Fitoterapia xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Oleanane-type saponins from *Anemone taipaiensis* and their cytotoxic activities

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ARTICLE INFO

Article history:
Received 14 March 2013
Received in revised form 6 June 2013
Accepted 9 June 2013
Available online xxxx

Keywords:
Anemone taipaiensis
Ranunculaceae
Triterpenoid saponin
Cytotoxicity

ABSTRACT

Phytochemical investigation of the *n*-BuOH extract of the rhizomes of *Anemone taipaiensis* led to the isolation of three new oleanane-type triterpenoid saponins (**1–3**), together with four known saponins (**4–7**). Their structures were elucidated on the basis of spectroscopic analysis and chemical derivatization. All the compounds were isolated for the first time from *A. taipaiensis*. The cytotoxicity of these compounds was evaluated in five human cancer cell lines including A549 (lung carcinoma), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), HL-60 (promyelocytic leukemia), and U87MG (glioblastoma). The monodesmosidic saponin **4** exhibited cytotoxic activity toward all cancer cell lines, with IC_{50} values ranging from 6.42 to 18.16 μM. In addition, the bisdesmosidic saponins **1** and **7** showed selective cytotoxicity against the U87MG cells.

1. Introduction

The genus *Anemone* belongs to the family Ranunculaceae, which has been proved to be a rich source of diverse saponin substances (such as oleanolic acid type, 27-hydroxy-oleanolic acid type, hederagenin type, 2-hydroxy-hederagenin type, *etc.*) with potentially useful biological properties including antitumor, antibacterial, antiperoxidation, insect deterrence, *etc.* [1–6]. Several species of *Anemone*, such as *Anemone flaccida*, *Anemone raddeana*, *Anemone tomentosa*, *Anemone anhuiensis* and *Anemone altaica*, have been used as Chinese folk medicines for a long time. *Anemone taipaiensis* is an endemic species in Shaanxi Province of China [7]. The rhizomes of this plant have been used in traditional medicine for the treatment of rheumatism and phlebitis. Our previous fragmentary investigations of *Anemone* species resulted in the isolation of a series of oleanane-type triterpenoid saponins [8–10]. As part of our ongoing search for new bioactive constituents from natural source [11–15], the further phytochemical study of the rhizomes of *A. taipaiensis* led to the isolation of three new oleanane-type saponins (**1–3**), along with four known saponins (**4–7**) (Fig. 1). We report herein the isolation and structural elucidation of these saponins, along with their cytotoxic results against five human cancer cell lines, lung carcinoma A549, cervical carcinoma HeLa, hepatocellular liver carcinoma HepG2, promyelocytic leukemia HL-60 and glioblastoma U87MG.

2. Experimental procedure

2.1. General

Optical rotations were measured on a Perkin–Elmer 343 polarimeter. NMR spectra were recorded on a Bruker AVANCE-500 spectrometer in pyridine-d_5 (99.95%, Sigma-Aldrich) with TMS as internal standard. The ESIMS and HRESIMS were obtained on a Micromass Quattro mass spectrometer. GC was performed on a Finnigan Voyager apparatus using an

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0367-326X/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.fitote.2013.06.008

L-Chirasil-Val column (0.32 mm × 25 m; injector temperature: 230 °C; column temperature: 100–180 °C, rate 5 °C/min; column head pressure: 12 Pa; carrier gas: He, 2 mL/min). HPLC was carried out on a Dionex P680 liquid chromatograph equipped with a UV 170 UV/Vis detector at 206 nm using a YMC-Pack R&D ODS-A column (20 × 250 mm i.d., 5 μm, YMC Co., Ltd. Japan). Materials for column chromatography (CC) were silica gel (10–40 μm, Qingdao Marine Chemical Inc., China), Sephadex LH-20 (40–70 μm, GE-Healthcare, Sweden), and reversed phase silica gel ODS-A (50 μm, YMC Co., Ltd, Japan). The Liebermann–Burchard test was made with acetic anhydride and sulfuric acid, and the Molish test was made with α-naphthol and sulfuric acid. TLC detection was achieved by spraying the silica gel plates (Qingdao Marine Chemical Inc., China) with 20% H_2SO_4–EtOH (v/v) solution followed by heating.

2.2. Plant material

A. taipaiensis was collected on Taibai Mountain, Shaanxi Province, China, in August 2009, and identified by Prof. Ji-Tao Wang (Department of Pharmacognosy, School of Pharmacy, Shaanxi University of Chinese Medicine). A voucher specimen (No.090918) has been deposited in the herbarium of Shaanxi University of Chinese medicine.

