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New ursane-type triterpenoid saponins from the stem bark of *Schefflera heptaphylla*

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ABSTRACT

Phytochemical investigation on the stem bark of *Schefflera heptaphylla* led to the isolation of five new ursane-type triterpenoid saponins (**1**–**5**). Their structures were determined on the basis of spectroscopic and chemical methods. It is noteworthy in this study that the genins of all compounds are reported for the first time. All compounds isolated from this plant were evaluated for their inhibitory activities on lipopolysaccharide-induced nitric oxide production in RAW264.7 cells, and compounds **2** and **5** showed weak anti-inflammatory activities under their non-cytotoxic concentrations.

1. Introduction

Schefflera heptaphylla (L.) Frodin (Araliaceae) is a medium-sized, evergreen tree up to 25 m tall, bole up to 80 cm in diameter. It is used as a folk remedy for the treatment of pain, inflammation, and common cold. It is also a principal ingredient of an herbal tea formulation widely used to treat common cold in southern China [1–3]. Previous phytochemical studies on *S. heptaphylla* showed that the plant is rich in triterpenoids and triterpenoid glycosides [1,4–10]. In the previous research, we had isolated and identified some triterpenoid saponins, including scheffursoside D, scheffursoside F, scheffoleoside A, scheffoleoside D, and acankoreoside A, from the stem bark of *S. heptaphylla* [5]. As part of our continuing search for bioactive constituents from *S. heptaphylla*, a 95% EtOH extract of the stem bark of *S. heptaphylla* had been investigated, and five new ursane-type triterpenoid saponins (**1**–**5**) were obtained (Fig. 1). In addition, all the new compounds were evaluated for their anti-inflammatory activities on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 cells. In this paper, we described the isolation, structural elucidation, and anti-inflammatory activities of these triterpenoid saponins.

2. Experimental

2.1. General methods

Optical rotations were carried out using a JASCO P-1030 automatic digital polarimeter. IR spectra were measured on a JASCO FT/IR-480 plus infrared spectrometer with KBr pellets. 1D and 2D NMR spectra were recorded on a Bruker AV-400 spectrometer with TMS as the internal standard, and chemical shifts were expressed in δ values (ppm). HRESIMS data were detected on an Agilent 6210 LC/MSD TOF mass spectrometer. Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), ODS silica gel (50 μm, YMC, Kyoto, Japan), and Sephadex LH-20 (Pharmacia, Uppsala, Sweden) were used for column chromatography (CC). Analytical high-performance liquid chromatography (HPLC) was carried out on a Waters

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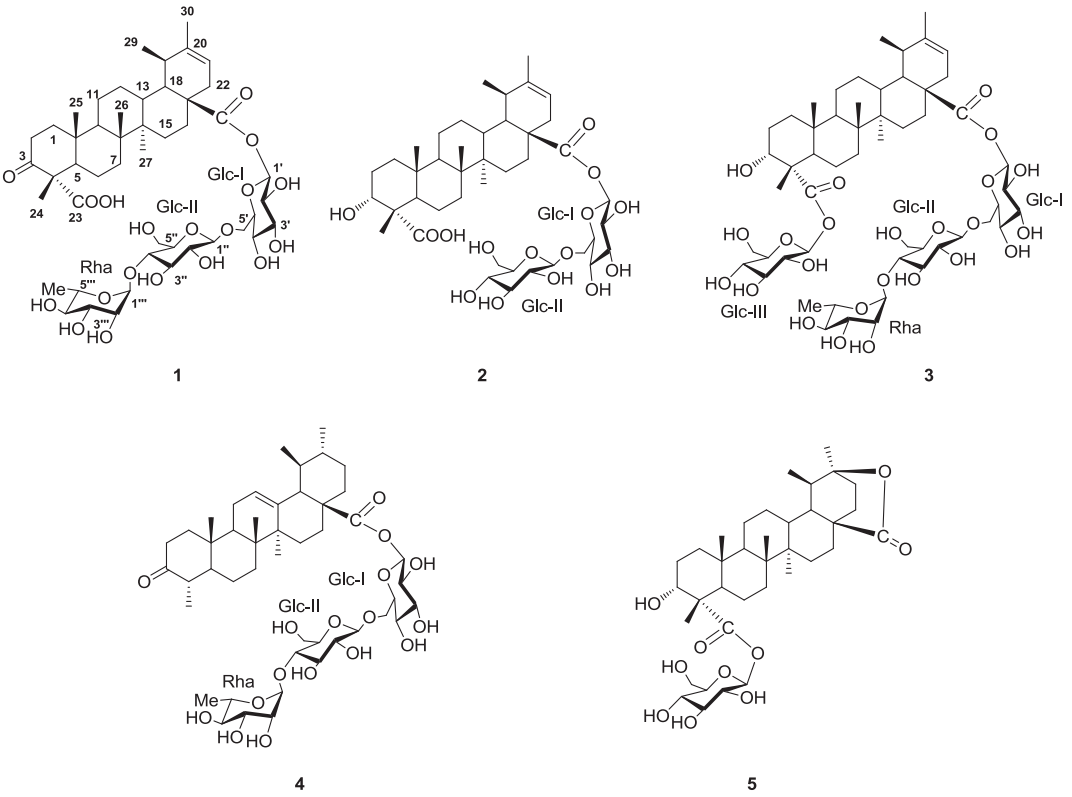

