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Sulfated neo-clerodane diterpenoids and triterpenoid saponins from *Sheareria nana* S. Moore

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astersaponins A (PubChemCID: 102321343)

ABSTRACT

Three novel neo-clerodane diterpenoids Sheareria A–C (**1–3**) together with three known triterpenoid saponins were isolated from the whole herb of *Sheareria nana* S. Moore. Their structures were established by spectroscopic and chemical method. This is the first natural sulfated neo-clerodane diterpenoids. This is the first report of all these compounds from this plant. These neo-clerodane diterpenoids and triterpenoid saponins from *S. nana* S. Moore may be considered as chemotaxonomic markers for the genus. The compounds isolated were evaluated for their cytotoxic effects against three cancer cell lines, the test substances demonstrated selectivity toward the cancer cells. To date, this is the first report on the phytochemical and biological activity of secondary metabolites from *S. nana* S. Moore.

1. Introduction

Sheareria nana S. Moore (Asteraceae), a Chinese endemic monotypic plant, distributed in northeast, southwest and central-south of China. As a traditional Chinese medicine, it has been used as a Diuresis Shenshi drug with a long history [1]. To date, no phytochemical and pharmacological research has been conducted on the title plant. This genus has been classified into two different tribes of Asteraceae: Heliantheae and Astereae [2–4]. Recently Li has supported the tribal delimitation of *S.* within the Astereae from the micromorphology, anatomy, and chromosome view [5]. In a search for bioactive metabolites from the family Asteraceae [6–8], the first phytochemical investigation on *S. nana* led to the isolation of 6 compounds (Fig. 1), including three new neo-clerodane diterpenoids, Shearerias A–C (**1–3**), and three known triterpenoid saponins. To the best of our knowledge, Compound **1** was the first natural sulfated neo-clerodane diterpenoids, while compounds **2** and **3** were the first natural sulfated 18-nor-neo-clerodane diterpenoids. In this paper, we report the isolation, structure elucidation, cytotoxicity, and chemotaxonomic significance of these compounds.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. Ultraviolet absorption spectra were recorded on a UV-2401 PC spectrophotometer. IR spectra were obtained from a Bio-Rad FtS-135 spectrometer. NMR spectra were measured on a Bruker AV-400 spectrometer with TMS as the internal standard. Mass spectrometry was performed on a Waters Q-TOF Premier instrument (Micromass MS Technologies, Manchester, UK) equipped with an ESI source in the positive/negative-ion mode. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd.), and reversed-phase C_{18} silica gel (250 mesh, Merck). Precoated TLC sheets of silica gel 60 GF_{254} (Qingdao Haiyang Chemical Co., Ltd.) were used. An Agilent 1200 Series machine equipped with Zorbax SB-C_{18} column (4.6 × 250 mm, 5 μm) was used for HPLC analysis, and a semi-preparative Zorbax SB-C_{18} column (9.4 × 250 mm, 5 μm) was used in sample preparation. Etoposide was purchased from Sigma-Aldrich Trading Co. Ltd. (Shanghai, People's Republic of China).

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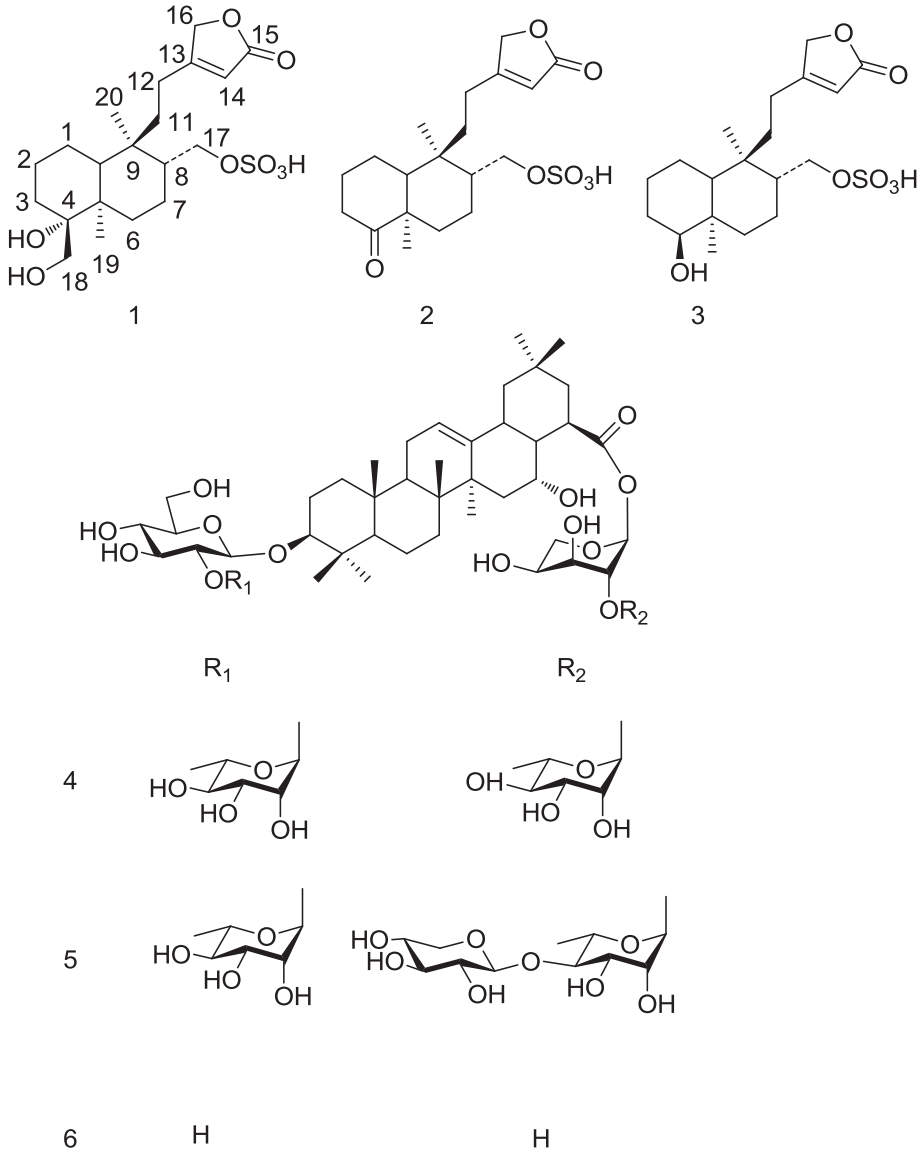

