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Chemical constituents of *Prunella vulgaris*

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Abstract

Nine compounds were isolated from the spikes of *Prunella vulgaris* by various kinds of chromatography. Their structures were established on the basis of spectral analysis as polygalacerebroside (**1**), ursolic acid (**2**), β -amyirin (**3**), quercetin (**4**), quercetin-3-O- β -D-galactoside (**5**), α -spinasterol (**6**), stigmasterol (**7**), β -sitosterol (**8**), daucosterol (**9**). Compound **1** was isolated from this genus for the first time. Phytochemical investigation on the spikes of *P. vulgaris* provided chemical constituents diversity, which were performed to facilitate further development and utilization of *P. vulgaris* pharmaceutical resource.

Key words: *Prunella vulgaris*; chemical constituents

Introduction

Prunella vulgaris L. (Labiatae) is widely distributed in the temperate zone and tropical mountains of Europe and Asia. It is a botanical source for various pharmaceutically active components, and their treatments of hypotensive, hypoglycemic, antibacterial, antiviral, anti-inflammatory, and antitumor activities have been corroborated by long term use in traditional Chinese medicine (Jiangsu College of New Medicine, 2004). Previous phytochemical studies have revealed that *P. vulgaris* mainly contains triterpenoid glycosides (Gu et al., 2008) and phenolic compounds (Gu et al., 2011). Increasing interest in *P. vulgaris* has led to further discoveries of many other kinds of compounds. In our continuous search for chemical constituents, we isolated nine known compounds, polygalacerebroside (**1**), ursolic acid (**2**), β -amyirin (**3**), quercetin (**4**), quercetin-3-O- β -D-galactoside (**5**), α -spinasterol (**6**), stigmasterol (**7**), β -sitosterol (**8**), and daucosterol (**9**), from the spikes of *P. vulgaris* (Fig. 1). Compound **1** was described for the first time from this genus. In the present paper, the isolation and structural elucidation of **1–9** are reported.

1 Experimental

1.1 General procedures

Melting point was determined on an XT4-A micro-melting point apparatus and was uncorrected. IR (infrared) spectra were measured on FT-IR-8900 spectrometer (Shi-

madzu, Japan). NMR spectra were recorded on a AV-500 spectrometer (Bruker Co., USA) with TMS as internal standard. Mass spectra were obtained on a Wiff Agilent time-of-flight mass (TOF-MS) and Micromass Qattro spectrometer (HR) (Agilent Co., USA) with an ESI source. Silica gel (200–300 mesh) for CC (column chromatography) and GF254 for TLC (thin layer chromatography) were produced by Qingdao Marine Chemical Group Co., China. Sephadex was produced by Merck Co., Germany. Macroporous resin D101 was supplied by the Shanghai Resin Factory of China.

1.2 Plant materials

The spikes of *P. vulgaris* were collected in Xuyi County, Jiangsu Province, China, and identified by Prof. Shihui Qian. A voucher specimen was deposited in the Department of Natural Product Chemistry, Jiangsu Provincial Institute of Traditional Chinese Medicine.

1.3 Extraction and isolation

Dried spikes (35 kg) of *P. vulgaris* were extracted two times with 50% ethanol at room temperature. The extract was concentrated to give the residue (4.4 kg), which was partitioned sequentially with petroleum ether, CHCl_3 and *n*-BuOH. The CHCl_3 extract was evaporated under reduced pressure to give a brown residue (120.0 g). The residue was subjected to a silica gel CC and eluted with CHCl_3 -MeOH of increasing polarity (100:0, 100:1, 50:1, 25:1, 15:1, 10:1, V/V) to provide eight fractions according to TLC detection on silica gel plates. Fraction 2 (60 g) was separated by CC on silica gel, eluting with petroleum ether-EtOAc (95:5, 90:10, 80:20, 70:30, V/V) to give compound

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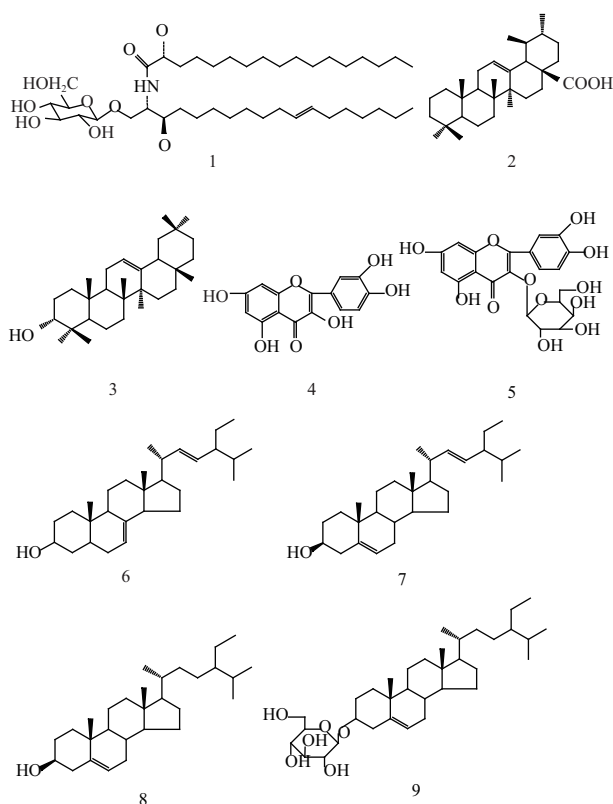


Fig. 1 Structures of compounds 1–9. (1) polygalacerebroside, (2) ursolic acid, (3) β -amyrin, (4) quercetin, (5) quercetin-3-O- β -D-galactoside, (6) α -spinasterol, (7) stigmasterol, (8) β -sitosterol, (9) daucosterol.

