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New steroidal saponins from the rhizomes of *Paris delavayi* and their cytotoxicity

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ABSTRACT

Four new furostanol saponins, named padelaosides C–F (**1**–**4**), together with four known spirostanol saponins **5**–**8** were isolated from the rhizomes of *Paris delavayi* Franchet. Their structures were elucidated on the basis of extensive spectroscopic analysis and chemical evidences. The discovery of the new compounds **1**–**4** extended the diversity and complexity of this furostanol saponin family. The cytotoxicity of all the saponins was evaluated for their cytotoxicity against human glioblastoma U87MG and human hepatocellular carcinoma Hep-G2 cell lines. The known spirostanol saponins **7** and **8** exhibited notable cytotoxicity against the two tumor cell lines with IC_{50} values of 1.13 and 3.42 μM, respectively, while the new furostanol saponins **3** and **4** showed moderate cytotoxicity with IC_{50} values of 15.28 to 16.98 μM.

1. Introduction

The genus *Paris* (family *Liliaceae*) is widely distributed in tropical and temperate regions of Europe and Asia. To data, 24 species were found in this genus, and 19 species are distributed in China [1]. *Rhizoma Paridis*, the rhizomes of *Paris polyphylla* var. *yannanensis* and *P. polyphylla* var. *chinensis*, recorded in the Chinese Pharmacopoeia, plays an important role in clinics for treating snake bite, fractures, tumors, traumatic bleeding, etc. [2]. The phytochemical and pharmacological investigations of the genus have demonstrated that steroidal saponins exhibited broad biological activities including hypocholesteremia, antitumor, anti-inflammatory, antifungal, analgesic and sedative effects, inhibitory activity against platelet aggregation and cAMP phosphodiesterase, etc. [3–6]. In dedatils, saponins extracted from *Paris fargesii* var. *brevipetala* exhibited cytotoxic activities on HepG2, A549, RPE and L929 cells [7]. A steroidal saponin isolated from *Paris polyphylla* was explored *anti*-angiogenic effects [8]. The steroid saponins obtained from *Paris polyphylla* Smith var. *chinensis* (Franch.) have been researched on anti-lung cancer activities [9], etc. The increasing application of *Rhizoma Paridis* resulted in the shortage of wildlife resources. To find the replacement plants of the crude drugs, one plant of the genus, *Paris delavayi* Franchet was chosen to investigate. *Paeonia delavayi*, a perennial herb, is mainly grown in Yunnan, Guizhou, Hubei and Hunan provinces of China, and the appropriate altitude for the living of the plant is about 1300 to 2000 m [1]. It has been reported that two new steroidal saponins, padelaosides A and B, several known steroidal saponins, two known triterpenoid saponins, one known pregnane saponin, one phytoecdysone and daucosterol have been isolated from *P. delavayi* [10–12]. Herein, we report the isolation, structure identification and cytotoxicity of four new furostanol saponins, named padelaosides C–F (**1**–**4**), and four known spirostanol saponins **5**–**8** from the rhizomes of *P. delavayi* (Fig. 1).

2. Experimental procedure

2.1. General

Optical rotations were measured on a Perkin-Elemr 241 MC digital polarimeter (German Perkin-Elmer Corporation). Melting points

