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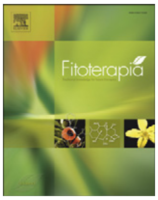

Chemical constituents of *Swertia yunnanensis* and their anti-hepatitis B virus activity

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ABSTRACT

Four new triterpenoids, sweriyunnangenin A (**1**), sweriyunnanosides A (**2**), B (**3**) and C (**4**), along with nineteen known compounds (**5**–**23**) were isolated from *Swertia yunnanensis*. Based on extensive spectroscopic analyses (1D- and 2D-NMR, HRESIMS, UV, IR, $[\alpha]_D$), the structures of sweriyunnangenin A (**1**), sweriyunnanosides A (**2**), B (**3**) and C (**4**) were elucidated as taraxer-14-ene-3α,6β-diol, oleanolic acid 28-O-β-D-glucopyranosyl-(1 → 2)-O-β-D-glucopyranoside, 2α,3β-di-hydroxyolean-12-en-28-oic acid 28-O-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranoside and hederagenin 28-O-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranoside, respectively. Twenty-two compounds were evaluated for their anti-HBV activities on the HepG 2.2.15 cell line *in vitro*, of which nine compounds showed potent anti-HBV activities. Compounds **1**, **5**–**6**, **14**–**16** and **19** showed activities against the secretion of HBsAg (IC_{50} values from 0.10 to 1.76 mM) and HBeAg (IC_{50} values from 0.04 to 1.41 mM), and compounds **11** and **13**–**16** exhibited significant inhibition on HBV DNA replication (IC_{50} values from 0.01 to 0.09 mM).

1. Introduction

The genus *Swertia* (Gentianaceae) contains about 170 species widely distributed in the world, 79 of which are found in China, and more than 30 species have been used as medicinal herbs for treating hepatitis, cholecystitis, pneumonia, osteomyelitis, dysentery and scabies in China [1–4]. The main constituents of this genus are iridoids, secoiridoids, xanthones, flavonoids, and triterpenoids, which possess hepatoprotective, antihepatotoxic, antioxidant, mutagenic, antidiabetic, anti-ulcer and antigastritic activities [4–7]. *Swertia mileensis* (Qing-ye-dan) is a famous traditional Chinese medicine (TCM) documented in Chinese Pharmacopeia for the treatment of hepatitis [8]. Our previous investigation on *S. mileensis* resulted in a series of novel skeletons with anti-hepatitis B virus (HBV) activity [9]. *Swertia yunnanensis*, the congener plant of *S. mileensis*, is also used for treating jaundice, hepatitis, and cholecystitis in Yunnan, Sichuan, and Guizhou provinces of China [2–4]. Previous phytochemical studies on *S. yunnanensis* resulted in eleven compounds, including xanthones, triterpenoids, secoiridoids, and steroids [10,11]. Our *in vitro* anti-HBV bioassay manifested that the ethanol extracts of *S. yunnanensis* showed significant inhibition on the secretion of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) with the IC_{50} values of 0.79 mg/mL (SI 2.41) and 0.34 mg/mL (SI 5.36), respectively, and on HBV DNA replication with the IC_{50} value of 0.08 mg/mL (SI 18.9). However, the active substances responsible for the anti-HBV property were unclear. In order to clarify its active constituents, extensive investigation on the ethanol extract of *S. yunnanensis* yielded four new triterpenoids (**1**–**4**) (Fig. 1),

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along with nineteen known compounds, including triterpenoids, xanthones, flavonoids, secoiridoids, steroids, nucleotide, and phenol. Herein, we described the isolation and structure elucidation of the isolates, as well as their anti-HBV activity.