2 (323 mg) and compound 3 (235 mg) at gradient 95:5 (V/V), compound 6 (65 mg), compound 7 (148 mg) and compound 8 (881 mg) at gradient 90:10 (V/V). Fraction 3 (30 g) was purified by CC on silica gel, eluting with CHCl_3 -MeOH of increasing polarity, to afford compound 4 (57 mg) at gradient 100:1 (V/V) and compound 9 (2 g) at gradient 50:1 (V/V), respectively. Compound 1 (100 mg) was isolated from fraction 4 (14 g) by Sephadex LH-20 column and elution with MeOH.

The *n*-BuOH extract was evaporated under reduced pressure to give a brown residue (1.2 kg). The residue was subjected to CC on macroporous resin D101 and eluted with 50% ethanol. Then, 50% fraction (380 g) was applied to a silica gel column and eluted with CHCl_3 -MeOH of increasing polarity to provide five fractions. Fraction 4 (36 g) was further chromatographed over a silica gel column, eluting with CHCl_3 -MeOH (80:20, V/V) to yield compound 5 (21 mg).

2 Results and discussion

Compound 1: white amorphous powder, mp. (melting point) 215–216°C; IR (KBr, cm^{-1}): 3380, 1645, 1540, 1080, 1030, 720; TOF-MS m/z (mass to charge ratio): 754.5454 $[\text{M}+\text{Na}]^+$. $^1\text{H-NMR}$ (Py- d_6) δ : 0.87 (3H, t, $J = 7.0$ Hz, H-16'), 0.88 (3H, t, $J = 7.0$ Hz, H-18), 1.25 (CH_2) $_n$, 2.01 (4H, H-10, 13), 3.85 (1H, m, H-5), 3.99 (1H,

m, H-2), 4.17 (2H, H-4, 3), 4.19 (1H, m, H-4), 4.28 (1H, m, H-3), 4.32 (1H, dd, $J = 6.0, 12.0$ Hz, H-6), 4.47 (1H, dd, $J = 2.0, 12.0$ Hz, H-6), 4.51 (1H, dd, $J = 6.0, 11.0$ Hz, H-1), 4.57 (1H, dd, $J = 3.0, 7.0$ Hz, H-2'), 4.70 (1H, dd, $J = 7.0, 11.0$ Hz, H-1), 4.94 (1H, d, $J = 8.0$ Hz, H-1), 5.27 (1H, m, H-2), 5.48 (1H, dt, $J = 6.0, 15.0$ Hz, H-11), 5.54 (1H, dt, $J = 6.0, 15.0$ Hz, H-12), 8.55 (1H, d, $J = 9.0$ Hz, NH); $^{13}\text{C-NMR}$ (Py- d_6) δ : 13.9 (C-18), 13.9 (C-16'), 32.6 (C-13), 51.4 (C-2), 62.3 (C-6), 70.1 (C-1), 71.1 (C-4), 72.1 (C-2'), 72.1 (C-4), 74.8 (C-2), 75.5 (C-3), 78.1 (C-3), 78.2 (C-5), 105.2 (C-1), 130.3 (C-12), 130.5 (C-11), 175.3 (C-1'). The detailed NMR and MS data analysis and comparison with reference data (Zhang et al., 2006) indicated that this compound was polygalacerebroside.

Compound 2: white amorphous powder, mp. 280–281°C; IR (KBr, cm^{-1}): ν_{max} : 3425, 2923, 2865, 1685, 1451, 1028, 996; ESI-MS m/z : 303.3 $[\text{M}+\text{H}]^+$; $^1\text{H-NMR}$ (DMSO- d_6) δ : 0.76, 0.81, 0.92, 0.96, 1.10 ($5 \times \text{CH}_3$, each s), 0.86 ($2 \times \text{CH}_3$, each d, $J = 6.6$ Hz), 3.27 (1H, br.s, H-3), 5.20 (1H, m, H-12). On comparing the spectral data with those in the literature (Wang et al., 1999), this compound was determined as ursolic acid.

