CHEMICAL CONSTITUENTS FROM *Viola yedoensis*

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The dried whole plants of *Viola yedoensis* Makino, a species Violaceae, are commonly used in Chinese medicine. *V. yedoensis* Makino is used to remove toxic heat, reduce swelling, and to treat carbuncle and boil, superficial infections, hieropyra, and venomous snake bites [1]. To date, 15 compounds have been isolated from this plant [2–4]. In this paper, we report the isolation and identification of an additional eight known compounds **1–8**; all these compounds were found in this species for the first time, while compounds **4–8** were isolated from the genus of *Viola* for the first time.

Powdered of dry plants (22 kg) were sequentially extracted overnight with petroleum ether, ethyl acetate, and 95% ethanol at room temperature (each solvent for three times; 5 mL of solvent was added per gram of dry plant weight). After removal of the solvent, three condensed extracts, including 262 g of the petroleum ether fraction, 679 g of the ethyl acetate fraction, and 972 g 95% of the ethanol fraction were obtained. According to our previous activity screening [5], the petroleum ether fraction and the ethyl acetate fraction had the strongest antimicrobial activity. Since the petroleum ether fraction has been studied before, the ethyl acetate fraction became the focus of this study. The condensed ethyl acetate fraction (265g) was separated repeatedly by silica gel chromatography with gradient elution by petroleum ether, ethyl acetate, or acetone, and over Sephadex LH-20, eluted by $CHCl_3$–MeOH (1:1) or MeOH. All fractions obtained were examined by thin layer chromatography (TLC); similar fractions were combined to produce compounds **1–8**.

Compound 1, white needle crystals ($CHCl_3$), mp 126–128°C. EI-MS, *m/z*: 414 [M^+], 396, 381, 303, 273, 255, 213, 107.

These data are in good agreement with those reported in the literature for β-sitostero1 [6].

The TLC R_f values of compound **1** agreed with that of the reference compound β-sitosterol (**1**) when developed in different solvent systems together on the same plate.

Compound 2, $C_9H_6O_4$, yellow needle crystals (MeOH), mp 258–260°C. It has strong blue fluorescence under ultraviolet light. EI-MS, *m/z*: 178 [M^+], 150, 121, 79, 69.

^{1}H NMR spectrum (600 MHz, CD_3OD, δ, ppm, J/Hz): 6.17 (1H, d, J = 9.6, H-3), 7.77 (1H, d, J = 9.6, H-4), 6.93 (1H, s, H-5), 6.74 (1H, s, H-8).

The spectral data agreed with those reported in the literature for esculetin [7].

Compound 3, $C_{10}H_8O_4$, yellow needle crystals (MeOH), mp 116–118°C. It has strong blue fluorescence under ultraviolet light. EI-MS, *m/z*: 192 [M^+], 177, 164, 149, 121, 79, 69.

^{1}H NMR spectrum (600 MHz, CD_3OD, δ, ppm, J/Hz): 3.90 (3H, s, OCH_3), 6.20 (1H, d, J = 9.6, H-3), 7.85 (1H, d, J = 9.6, H-4), 7.11 (1H, s, H-5), 6.77 (1H, s, H-8).

^{13}C NMR spectrum (150 MHz, CD_3OD, δ, ppm): 56.8 (C-6-OCH_3), 164.0 (C-2), 152.9 (C-7), 151.4 (C-9), 147.1 (C-6), 146.1 (C-4), 112.6 (C-10), 103.9 (C-8), 109.9 (C-5).

The spectral data agreed with those reported in the literature for scopoletin [7].

Compound 4, $C_{10}H_8O_4$, yellow needle crystals (MeOH), mp 154–156°C. It has strong blue fluorescence under ultraviolet light. EI-MS, *m/z*: 192 [M^+], 177, 164, 149, 121, 79, 69.

^{1}H NMR spectrum (600 MHz, CD_3OD, δ, ppm, J/Hz): 3.90 (3H, s, OCH_3), 6.20 (1H, d, J = 9.6, H-3), 7.85 (1H, d, J = 9.6, H-4), 6.97 (1H, s, H-5), 6.96 (1H, s, H-8).

The spectral data agreed with those reported in the literature for isoscopoletin [8].

Compound 5, $C_{32}H_{64}O_2$, white powder (petroleum ether–ethyl acetate), mp 66–68°C. IR spectrum (KBr, ν_{max}, cm^{-1}): 3400–3800 (br, COOH), 2917, 2849, 1708 (COO), 1472, 1463, 729, 719.