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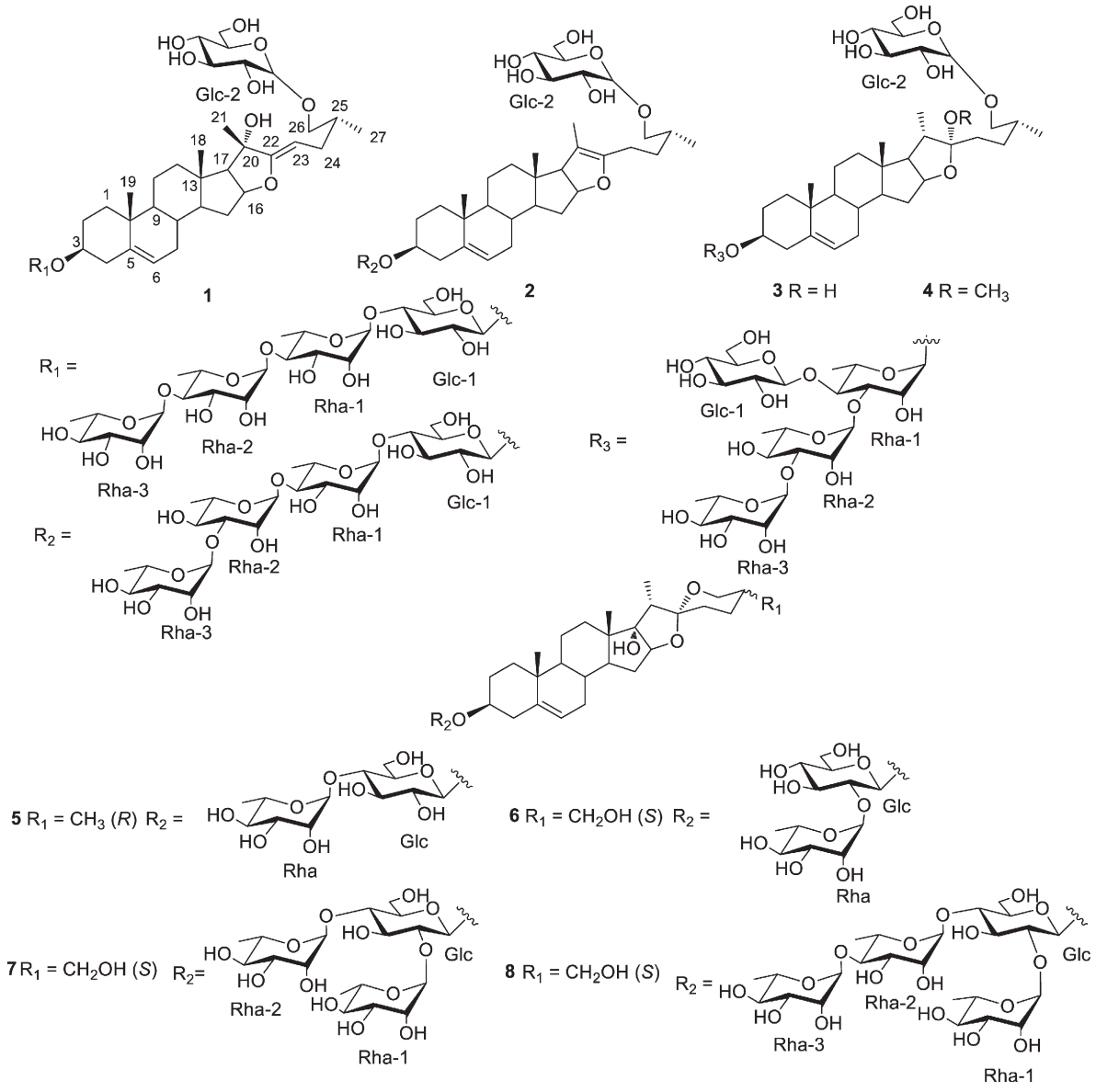

Fig. 1. Structures of compounds **1–8** from *Paris delavayi* Franchet.

were determined on an XT5-XMT apparatus and uncorrected. UV spectra were obtained using a Shimadzu UV-2401PC spectrophotometer. IR spectra were recorded on a Shimadzu IRPrestige-21 spectrophotometer. 1D and 2D NMR spectral experiments were measured in C_5D_5N on a Bruker AVANCE-500 spectrometer, with TMS as internal standard. ESI-MS and HR-ESI-MS spectra were carried out on a Waters Quattro Micromass mass spectrometer. GC analysis was performed on an Agilent 6890 N apparatus using an HP-5 capillary column (30 m × 0.32 mm, 0.25 μm) and an FID detector with an initial temperature of 120 °C for 2 min and then temperature programming to 280 °C at the rate of 10 °C/min. Column chromatographies (CC) were performed on silica gel H (10–40 μm, Qingdao Marine Chemical Inc., Qingdao, China), reversed-phase Si gel (Lichroprep RP-18, 40–63 μm, Merck Inc., Darmstadt, Germany) and Sephadex LH-20 (GE-Healthcare, Sweden). HPLC were 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 (250 × 20 mm i.d., 5 μm, YMC, Kyoto, Japan) for semi-preparation. TLC detections were carried out on precoat plates with RP-18 (Merck) and silica GF_{254} (Qingdao Marine Chemical Inc., Qingdao, China) with 20% H_2SO_4 followed by heating for three minutes. Standards D-glucose (D-Glc) and L-rhamnose (L-Rha) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Plant material

The rhizomes of *Paris delavayi* were collected from Wen County, Gansu Province of China in October 2012, and were identified by one of the authors, Prof. Haifeng Tang. A voucher specimen (No. 20121002) was deposited in the Herbarium of Institute of Materia Medica, School of Pharmacy, Fourth Military Medical University, Xi'an, China.

2.3. Extraction and isolation

The air-dried rhizomes of *P. delavayi* (10 kg) were crushed and refluxed with 70% EtOH (86 L × 3, 2 h/time). The extract was condensed in a rotary evaporator to obtain a syrupy residue (780 g). The residue was suspended in H_2O (5 L) and extracted successively with petroleum ether (5 L × 3) and *n*-BuOH (5 L × 3). The *n*-BuOH phase was evaporated under reduced pressure to give a dark gummy residue (140 g). The residue was subjected to silica gel column chromatography (CC) and