Fig. 1. The chemical structures of compounds **1–6**.

2.2. Plant material

The whole herb of *S. nana* S. Moore were collected in October 2015 in Jishou, Hunan Province, PR China, and identified by Professor Gongxi Chen (Jishou University). A voucher sample (S. X. 0001) was deposited in was deposited in the translational Medicine Laboratory, Shanghai First Maternity and Infant Hospital, Tongji University School of medicine.

2.3. Extraction and isolation

Dried and powered whole herb of *S. nana* S. Moore (3 kg) were extracted with 85% aqueous EtOH (v/v) (3 × 30 L, each for 4 h) at 80 °C. After evaporation of the solvent, the residue (280 g) was dissolved in H_2O (3 × 8 L) and extracted successively with petroleum ether (3 × 10 L), EtOAc (3 × 10 L), and *n*-BuOH (3 × 10 L). The EtOAc extract (140 g) was subjected to silica gel (2 kg, 200–300 mesh, 10 × 90 cm) and eluted with $CHCl_3$–MeOH (9:1, 7:3, 1:1, 0:1, each 15 L) to produce four fractions (A–D). Fraction C (48 g) was subjected to an RP-18 column (300 g, 5 × 60 cm) by eluted with MeOH/H_2O at 30, 45, 60, 75, 90%, each 3 L to produce five fractions (C1–C5). Fraction C3 was purified by Sephadex LH-20 (100 g, 2 × 140 cm) with MeOH (600 mL) to produce fractions C3-1, C3-2, and C3-3. Fraction C3–1 (23 mg) was subjected to was separated by semi-preparative HPLC, using methanol–water (76:24, containing 0.1% trifluoroacetic acid, 2 mL/min) as mobile phase, to yield **2** (7 mg), and **3** (5 mg), whereas fraction C3–2 (310 mg) was chromatographed on silica gel (30 g, 200–300 mesh, 2 × 50 cm) eluted with $CHCl_3$–MeOH (8:2, 300 mL; 7:3, 230 mL) to produce fractions C3-2-1, C3-2-2, and C3-3-3. Fraction C3-2-2 (26 mg) was subjected to was separated by semi-preparative HPLC, using methanol–water (70:30, containing 0.1% trifluoroacetic acid, 2 mL/min) as mobile phase, to yield **1** (6 mg).

The *n*-BuOH extract (10 g) was subjected to silica gel (200 g, 200–300 mesh, 6 × 60 cm) and eluted with $CHCl_3$–MeOH (9:1, 7:3, 1:1, 0:1, each at 1 L) to produce four fractions (A–D). Fraction C (4 g) was subjected to column chromatography over ODS (80 g, 3 × 60 cm) using MeOH–H_2O (70:30, 80:20, 90:10, each 500 mL) to yield **4** (6 mg), **5** (4 mg) and **6** (8 mg).

2.3.1. 4,18-dihydroxy-17-sulfated-13(14)-neoclerodan-15,16-olide (1)

Yellow gum; $[\alpha]_D^{20}$ –40.4 (*c* 0.2, MeOH); IR ν_{max} (KBr) cm^{-1}: 3450, 1732, 1637, 1563, 1412, 1382, 1247, 565; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 431.1733 $[M–H]^-$ (calcd for $C_{20}H_{31}O_8S$, 431.1745), ESIMS *m/z* 450 $[M + NH_4]^+$, 431 $[M–H]^-$.