EI-MS, *m/z*: 480 [M^+], 452, 438, 424, 410, 396, 382, 368, 353, 297, 241, 185, 129, 111, 9, 83, 73, 57.

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The spectral data agreed with those reported in the literature for lacceroic acid [9, 10].

Compound 6, $C_{30}H_{48}O_3$, white crystals ($CHCl_3$–MeOH), mp 260–262°C. EI-MS, *m/z*: 456 [M^+], 438, 411, 248, 203, 189, 175.

^{1}H NMR (δ): 5.22 and ^{13}C NMR (δ): 139.6 (C-13), 126.9 (C-12); these are the typical signals of urs-12-ene type triterpenoids. Signal δ 3.14 (1H, m) is H-3α.

^{13}C NMR spectrum (150 MHz, CD_3OD, δ, ppm): 36.3 (C-1), 26.4 (C-2), 79.7 (C-3), 35.3 (C-4), 56.1 (C-5), 18.7 (C-6), 32.8 (C-7), 38.2 (C-8), 43.2 (C-9), 31.6 (C-10), 24.3 (C-11), 126.9 (C-12), 139.6 (C-13), 40.4 (C-14), 27.9 (C-15), 25.3 (C-16), 44.3 (C-17), 54.4 (C-18), 37.3 (C-19), 34.0 (C-20), 31.0 (C-21), 32.6 (C-22), 29.1 (C-23), 16.2 (C-24), 17.7 (C-25), 19.4 (C-26), 21.6 (C-27), 181.6 (C-28), 19.9 (C-29), 20.8 (C-30). The spectral data agreed with those reported in the literature for ursolic acid [11].

The TLC R_f value of compound **6** agreed with the reference compound ursolic acid.

Compound 7, $C_7H_6O_4$, colorless needle crystals (MeOH), mp 172–174°C. EI-MS, *m/z*: 154 [M^+], 137, 109, 97, 92, 81, 63.

^{1}H NMR spectrum (600 MHz, CD_3OD, δ, ppm, J/Hz): 6.78 (1H, d, J = 8.4, H-5), 7.40 (1H, d, J = 1.8, 8.4, H-6), 7.42 (1H, dd, J = 8.4, H-2).

^{13}C NMR spectrum (150 MHz, CD_3OD, δ, ppm): 170.3 (C-7), 151.5 (C-4), 146.0 (C-3), 123.9 (C-6), 123.3 (C-1), 117.7 (C-2), 115.7 (C-5).

The spectral data agreed with those reported in the literature for protocatechuic acid [12].

Compound 8, white needle crystals ($CHCl_3$), mp 168–174°C. EI-MS, *m/z*: 444 [M^+], 384, 353, 293, 252, 224, 176, 131, 105, 91, 77.

^{1}H NMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.03 (3H, s, CH_3-CO), 3.81 (dd, J = 11.4, 4.2, H-1), 3.93 (dd, J = 11.4, 4.8, H-1), 4.34 (1H, m, H-2), 2.75 (2H, m, H-3), 4.76 (1H, m, H-2′), 3.05 (dd, J = 13.8, 8.4, H-3′), 3.22 (dd, J = 13.2, 6.0, H-3′), 5.94 (d, J = 8.4, CH-CO-NH-CH), 6.74 (d, J = 7.2, benzoyl-NH). Benzyl: 7.07 (2H, d, J = 7.2), 7.15 (2H, m), 7.17 (1H, m). Another benzyl: 7.22 (2H,m), 7.27 (2H, m), 7.29 (1H, m). Benzoyl: 7.72 (2H, d, J = 7.8), 7.44 (2H, t, J = 7.8), 7.52 (1H, t, J = 7.2).

^{13}C NMR spectrum (150 MHz, CD_3OD, δ, ppm): 64.5 (C-1), 49.4 (C-2), 37.4 (C-3), 170.2 (C-1′), 55.0 (C-2′), 38.4 (C-3′), 167.1 (CO), 20.8 (CH_3CO), 170.7 (CH_3CO). Benzyl: 136.7, 129.1, 128.6, 126.7. Another benzyl: 136.6, 128.7, 129.3, 127.1. Benzoyl: 136.6, 127.0, 128.6, 131.9.

The spectral data agreed with those reported in the literature for *N*-benzoyl-L-phenylalanyl-L-phenylalaniol acetate (aurantiamide acetate) [13].

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