2. Experimental procedure

2.1. General

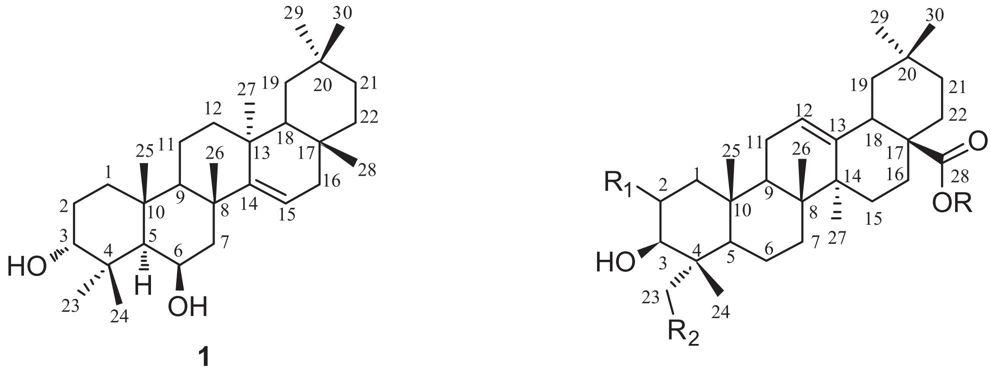
Optical rotations were obtained on a JASCO Model 1020 Digital Polarimeter (HORIBA, Tokyo, Japan) at room temperature. UV spectra were recorded using a Shimadzu UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets (Bio-Rad, Hercules, California, USA). 1D (^{1}H, ^{13}C) and 2D NMR experiments (^{1}H–^{1}H COSY, HSQC, HMBC and ROESY) were recorded on Bruker AM-400, DRX-500 or AVANCE III-600 spectrometers (Bruker, Bremerhaven, Germany). The chemical shifts were given in δ (ppm) scale and referenced to the solvent signal. Mass spectra were acquired on a LCMS-IT-TOF spectrometer (Shimadzu, Kyoto, Japan). Column chromatography was performed on silica gel (200–300 mesh; Qingdao Makall Chemical Company, Qingdao, PR China). Semi-preparative HPLC was carried out by Waters Alliance 2695 liquid chromatography with a ZORBAX SB-C_{18} (5 μm, 9.4 × 250 mm) column (Agilent, USA) at a flow rate of 3.0 mL/min. Sephadex LH-20 (20–150 μm) for chromatography was purchased from Pharmacia Fine Chemical Co. Ltd. (Pharmacia, Uppsala, Sweden), and Rp-18 (40–63 μm) from Fuji Silysia Chemical Ltd. (Fuji, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

2.2. Plant material

The whole plants of *S. yunnanensis* were collected in Lijiang, Yunnan province, PR China, in September 2008 and identified by Prof. Dr. Li-Gong Lei, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 20081118) was deposited at the Laboratory of Antivirus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried whole plants of *S. yunnanensis* (5.2 kg) were powdered and extracted with 90% EtOH (50 L) at room temperature for 3 times. The combined EtOH extract was concentrated under reduced pressure to give a dark-brown residue, which was suspended in H_2O (5 L) and partitioned with petroleum ether (PE, 5 L × 3), EtOAc (5 L × 4) and *n*-butanol (4 L × 3), successively. The EtOAc part (217 g) was subjected to silica gel column chromatography (CC, 18 × 28 cm, 2500 g), eluting with a gradient of increasing MeOH in $CHCl_3$, to give 5 fractions (Frs. A1–A5). Fr. A1 was further divided into 3 subfractions (Frs. A1-1–A1-3), by silica gel CC (9 × 30 cm, 750 g) using PE–EtOAc (90:10, 80:20, 70:30, *v*/*v*) as the eluent. Fr. A1-2 was chromatographed on silica gel CC (2 × 36 cm, 36 g, PE–Me_2CO, 92:8) to yield compounds **1** (64 mg), **5** (156 mg) and **20** (342 mg). Fr. A1-3 was subjected to silica gel CC (2 × 30 cm, 30 g) eluted with PE–EtOAc (75:25) and further purified over a Sephadex LH-20 CC (1.3 × 135 cm, 53 g) with an isocratic solvent system of $CHCl_3$–MeOH (50:50) to yield compounds **10** (17 mg), **11** (32 mg) and **18** (7 mg). Compounds **6** (31 g) and **19** (587 mg) were obtained from Fr. A2 by silica gel CC (9 × 30 cm, 750 g) using $CHCl_3$–Me_2CO (95:5, 90:10) as the eluent and recrystallized from $CHCl_3$–MeOH (50:50). Fr. A3 and Fr. A4 were further separated by CC over Rp-18 (2.54 × 50 cm, 125 g), eluting with a gradient of increasing MeOH in H_2O (25:75–100:0) and further purified using preparative HPLC (SB-C_{18}, 5 μm, 9.4 × 250 mm, Agilent) to afford compounds **12** (27 mg), **13** (9 mg), **14** (16 mg), **15** (14 mg) and **16** (23 mg) eluted with MeOH–H_2O. Fr. A5 was subjected to silica gel CC (4 × 135 cm, 150 g) eluted with



1

	R_1	R_2	R_3
2	—H	H	glc$\overset{2}{—}$glc
3	····OH	H	glc$\overset{2}{—}$glc$\overset{6}{—}$glc$\overset{6}{—}$glc
4	—H	OH	glc$\overset{2}{—}$glc$\overset{6}{—}$glc$\overset{6}{—}$glc

20

Fig. 1. Chemical structure of compounds **1**–**4**.