2.3. Extraction and isolation

The air-dried rhizomes of *A. taipaiensis* (5 kg) were powdered and extracted with 70% EtOH (5 L) under reflux for three times (each for 2 h). The extract was evaporated *in vacuo* to yield a residue (650 g) which was suspended in water (8 L) and partitioned successively with petroleum ether (8 L × 2) and *n*-BuOH (8 L × 3). The *n*-BuOH extract (110 g) was separated by silica gel CC using a stepwise gradient of $CHCl_3$–MeOH–H_2O (10:1:0.05–6: 4:0.8) to give nine fractions (Fr. 1–Fr. 9). Fr. 5 (3.2 g) was subject to silica gel CC with a $CHCl_3$–MeOH–H_2O gradient (10:1:0.1–6:4:0.8) to give three sub-fractions (Fr. 5.1–Fr. 5.3). Compound **4** (42 mg) was obtained from Fr. 5.2 by semipreparative HPLC [MeOH–H_2O (84:16), 8 mL/min, t_R 14.9 min]. Fr. 9 (29.2 g) was chromatographed on silica gel CC with a stepwise gradient of $CHCl_3$–MeOH–H_2O gradient (8:2:0.2–6:4:0.5) to yield five fractions (Fr. 9.1–Fr. 9.5). Fr. 9.2 (9.5 g) was subjected to ODS CC using a stepwise gradient [MeOH–H_2O (1:4–4:1)] to afford eight fractions (Fr. 9.2.1–Fr. 2.9.8). Fr. 9.2.3 (2.0 g) and Fr. 9.2.5 (1.0 g) were submitted to gel permeation chromatography on Sephadex LH-20 in MeOH to remove the pigments and carbohydrates. Compounds **5** [65 mg, t_R 26.6 min] and **6** [250 mg, t_R 33.7 min] were obtained from Fr. 9.2.3 by semipreparative HPLC [MeOH–H_2O (59:41), 7.2 mL/min]. Fr. 9.2.5 was purified by semipreparative HPLC to yield compound **7** [45 mg, MeOH–H_2O (65:35), 7.8 mL/min, t_R 32.0 min]. Fr. 9.3 (8.3 g) was separated by ODS CC eluting with a gradient of MeOH–H_2O (1:10–3:1) to afford six fractions (Fr. 9.3.1–Fr. 9.3.6). Fr. 9.3.2 (1.2 g) and Fr. 9.3.3 (2.0 g) were further purified by semipreparative HPLC after CC over Sephadex LH-20 (MeOH), to give compound **1** [28 mg, MeOH–H_2O (54:46), 6.0 mL/min, t_R 25.0 min from Fr. 9.3.2], compound **2** [44 mg, MeOH–H_2O (55:45), 6.0 mL/min, t_R 36.5 min from Fr. 9.3.3], and compound **3** [250 mg, MeOH–H_2O (55:45), 6.0 mL/min, t_R 47.8 min from Fr. 9.3.3]. Purities of these compounds were determined by HPLC > 95%.

Compound **1**: white amorphous powder; $[\alpha]_D^{22}$ −8.6 (*c* 0.15, MeOH); for ^1H and ^{13}C NMR spectroscopic data, see Table 1; key HMBC and NOESY correlations, see Fig. 1; ESIMS (pos. ion mode) *m/z* 1405 $[M + Na]^+$, 935 [1405 − 146 − 162 − 162]$^+$; ESIMS (neg. ion mode) *m/z* 1381

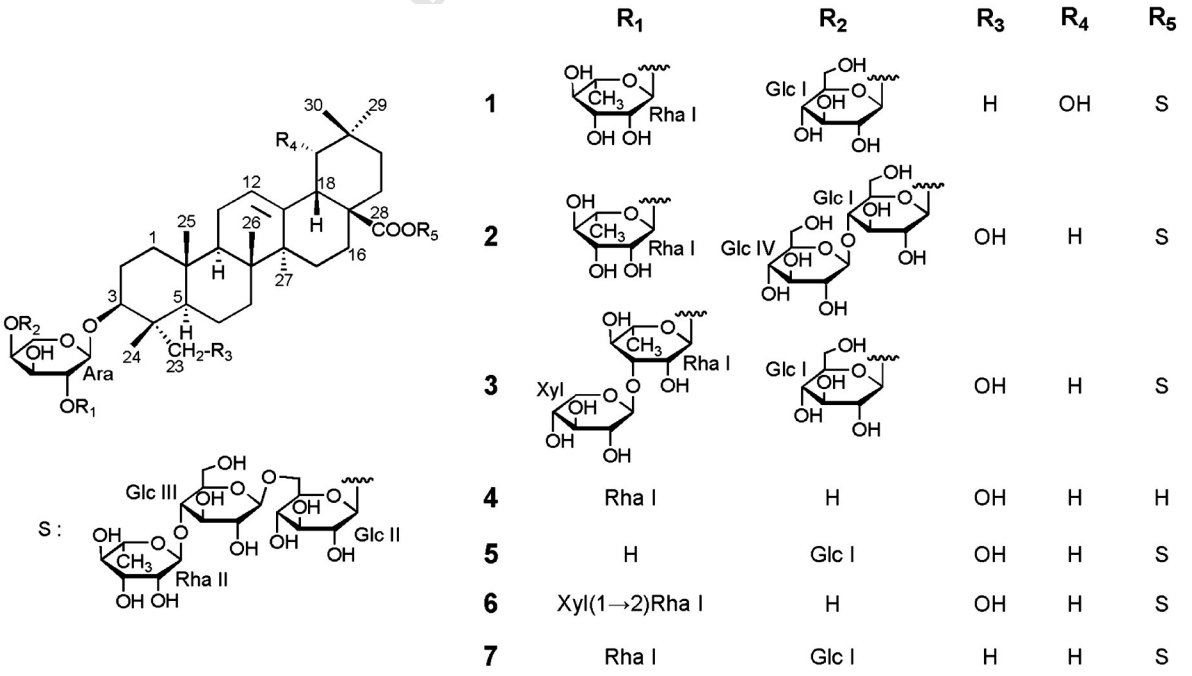

Fig. 1. Structures of compounds **1–7**.

Please cite this article as: Wang X, et al, Oleanane-type saponins from *Anemone taipaiensis* and their cytotoxic activities, Fitoterapia (2013), http://dx.doi.org/10.1016/j.fitote.2013.06.008