1H, H-3), 3.35 (1H, H-4), 1.93 (2H, H-5), 5.36 (1H, H-6), 5.36 (1H, H-7), 1.93 (2H, H-8), 0.85 (3H, J = 7.0, H-9), 7.50 (1H, d, J = 9.0, H-2-NH-), 3.86 (1H, m, H-2′), 0.85 (3H, J = 7.0, H-16′), 1.24~1.60, (44H, H-5–9, 14–17,

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3′–15′), 4.15 (1H, d, J = 7.0, H-1″), 2.95 (1H, m, H-2″), 3.15 (1H, m, H-3″), 3.34 (1H, H-4″), 3.10 (1H, m, H-5″), 3.65 (1H, H-6″a), 3.45 (1H, dd, J =6.0, 12.0, H-6″b); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 68.8 (C-1), 49.8 (C-2), 74.1 (C-3), 70.0 (C-4), 31.2 (C-10), 130.2 (C-11), 129.6 (C-12), 31.9 (C-13), 13.8 (C-18), 173.7 (C-1′), 70.9 (C-2′), 13.8 (C-16′), 21.0, 24.4, 28.5 (C-5–9), 28.6–28.9 (C-14–17), 28.9–29.0, 32.2 (C-3′–15′), 103.4 (C-1″), 73.4 (C-2″), 76.5 (C-3″), 70.5 (C-4″), 76.8 (C-5″), 61.0 (C-6″).

Schizandrin (5). Yellow needle, ESI-MS *m/z:* 433.0 $[M+H]^+$; PMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 6.61 (1H, s, H-4), 2.82 (d, J = 13.1, H-6*α*), 2.32 (dd, J = 13.1, 1.1, H-6*β*), 1.90 (1H, m, H-8), 2.52 (dd, J = 14.1, 6.5, H-9*α*), 2.55 (dd, J = 14.1, 3.1, H-9*β*), 6.54 (1H, s, H-11), 1.19 (3H, s, CH_3-7), 0.88 (3H, d, J = 7.1, CH_3-8), 3.55, 3.56, 3.89 (× 2), 3.88 (× 2) (6 × OCH_3); ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 151.7 (C-1), 140.6 (C-2), 152.0 (C-3), 110.6 (C-4), 131.7 (C-5), 41.0 (C-6), 71.5 (C-7), 41.5 (C-8), 34.5 (C-9), 133.6 (C-10), 110.2 (C-11), 152.1 (C-12), 140.0 (C-13), 151.6 (C-14), 122.4 (C-15), 124.1 (C-16), 16.0 (C-17), 29.9 (C-18), 55.8 (× 2) ($\underline{C}H_3$-3, 12), 60.6 (× 2) ($\underline{C}H_3$-1, 14), 60.9 (× 2) ($\underline{C}H_3$-2, 13).

1-*O*-Caffeoylglycerol (6). White needle, ESI-MS *m/z*: 253 $[M–H]^–$; PMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.6 (1H, d, J = 16.0, H-*β*), 7.1 (1H, d, J = 2.0, H-2), 7.0 (1H, dd, J = 8, 2, H-6), 6.8 (1H, d, J = 8.0, H-5), 6.3 (1H, d, J = 16.0, H-*α*), 4.4 (1H, dd, J = 11.0, 4.5, H-1′a), 4.3 (1H, dd, J = 11.5, 6.0, H-1′b), 4.0 (1H, m, H-2′), 3.7 (2H, dd, J = 6.0, 2.0, H-3′); ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 127.8 (C-1), 115.2 (C-2), 146.8 (C-3), 147.1 (C-4), 116.5 (C-5), 123.0 (C-6), 169.2 (C=O), 115.0 (C-*α*), 149.6 (C-*β*), 66.5 (C-1′), 71.2 (C-2′), 64.1 (C-3′).

