CHEMICAL CONSTITUENTS OF STEM BARK FROM *Periploca sepium*

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The root bark of *Periploca sepium* (Asclepiadaceae), which contains many kinds of compounds such as cardenoides, pregnane glycosides, etc. [1], has been used as a traditional Chinese herbal medicine for more than two thousand years [2]. In order to find a new drug resource, the present paper describes the isolation and structural elucidation of four compounds from the stem bark of *Periploca sepium*.

Two kilograms dried stem bark powder of *P. sepium* collected from Qinling Mountain, Shaanxi, China, in August 2004 was extracted exhaustively with 95% ethanol at room temperature. The ethanol extract was concentrated in vacuum at 60°C to give a residue. The residue was suspended in water and successively treated with petroleum ether, ethyl acetate, and *n*-butanol.

The ethyl acetate extract (53 g) was subjected to chromatography on silica gel eluting successively with chloroform-methanol gradient (1:0, 20:1, 10:1, 5:1, 2:1, 0:1) to give six fractions (I–VI). Repeated chromatography of fraction I on silica gel with a gradient of chloroform in petroleum ether and Sephadex LH-20 with chloroform–methanol (1:1) afforded compound **1** (100 mg). Fractions II and IV were repeatedly subjected to column chromatography on silica gel with a gradient of methanol in chloroform and Sephadex LH-20 with chloroform–methanol (1:1) afforded compound **2** (50 mg) from fraction II and compounds **3** (80 mg) and **4** (65 mg) from fraction IV. The structures of these compounds were confirmed using a combination of spectral analyses and by comparison with reported spectral data in the literature.

***β*-Amyrin Acetate (1)**. $C_{32}H_{52}O_2$, mp 234–235°C, colorless crystals. EI-MS spectrum (70 eV): *m/z* 468 $[M]^+$ (20), 453 (8), 257 (6), 218 (100), 203 (60), 189 (26), 135 (18), 107 (10). ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm, J/Hz): 5.17 (1H, t, J = 3.6, H-12), 4.49 (1H, m, H-3), 2.05 (3H, s, H-32), 1.12 (3H, s), 0.97 (3H, s), 0.92 (3H, s), 0.87 (6H, s), 0.81 (3H, s), 0.78 (6H, s). ^{13}C NMR ($CDCl_3$), 100 MHz, δ, ppm): 38.2 (C-1), 23.7 (C-2), 80.9 (C-3), 37.7 (C-4), 55.2 (C-5), 18.2 (C-6), 32.6 (C-7), 39.8 (C-8), 47.5 (C-9), 36.8 (C-10), 23.5 (C-11), 121.6 (C-12), 145.2 (C-13), 41.7 (C-14), 28.4 (C-15), 26.1 (C-16), 32.5 (C-17), 47.2 (C-18), 46.8 (C-19), 31.1 (C-20), 34.7 (C-21), 37.1 (C-22), 28.0 (C-23), 16.7 (C-24), 15.5 (C-25), 16.8 (C-26), 25.9 (C-27), 26.9 (C-28), 33.3 (C-29), 23.6 (C-30), 171.0 (C-31), 21.3 (C-32) [3].

***β*-Amyrin (2)**, $C_{30}H_{50}O$, colorless crystals, mp 196–197°C. EI-MS spectrum (70 eV): *m/z* 426 $[M]^+$ (47), 411 (28), 257 (14), 247 (12), 218 (100), 203 (62), 189 (49), 135 (55). ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm, J/Hz): 5.10 (1H, t, J = 6.9, H-12), 3.18 (1H, m, H-3), 1.12 (3H, s), 0.98 (3H, s), 0.96 (3H, s), 0.92 (3H, s), 0.87 (3H, s), 0.85 (3H, s), 0.81 (3H, s), 0.79 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 38.3 (C-1), 27.1 (C-2), 79.1 (C-3), 38.7 (C-4), 55.4 (C-5), 18.4 (C-6), 32.6 (C-7), 39.6 (C-8), 47.8 (C-9), 36.7 (C-10), 23.4 (C-11), 121.8 (C-12), 145.3 (C-13), 41.6 (C-14), 28.2 (C-15), 26.1 (C-16), 32.7 (C-17), 47.3 (C-18), 46.8 (C-19), 31.3 (C-20), 34.9 (C-21), 37.2 (C-22), 28.2 (C-23), 15.7 (C-24), 15.5 (C-25), 16.9 (C-26), 26.1 (C-27), 27.1 (C-28), 33.3 (C-29), 23.7 (C-30) [3].

Periplogenin (3), colorless needles, mp 135–137°C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm, J/Hz): 5.89 (1H, s, H-22), 4.99 (1H, dd, J = 18.0, J = 1.6, H-21), 4.82 (1H, dd, J = 18.0, J = 1.6, H-21), 4.19 (1H, br.s, H-3), 2.79 (1H, dd, J = 9.2, J = 5.6, H-17), 0.95 (3H, s, H-19), 0.88 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 24.83 (C-1), 27.81 (C-2), 67.94 (C-3), 36.79 (C-4), 74.77 (C-5), 35.08 (C-8), 23.70 (C-7), 40.64 (C-8), 38.95 (C-9), 40.66 (C-10), 21.50 (C-11), 39.96 (C-12), 49.54 (C-13), 85.36 (C-14), 32.91 (C-15), 26.82 (C-16), 50.66 (C-17), 15.76 (C-18), 16.75 (C-19), 174.96 (C-20), 73.62 (C-21), 117.58 (C-22), 174.86 (C-23) [4].

***Δ*^{5}-Pregnene-3*β*,17*α*,20(*S*)-triol-20-*O*-*β*-D-canaropyranoside (4)**, colorless needles, mp 238–240°C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm, J/Hz): 5.36 (1H, br.s, H-6), 4.62 (1H, dd, J = 9.6, J = 1.6, H-1-can), 3.75 (1H, q, J = 6.8, H-20), 3.62 (1H, m, H-3), 1.33 (3H, d, J = 6.0, H-6-can), 1.31 (3H, d, J = 6.8, H-21), 0.98 (3H, s, H-19), 0.73 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 37.7 (C-1), 31.9 (C-2), 71.9 (C-3), 42.9 (C-4), 140.8 (C-5), 121.4 (C-6), 31.9 (C-7), 31.7 (C-8),

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50.2 (C-9), 36.6 (C-10), 20.8 (C-11), 39.6 (C-12), 45.5 (C-13), 51.1 (C-14), 23.6 (C-15), 31.1 (C-16), 85.3 (C-17), 14.4 (C-18), 19.5 (C-19), 82.8 (C-20), 18.3 (C-21), 101.8 (C-1-can), 39.1 (C-2-can), 71.4 (C-3-can), 77.8 (C-4-can), 71.5 (C-5-can), 17.5 (C-6-can) [5].

All four compounds are isolated for the first time from the stem bark of *Periploca sepium*.

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