Fig. 1. Chemical structures of compounds **1**–**5**.

chromatograph equipped with an evaporative light-scattering detector, a P680 pump, and a reversed phase (RP) C_{18} column (5 μm, 4.6 mm × 250 mm, Cosmosil, Kyoto, Japan). Semi-preparative HPLC was performed on an Agilent 1200 unit with DAD detector and a RP C_{18} column (5 μm, 10 mm × 250 mm; Cosmosil, Kyoto, Japan). Preparative HPLC was carried out on a Varian chromatograph equipped with a Prostar 215 pump and a Prostar 325 UV–Vis detector with a RP C_{18} column (5 μm, 20 mm × 250 mm; Cosmosil, Kyoto, Japan). Thin-layer chromatography (TLC) was performed using pre-coated silica-gel plates (GF_{254}, Yantai Chemical Industry Research Institute, Yantai, China). All the reagents were purchased from Tianjin Damao Chemical Company (Tianjin, China). L-cysteine methyl ester and standard sugars D-glucose (D-Glc), L-glucose (L-Glc), and L-rhamnose (L-Rha) in the analysis of HPLC experiments were purchased from Adamas-beta Company (Basel, Switzerland). *O*-Tolyl isothiocyanate and dexamethasone were purchased from Sigma Company (Sigma, St. Louis, MO, USA).

2.2. Plant material

The dried stem bark of *S. heptaphylla* was collected from Yulin, Guangxi, China, in August 2008, and was authenticated by Mr Zhen-Qiu Mai, a senior herbalist at the Chinese Medicinal Material Company, Guangdong, China. A voucher specimen with accession (No. SH20090301) has been deposited in the herbarium of College of Pharmacy, Jinan University.

2.3. Extraction and isolation

The dried and powdered stem bark of *S. heptaphylla* (10 kg) was soaked in 95% EtOH at room temperature for five times. The solution was evaporated under reduced pressure to obtain an extract (1.3 kg). This extract was suspended in distilled water, and then partitioned with petroleum ether, EtOAc, and n-BuOH, respectively. The n-BuOH-soluble residue (100 g) was subjected to silica gel column and eluted with $CHCl_3$–MeOH (100:0; 90:10; 80:20; 70:30; 60:40; 50:50; 30:70; 0:100) in gradient to yield 40 fractions (Fr 1–40) based on their TLC patterns. Fr 12 (5.3 g) was separated on an ODS gel column (180 g, 3.5 cm × 40 cm) eluted with H_2O–MeOH (80:20, 70:30, 60:40, 40:60, 20:80, 0:100, each 3 L), to give 15 subfractions (Sfr 1–15). Sfr 12 (250 mg) was subjected to preparative HPLC using 70% MeOH–H_2O (7 mL/min) to give compounds **1** (7.4 mg) and **2** (5.1 mg). Sfr 10 (210 mg) was subjected to semi-preparative HPLC using 63% MeOH–H_2O (3 mL/min) to give compound **3** (11.3 mg). Sfr 6 (318 mg) was subjected to preparative HPLC using 62% MeOH–H_2O (7 mL/min) to give compounds **4** (13.4 mg) and **5** (21.9 mg).

2.3.1. 3-oxo-urs-20-en-23,28-dioic acid 28-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**1**)

Amorphous powder; $C_{48}H_{76}O_{19}$; $[\alpha]^{25}_{D}$ −2.35° (*c* 1.03, MeOH); IR (KBr) ν_{max}: 3420, 2935, 1724, 1070 cm^{-1}; HRESIMS (positive-ion mode) *m/z* 979.4880 $[M + Na]^+$ (calcd. for $C_{48}H_{76}O_{19}Na$: 979.4878); ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) data: see Tables 1 and 2.