Compound 3: white amorphous powder, mp. 197–198°C; IR (KBr, cm^{-1}): ν_{max} : 3400, 2920, 2840, 1460, 1380, 1358, 1040; ESI-MS m/z : 449.3 $[\text{M}+\text{Na}]^+$; $^1\text{H-NMR}$ (Py- d_6) δ : 0.78, 0.82, 0.85, 0.92, 0.96, 0.98, 1.23, 1.23 (3H, s, CH_3), 3.26 (1H, m, H-3), 5.24 (1H, m, H-12). The compound was confirmed by comparison of its NMR and MS data with literature values (Mabate and Kundu, 1994). Thus, its structure was identified as β -amyrin.

Compound 4: yellow amorphous powder, mp. 308–310°C; ESI-MS m/z : 301.2 $[\text{M}-\text{H}]^-$; $^1\text{H-NMR}$ (CD_3OD , 500 MHz) δ : 7.72 (1H, d, $J = 2.0$ Hz, H-2'), 7.61 (1H, dd, $J = 2.0, 8.5$ Hz, H-6'), 6.88 (1H, d, $J = 8.5$ Hz, H-5'), 6.38 (1H, d, $J = 2.0$ Hz, H-8), 6.17 (1H, d, $J = 2.0$ Hz, H-6); $^{13}\text{C-NMR}$ (CD_3OD , 125 MHz) δ : 148.5 (C-2), 137.7 (C-3), 177.8 (C-4), 163.0 (C-5), 99.7 (C-6), 166.1 (C-7), 94.0 (C-8), 158.7 (C-9), 105.0 (C-10), 124.7 (C-1'), 116.2 (C-2'), 146.7 (C-3'), 1493 (C-4'), 116.7 (C-5'), 122.1 (C-6'). Comparing the data and features of the ^1H - and ^{13}C -NMR spectra with the known compound (Wang et al., 2008), a structure based on quercetin was inferred.

Compound 5: yellow needle-like crystal, mp. 284–286°C; ESI-MS m/z : 487.2 $[\text{M}+\text{Na}]^+$; $^1\text{H-NMR}$ (DMSO- d_6) δ : 6.19 (1H, br.s, H-6), 6.40 (1H, br.s, H-8), 7.52 (1H, br.s, H-2'), 6.81 (1H, d, $J = 8.3$ Hz, H-5'), 7.66 (1H, d, $J = 8.3$ Hz, H-6'), 5.36 (1H, d, $J = 7.6$ Hz, H'-Gal); $^{13}\text{C-NMR}$ (DMSO- d_6) δ : 156.3 (C-2), 133.8 (C-3), 177.5 (C-4), 161.2 (C-5), 98.6 (C-6), 164.0 (C-7), 93.4 (C-8), 156.3 (C-9), 104.0 (C-10), 121.3 (C-1'), 115.2 (C-2'), 144.7 (C-3'), 148.3 (C-4'), 116.2 (C-5'), 121.7 (C-6'), 102.3 (C-1''), 71.3 (C-2''), 73.4 (C-3''), 68.0 (C-4''), 75.8 (C-5''), 60.8 (C-6''). This compound was identified as quercetin-3-O- β -D-galactoside by comparison of data with the reported in the literature (Markham et al., 1978).

Compound **6**: white plate crystal, mp. 160–162°C; ESI-MS m/z : 413.1 $[M+H]^+$. 1H -NMR ($CDCl_3$, 500 MHz) δ : 5.0–5.2 (3H, m, H-7, H-22, H-23), 3.59 (1H, m, H-3), 2.16 (1H, s, –OH), 1.02 (3H, d, $J = 6$ Hz, H-19), 0.80–0.55 (3H \times 4, –CH₃). On comparing NMR and ESI-MS data with literature values (Kojima et al., 1990), the compound was identified as α -spinasterol by co-TLC with reference substance.

Compound **7**: white plate crystal, mp. 168–171°C; ESI-MS m/z : 413.3 $[M+H]^+$. 1H -NMR (500 MHz, $CDCl_3$) δ : 0.71 (3H, s, CH₃), 0.79 (3H, s, CH₃), 0.81 (3H, s, CH₃), 0.84 (3H, s, CH₃), 1.00 (3H, s, CH₃), 1.02 (3H, s, CH₃), 3.48 (1H, m, H-23), 5.03 (1H, m, H-23), 5.15 (1H, m, H-22), 5.32 (1H, m, H-6). The compound was characterized by detailed 1H -NMR analyses to be α -spinasterol (Ren et al., 2008).

Compound **8**: white plate crystal, mp. 288–289°C; showed positive Lieberman-Burchard reaction. It was elucidated as β -sitosterol by TLC comparison with authentic sample.

Compound **9**: white plate crystal, mp. 136–138°C; showed positive Lieberman-Burchard and Molish reactions. It was determined to daucosterol by TLC comparison with authentic sample.

3 Conclusions

In continuation with our studies on *P. vulgaris* chemistry, increasing interest in medicinal resources has led to additional discoveries of cerebroside, flavonoids, sterols and triterpenoids. We report here on the isolation and structural elucidation of these nine compounds by chemical and spectroscopic analysis. Compound **1** was isolated from this genus for the first time. The present investigation provided pharmaceutical chemistry ingredient diversity.

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