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CHEMICAL CONSTITUENTS FROM *Verbena officinalis*

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Verbena officinalis L. is a member of the Verbenaceae family, which is widely distributed in all temperate regions of the world. This plant has been used in folk medicine extensively in China for its diuretic, expectorant, and anti-rheumatic activities [1]. Previous phytochemical studies on this plant revealed the presence of triterpenes [2–4], flavonoids [5], iridoids [6, 7], and phenylpropanoids [8]. During the course of our continuing search for biologically active compounds from *V. officinalis* L., 21 compounds were isolated and identified. Seven compounds were obtained from this plant for the first time.

The stems and barks of *V. officinalis* were collected in Huaxi Gaopoxiang, Guizhou Province in China, in July, 2007. The plant material was identified by Prof. Huang Baokang and Zheng Hanchen, Department of Phytochemistry, Second Military Medical University. The voucher specimen is deposited at the School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China.

Air-dried stems and barks of *V. officinalis* (24 kg) were extracted with 70% ethanol three times at room temperature. The ethanol extract was concentrated and then the concentrated solution was dissolved in water to form a suspension. The subsequent suspension was successively partitioned with petroleum ether, dichloromethane, ethyl acetate, and *n*-butyl alcohol, yielding 304.5 g, 209.9 g, 196.2 g, and 396.4 g of extract, respectively. These fractions were subjected to a series of chromatographic techniques, such as chromatography using silica gel (mesh 200–300), and Sephadex LH-20, and HPLC, yielding compounds **1–21**, and their structures were elucidated on the basis of physicochemical and spectral analysis as 5α,8α-epidioxyergosta-6,22-dien-3 β-ol (**1**) [9], 3α,24-dihydroxy-urs-12-en-28-oic acid (**2**) [3], 3α,24-dihydroxy-olean-12-en-28-oic acid (**3**) [3], barbinervic acid (**4**) [10], oleanoic acid (**5**) [2], ursolic acid (**6**) [2], β-sitosterol (**7**) [2], daucosterol (**8**) [11], apigenin (**9**) [5], luteolin (**10**) [5], castanoside (**11**) [12], verbenalin (**12**) [6], hastatoside (**13**) [7], 3,4-dihydroverbenal (**14**) [6], martinoside (**15**) [13], acteoside (**16**) [7], isoverbascoside (**17**) [7], cistanoside E (**18**) [14], 1-hydroxyltetracontane (**19**) [15], tridecane (**20**) [15], and 4-dodecyl-1,2-benzenediol (**21**) [15].

5α,8α-Epidioxyergosta-6,22-dien-3β-ol (1): $C_{28}H_{44}O_3$, white needle crystal, mp 186–187°C; ESI-MS *m/z* 451.4 $[M + Na]^+$. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.81 (3H, d, J = 7.0, H-26), 0.83 (3H, d, J = 7.0, H-27), 0.84 (3H, s, H-19), 0.88 (3H, s, H-18), 0.90 (3H, d, J = 7.0, H-28), 1.00 (3H, d, J = 6.6, H-21), 3.98 (1H, m, H-3), 5.14 (1H, dd, J = 15.6, 8.3, H-23), 5.22 (1H, dd, J = 15.6, 7.8, H-22), 6.24 (1H, d, J = 8.5, H-6), 6.50 (1H, d, J = 8.5, H-7). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 34.7 (C-1), 30.0 (C-2), 66.4 (C-3), 36.9 (C-4), 82.1 (C-5), 135.2 (C-6), 130.7 (C-7), 79.4 (C-8), 51.7 (C-9), 36.9 (C-10), 23.4 (C-11), 39.3 (C-12), 44.5 (C-13), 51.1 (C-14), 20.6 (C-15), 28.6 (C-16), 56.2 (C-17), 12.8 (C-18), 18.1 (C-19), 39.7 (C-20), 19.6 (C-21), 135.4 (C-22), 132.3 (C-23), 42.7 (C-24), 38.0 (C-25), 17.5 (C-26), 20.8 (C-27), 19.9 (C-28) [9].

Barbinervic acid (6): $C_{30}H_{48}O_5$, white needle crystal, mp 278–280°C. EIS-MS *m/z* 511.5 $[M + Na]^+$. ^{1}H NMR (500 MHz, DMSO-d_6 + $CDCl_3$, δ, ppm, J/Hz): 0.72 (3H, s, H-25), 0.86 (3H, s, H-26), 0.88 (1H, d, J = 5.0, H-30), 0.97 (3H, s, H-23), 1.13 (3H, s, H-29), 1.37 (3H, s, H-27), 2.56 (1H, s, H-18), 3.30 (1H, d, J = 11.0, H-24a), 3.55 (1H, d, J = 11.0, H-24b), 3.70 (1H, s, H-3), 5.23 (1H, s, H-12). ^{13}C NMR (125 MHz, DMSO-d_6 + $CDCl_3$, δ): 32.8 (C-1), 25.0 (C-2), 68.5 (C-3), 42.2 (C-4), 46.4 (C-5), 18.0 (C-6), 32.6 (C-7), 39.8 (C-8), 48.7 (C-9), 36.3 (C-10), 23.1 (C-11), 127.0 (C-12), 138.2 (C-13), 40.9 (C-14), 28.9 (C-15), 27.8 (C-16), 46.8 (C-17), 53.0 (C-18), 71.6 (C-19), 41.1 (C-20), 25.7 (C-21), 37.1 (C-22), 22.1 (C-23), 64.3 (C-24), 15.1 (C-25), 16.2 (C-26), 23.8 (C-27), 179.8 (C-28), 26.3 (C-29), 15.9 (C-30) [10].

