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Anti-inflammatory activities of triterpenoid saponins from *Polygala japonica*

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Abstract

Bioassay-guided investigation was performed to identify the active constituents from a methanol extract of *Polygala japonica*, a folk medicinal plant widely used in China to treat inflammatory diseases. The *n*-BuOH and EtOAc fractions of the *P. japonica* methanol extract, which show significant anti-inflammatory activity in *in vivo* test, were further subjected to column chromatography to afford six triterpene glycosides, marked here as saponins **1–6**. All compounds were evaluated for their anti-inflammatory activity in the carageenan-induced mouse paw edema test, and saponins **1, 4** and **5** showed significantly anti-inflammatory effects on both phases of carageenan-induced acute paw edema in mice. Saponin **5** was also found to significantly inhibit the production of inflammatory mediators – nitric oxide (NO) in LPS-stimulated RAW264.7 macrophages, with no obvious effects on macrophage viability.

Keywords: *Polygala japonica*; Polygalaceae; Triterpenoid saponins; Anti-inflammatory activity

Introduction

Polygala japonica Houtt (Polygalaceae, Gua-zi-jin in Chinese) is widely distributed in Asia, especially in Eastern China. It has long history of using *P. japonica* in traditional medicine for the treatment of various inflammatory disorders, such as acute tonsillitis, pharyngitis, myelitis and nephritis (Liu and Li, 1998; Teng and Teng, 1994; Wan et al., 1997). Previous chemical studies have reported triterpenoid saponins, flavones isolated from *Polygala japonica* (Fang and Yin, 1989; Keun et al., 1993; Zhang et al., 1995a, b, 1996a, b; Li et al., 2006). However, no report has yet been published on the isolation and identification of anti-inflammatory bioactive chemical constituents from the plant. Our preliminary test has confirmed the anti-inflammation activity of MeOH extract of *Polygala japonica* in carageenan-induced rat paw edema. In this article, we extended our efforts to isolate and identify the bioactive compounds from MeOH extract. We report herein the anti-inflammatory activities of isolated triterpene glycosides on carageenan-induced mouse paw edema and nitric oxide (NO) production in LPS-stimulated RAW264.7 macrophages, which may support the wide utility of *Polygala japonica* in traditional medicine of China.

Materials and methods

Plant materials

The aerial part of *Polygala japonica* was purchased from Jiangsu Medical Material Company (Jiangsu,

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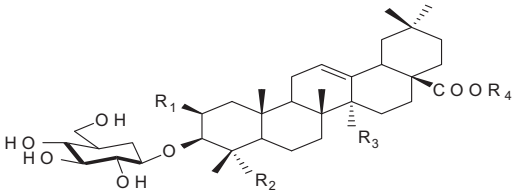
China), and was authenticated as *Polygala japonica* Houtt by Dr. Zenglai Xu (Jiangsu Zhongshan Arboretum, Nanjing, China). A voucher specimen (ZDN020518) has been deposited at the Herbarium of China Pharmaceutical University.

