CHEMICAL COMPOSITION OF THE LEAVES OF *Periploca sepium*

Ma Yang-Min,[1*] Wang Pei,[1] Chen Ling,[2] and Feng Cheng-Liang[1]

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The genus *Periploca* (Asclepiadaceae) contains 12 species, of which there are four species in China. *Periploca sepium* Bage, widely distributed in the northeast, southeast, northwest, and southwest of China, is one of the four species. The root of *Periploca sepium* Bage has been used as a traditional Chinese herbal medicine for more than two thousand years [1]. Previous phytochemical studies of this species have isolated, from the root of *P. sepium*, cardenoides, pregnane glycosides, etc. [2, 3]. For many years, research on the plant has mainly concentrated on the underground part. To continue searching for novel agents from *P. sepium*, the leaves of *P. sepium* were chosen for phytochemical investigation. In this paper, we report the isolation and identification of four compounds.

The leaves of *P. sepium* Bage were collected from Qinling Mountain, Shaanxi, China, in July, 2006 and identified by Prof. E. X. Han of Northwest A & F University. The voucher specimen was deposited in the Forestry College, Northwest A & F University.

Air-dried and powdered leaf of *P. sepium* Bage (3.9 kg) 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 extracted with petroleum ether, ethyl acetate, and *n*-butanol.

The ethyl acetate extract (47.0 g) was subjected to repeated silica gel column chromatography with Sephadex LH-20 using different percentages of chloroform–methanol mixtures, and compounds **1** and **2** were obtained.

Repeated chromatography of the *n*-butanol extract (47.3 g) on silica gel with chloroform–methanol and Sephadex LH-20 afforded compounds **3** and **4**.

The structures of these compounds were elucidated by a combination of spectral analysis and were determined as oleanolic acid (**1**) [4], quercetin (**2**) [5], 6′-methyl ester of quercetin-3-*O*-*β*-D-glucuronide (**3**) [6], and isoquercitrin (**4**) [7] by comparison with the data in the literature. All these four compounds were isolated for the first time from *P. sepium,* and compound **2** was isolated for the first time from the genus of *Periploca*.

Oleanolic acid (1) white powder, mp > 300°C. IR (KBr, ν_{max}, cm^{-1}): 3446 (OH), 2941, 2861, 1693 (C=O), 1636 (C=C), 1462, 1385, 1274, 1084, 1036, 749. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 0.74 (3H, s, CH_3, H-26), 0.77 (3H, s, CH_3, H-23), 0.90 (3H, s, CH_3, H-24), 0.91 (3H, s, CH_3, H-29), 0.92 (3H, s, CH_3, H-30), 0.98 (3H, s, CH_3, H-25), 1.13 (3H, s, CH_3, H-27), 5.28 (1H, br.s, H-12), 3.24 (1H, br.s, H-3), 2.81 (1H, br.s, H-18).

Quercetin (2) yellow needle crystals, mp > 300°C. UV (CH_3OH, λ_{max}, nm): 381.0, 257.0, (CH_3OH + NaOH, λ_{max}, nm): 419.0, 330.0, 280.0, (CH_3OH + $AlCl_3$, λ_{max} nm): 448.0, 271.0. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.19 (1H, d, J = 1.8, H-6), 6.41 (1H, d, J = 1.8, H-8), 6.88 (1H, d, J = 8.4, H-5′), 7.54 (1H, dd, J = 2.0, 8.4, H-6′), 7.68 (1H, d, J = 2.0, H-2′), 9.34 (1H, s, 3′-OH), 9.40 (1H, s, 3-OH), 9.62 (1H, s, 4′-OH), 10.80 (1H, s, 7-OH), 12.51 (1H, s, 5-OH). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 147.24 (C-2), 136.19 (C-3), 176.29 (C-4), 161.17 (C-5), 98.63 (C-6), 164.34 (C-7), 93.79 (C-8), 156.58 (C-9), 103.46 (C-10), 122.40 (C-1′), 115.51 (C-2′), 145.51 (C-3′), 148.15 (C-4′), 116.05 (C-5′), 120.42 (C-6′).

6′-Methyl ester of quercetin-3-*O*-*β*-D-glucuronide (3) yellow needle crystals, mp 181–183°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.21 (1H, d, J = 1.1, H-6), 6.41 (1H, d, J = 1.1, H-8), 6.83 (1H, d, J = 8.4, H-5′), 7.51 (1H, d, J = 2.5, H-2′), 7.56 (1H, dd, J = 2.5, 8.4, H-6′), 5.46 (IH, d, J = 6.9, H-1″), 3.56–3.73 (m, sugar protons), 3.57 (3H, s, COO$\underline{CH_3}$).

1) Key Laboratory of Auxiliary Chemistry & Technology for Chemical Industry, Ministry of Education, College of Chemistry and Chemical Engineering, Shaanxi University of Science and Technology, Xi′an, 710021, P. R. China, e-mail: mym63@sina.com; 2) College of Forestry, Northwest A & F University, Yangling, 712100, P. R. China. Published in Khimiya Prirodnykh Soedinenii, No. 3, p. 391, May–June, 2010. Original article submitted December 25, 2008.

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^{13}C NMR (75 MHz, DMSO-d_6, δ): 156.77 (C-2), 133.62 (C-3), 177.59 (C-4), 161.67 (C-5), 99.27 (C-6), 164.79 (C-7), 94.07 (C-8), 156.91 (C-9), 104.36 (C-10), 121.36 (C-1′), 115.63 (C-2′), 145.37 (C-3′), 149.10 (C-4′), 116.57 (C-5′), 122.14 (C-6′), 101.88 (C-1″), 74.23 (C-2″), 76.15 (C-3″), 71.85 (C-4″), 76.15 (C-5″), 169.35 (C$\underline{\text{C}}$OOCH$_3$), 52.29 (COO$\underline{\text{CH}}_3$).

Isoquercitrin (4) yellow needle crystal, mp 233–235°C. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.20 (1H, d, J = 1.7, H-6), 6.41 (1H, d, J = 1.7, H-8), 6.83 (1H, d, J = 8.9, H-5′), 7.57 (1H, d, J = 1.9, H-2′), 7.58 (1H, dd, J = 1.9, 8.9, H-6′), 5.46 (1H, d, J = 7.1, H-1″), 3.10–3.61 (m, sugar protons). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 156.68 (C-2), 133.86 (C-3), 177.93 (C-4), 161.73 (C-5), 99.15 (C-6), 164.62 (C-7), 93.98 (C-8), 156.81 (C-9), 104.46 (C-10), 121.68 (C-1′), 115.69 (C-2′), 145.28 (C-3′), 148.93 (C-4′), 116.72 (C-5′), 122.07 (C-6′), 101.45 (C-1″), 74.59 (C-2″), 77.02 (C-3″), 70.46 (C-4″), 78.00 (C-5″), 61.49 (C-6″).

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