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Castanoside A (11): $C_{30}H_{26}O_{12}$, yellow powder, mp 247–249°C; ESI-MS *m/z* 617.2 $[M + Na]^+$. ^1H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 4.03 (1H, dd, J = 11.5, 6.5, H-6″a), 4.27 (1H, d, J = 11.5, H-6″b), 5.44 (1H, d, J = 5.0, H-1″), 6.09 (1H, d, J = 16.0, H-8‴), 6.15 (1H, d, J = 2.0, H-6), 6.38 (1H, d, J = 2.0, H-8), 6.78 (2H, d, J = 8.5, H-3‴, 5‴), 6.85 (2H, d, J = 8.5, H-3′, 5′), 7.33 (1H, d, J = 16.0, H-7‴), 7.36 (2H, d, J = 8.5, H-2‴, 6‴), 8.00 (2H, d, J = 8.5, H-2′, 6′). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 156.4 (C-2), 133.0 (C-3), 177.4 (C-4), 161.1 (C-5), 98.7 (C-6), 164.1 (C-7), 93.6 (C-8), 156.3 (C-9), 103.8 (C-10), 120.7 (C-1′), 130.8 (C-2′), 115.7 (C-3′), 159.9 (C-4′), 115.7 (C-5′), 130.8 (C-6′), 100.9 (C-1″), 74.2 (C-2″), 74.1 (C-3″), 69.9 (C-4″), 76.2 (C-5″), 62.9 (C-6″), 124.9 (C-1‴), 130.1 (C-2‴), 115.1 (C-3‴), 159.8 (C-4‴), 115.1 (C-5‴), 130.1 (C-6‴), 144.6 (C-7‴), 113.6 (C-8‴), 166.1 (C=O) [12].

Isoverbascoside (16): $C_{29}H_{36}O_{15}$, amorphous yellow powder; ESI-MS *m/z* 647.2 $[M + Na]^+$. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 0.87 (3H, d, J = 6.1, H-6″), 2.57 (2H, t, J = 6.8, H-7β), 3.59 (1H, d, J = 8.4, H-7α), 3.84 (1H, d, J = 8.4, H-8α), 4.16 (1H, d, J = 7.9, H-1′), 4.97 (1H, s, H-1″), 6.05 (1H, d, J = 15.8, H-8‴), 6.34 (1H, d, J = 8.0, H-6), 6.45 (1H, s, H-2), 6.47 (1H, d, J = 8.0, H-5), 6.48 (1H, d, J = 8.0, H-5), 6.55 (1H, d, J = 8.2, H-5‴), 6.73 (1H, d, J = 8.2, H-6‴), 6.84 (1H, s, H-2‴), 7.37 (1H, d, J = 15.8, H-7‴). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 131.5 (C-1), 116.3 (C-2), 146.1 (C-3), 144.7 (C-4), 117.1 (C-5), 121.3 (C-6), 36.5 (C-7), 72.2 (C-8), 104.2 (C-1′), 76.2 (C-2′), 81.6 (C-3′), 70.6 (C-4′), 76.0 (C-5′), 62.4 (C-6′), 103.0 (C-1″), 70.4 (C-2″), 73.8 (C-3″), 72.3 (C-4″), 72.1 (C-5″), 18.4 (C-6″), 127.6 (C-1‴), 114.6 (C-2‴), 148.0 (C-3‴), 149.9 (C-4‴), 116.5 (C-5‴), 123.2 (C-6‴), 115.2 (C-7‴), 146.9 (C-8‴), 168.3 (C=O) [7].

Cistanoside E (18): $C_{21}H_{32}O_{12}$, amorphous yellow powder; ESI-MS *m/z* 499.3 $[M + Na]^+$. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 1.24 (3H, d, J = 6.2, H-6″), 2.81 (1H, m, H-β), 3.81 (3H, s, CH_3O-4), 4.30 (1H, d, J = 7.9, H-1′), 5.14 (1H, br.s, H-1″), 6.66 (1H, dd, J = 8.2, 1.8, H-6), 6.72 (1H, d, J = 1.8, H-2), 6.81 (1H, d, J = 8.2, H-5). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 132.7 (C-1), 112.9 (C-2), 147.6 (C-3), 145.0 (C-4), 117.1 (C-5), 121.1 (C-6), 104.3 (C-1′), 75.6 (C-2′), 84.5 (C-3′), 70.2 (C-4′), 77.8 (C-5′), 62.9 (C-6′), 102.8 (C-1″), 70.1 (C-2″), 72.4 (C-3″), 74.0 (C-4″), 72.3 (C-5″), 17.9 (C-6″), 72.0 (C-α), 36.6 (C-β), 56.6 (CH_3O-4) [14].

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