Extraction, isolation and identification

The dried aerial part of the plant (9 kg) was extracted with methanol for 12 h and evaporated to dryness in vacuo to yield a total (MeOH ext., 1651 g, yield 18.34%). About 95% of the total extract was suspended in water, then subjected to liquid–liquid partition by adding petroleum ether, ethyl acetate and *n*-butanol successively, yielding three fractions, i.e., a petroleum ether fraction (MSO fr., 148.0 g, yield 1.73%), an ethyl acetate fraction (EtOAc fr., 1103.6 g, yield 12.9%) and an *n*-butanol fraction (*n*-BuOH fr., 108.28 g, yield 1.27%). The residual part that suspended in water was the water residue fraction (water fr., 42.29 g, yield 0.49%). The EtOAc fraction (103.6 g) was further fractionated via silica gel CC, using a gradient of $CHCl_3$/MeOH (100/0 to 0/100). Frs. [64–75] = IV eluted with $CHCl_3$/MeOH (8/1) were purified on a reversed-phase RP18 CC using a gradient of MeOH/H_2O (8/2 to 9/1) to yield bayogenin-3-*O*-*β*-D-glucopyranoside (saponin **5**, 15 mg). Frs. [76–81] = V eluted with $CHCl_3$/MeOH (5/1) was purified on a reversed-phase RP18 column using a gradient of MeOH/H_2O (7/3 to 9/1) to yield tenuifolin (saponin **6**, 11 mg). The *n*-BuOH fraction (58.28 g) was fractionated on silica gel CC, using a gradient of $CHCl_3$/MeOH (100/4 to 0/100). Frs. [76–83] = F eluted with $CHCl_3$/MeOH (3/1) were purified on a reversed-phase RP18 column using a gradient of MeOH/H_2O (6/4 to 8/2) to yield 3-*O*-*β*-D-glucopyranosyl bayogenin 28-*O*-*β*-D-xylopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)-*β*-D-glucopyranosyl ester (saponin **1,** 25 mg) and 3-*O*-*β*-D-glucopyranosyl medicagenic acid 28-*O*-{*β*-D-xylopyranosyl (1→4)–[*β*-D-apiofuranosyl (1→3)]-α-L-rhamnopyranosyl (1→2)-*β*-D-glucopyranosyl} ester (saponin **2,** 36 mg). Frs [92–97] = J eluted with $CHCl_3$/MeOH (2/1) was purified on a reversed-phase RP18 CC using a gradient of MeOH/H_2O (5/5 to 7/3) to give 3-*O*-*β*-D-glucopyranosyl 2-oxo-olean-12-en-23,28-dioic acid 28-*O*-{*β*-D-xylopyranosyl (1→4)–[*β*-D-apiofuranosyl(1→3)]-α-L-rhamnopyranosyl (1→2)-*β*-D-glucopyranosyl} ester (saponin **3,** 27 mg) and polygalasaponin V (saponin **4,** 30 mg) (see structures in Fig. 1).

Spectroscopy and chromatography

^1H and ^{13}C-NMR spectra were recorded at 500 and 125 MHz, respectively, using an AV-500 spectrometer in C_5D_5N with TMS as internal standard. HRESI-MS was performed on an IonSpec4.7 Tesla mass spectrometer, ESI-MS and MS-MS experiments were recorded on a LC-MSD-Trap mass spectrometer. TLC was carried out on silica gel 60 F_{254}, and spots were visualized by spraying with 10%H_2SO_4 solution followed by heating. Silica gel (silica gel 60, Merck) and RP-18 (40–75 μm, Merck) were used for column chromatography. HPLC was performed on a Shimadzu apparatus equipped with a LC-10AT pump, an Alltech ELSD 500 detector and a Class VP software using carbohydrate analysis column (Cosmosil, 4.6 × 250 mm, 5 μm), 90 °C drift tube temperature, CH_3CN–H_2O (85:15) as solvent with a flow rate of 1 ml/min.



Saponin	R_1	R_2	R_3	R_4
1	OH	CH_2OH	CH_3	Glc-Rha–Xyl
2	OH	COOH	CH_3	Glc-Rha–Xyl | Api
3	=O	COOH	CH_3	Glc-Rha–Xyl | Api
4	OH	CH_2OH	CH_3	Glc-Rha–Xyl | Api
5	OH	CH_2OH	CH_3	H
6	OH	COOH	CH_2OH	H

Fig. 1. Structures of isolated triterpene glycosides **1–6**.

Chemicals

Lipopolysaccharide (LPS), carageenan, 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT), and the reference drug indomethacin were purchased from Sigma Chemical Co. (St. Louis, MO, USA); RPMI 1640 medium and Newborn bovine serum were from Gibco BRL, Life Technologies, Inc. (New York, USA); and other chemicals were from Shanghai Chemical Company (Shanghai, China).

Cell culture and sample treatment

RAW 264.7, a murine macrophage cell line, was obtained at passage 4 from Shanghai Institute of Biochemistry and Cell Biology. Cells were cultured at 37 °C in Dulbecco's Modified Eagle Medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 units/ml), and streptomycin sulfate (100 μg/ml) in a humidified atmosphere of 5% CO_2. The medium was changed about once a day and