Isoacteoside (8). Yellow powder ESI-MS *m/z:* 647 $[M+Na]^+$, 623 $[M–H]^–$; PMR (300 MHz, CD_3OD, δ, ppm, J/Hz): 7.58 (1H, d, J = 15.8, H-*β*), 6.31 (1H, d, J = 15.8, H-*α*), 6.54–7.06 (6H, Ar-H), 5.21 (1H, s, H-1 of rhamnose), 4.35 (1H, d, J = 7.5, H-1 of glucose), 3.48, (2H, m, CH_2-7), 2.79 (2H, m, CH_2-8), 1.26 (3H, d, J = 6.0, CH_3 of rhamnose); ^{13}C NMR (75 MHz, CD_3OD, δ, ppm): 2,4-dihydroxyphenyl ethanol moiety: 131.3 (C-1), 116.3 (C-2), 144.6 (C-3), 146.1 (C-4), 117.1 (C-5), 121.3 (C-6), 72.4 (C-7), 36.6 (C-8); caffeoyl moiety: 127.6 (C-1), 114.8 (C-2), 149.6 (C-3), 146.7 (C-4), 116.5 (C-5), 123.2 (C-6), 169.4 (C=O), 115.1(C-*α*), 147.2 (C-*β*); glucose moiety: 104.3 (C-1), 75.3 (C-2), 83.9 (C-3), 70.0 (C-4), 75.6 (C-5), 64.6 (C-6); rhamnose moiety: 102.7 (C-1), 72.3 (C-2), 72.2 (C-3), 74.0 (C-4), 70.3 (C-5), 17.9 (C-6).

Eutigoside A (9). White powder, ESI-MS *m/z:* 469 $[M+Na]^+$, 447 $[M+H]^+$; PMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.59 (d, J = 16.0, C-*β*″), 6.27 (d, J = 16.0, C-*α*″), 7.38 (2H, dd, J = 8.0, 2.0, H-2″, H-6″), 6.73 (2H, dd, J = 8.0, 2.0, H-3″, H-5″), 7.03 (2H, d, J = 8.0, 2.0, H-2, H-6), 6.64 (2H, d, J = 8.0, 2.0, H-3, H-5), 2.83 (2H, CH_2-*β*), 3.72 (2H, CH_2-*α*), 4.38 (1H, d, J = 7.5, H-1′), 3.38–3.50 (2H, m, H-2′, 3′, 5′), 3.24 (1H, m, H-4′), 4.54 (1H, d, J = 11.5, H-6b′), 4.36 (1H, d, J = 11.5, H-6a′); ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 127.7 (C-1″), 115.8 (C-2″), 130.6 (C-3″), 160.9 (C-4″), 130.6 (C-5″), 115.8 (C-6″), 169.5 (C=O), 115.1 (C-*α*), 147.2 (C-*β*), 130.8 (C-1), 130.6 (C-2), 116.6 (C-3), 156.5 (C-4), 116.6 (C-5), 130.6 (C-6), 72.1 (C-*α*), 36.4 (C-*β*), 104.3 (C-1′), 75.3 (C-2′), 77.8 (C-3′), 72.0 (C-4′), 75.6 (C-5′), 64.6 (C-6′).

Quercetin-7-*O*-rhamnoside (13). Yellow powder, ESI-MS *m/z:* 471 $[M+Na]^+$, 449 $[M+H]^+$, 447 $[M–H]^–$; PMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz), 12.48 (1H, s, OH-5), 9.61–9.39 (4H, s, -OH × 3), 6.41 (1H, d, J = 2.0, H-6), 6.77 (1H, d, J = 2.0, H-8), 7.73 (1H, d, J = 1.0, H-2′), 6.89 (1H, d, J = 9.0, H-5′), 7.59 (1H, dd, J = 9.0, 2.0, H-6′), 5.55 (1H, s, H-1″), 3.87 (1H, d, J = 4.0, H-2″), 3.66 (1H, dd, J = 9.5, 5.0, H-3″), 3.33 (1H, m, H-4″), 3.47 (1H, m, H-5″), 1.14 (3H, d, J = 6.0, Rha-Me); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm), 147.8 (C-2), 136.0 (C-3), 175.9 (C-4), 160.3 (C-5), 98.8 (C-6), 161.4 (C-7), 94.2 (C-8), 155.7 (C-9), 104.6 (C-10), 121.7 (C-1′), 115.6 (C-2′), 145.0 (C-3′), 147.5 (C-4′), 115.3 (C-5′), 120.1 (C-6′), 98.5 (C-1″), 71.6 (C-4″), 70.2 (C-3″), 70.0 (C-2″), 69.8 (C-5″), 17.9 (C-6″).

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