Carbohydrate Research 368 (2013) 1–7

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

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

Triterpenoid saponins from rhizomes of *Paris polyphylla* var. *yunnanensis*

Xia Wu [a,b], Lei Wang [a,b], Guo-Cai Wang [a,b], Hui Wang [c], Yi Dai [a,b], Xin-Xin Yang [a,b], Wen-Cai Ye [a,b,*], Yao-Lan Li [a,b,*]

[a] Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, PR China
[b] Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University, Guangzhou 510632, PR China
[c] School of Medicine, Jinan University, Guangzhou 510632, PR China

ARTICLE INFO

Article history:
Received 28 September 2012
Received in revised form 28 November 2012
Accepted 30 November 2012
Available online 8 December 2012

Keywords:
Paris polyphylla var. *yunnanensis*
Liliaceae
Triterpenoid saponins
Cytotoxic activities

ABSTRACT

Phytochemical investigation of the rhizomes of *Paris polyphylla* var. *yunnanensis* resulted in the isolation of six new oleanane-type triterpenoid saponins, paritrisides A–F (**1**–**6**), along with nine known triterpenoid saponins (**7**–**15**). The structures of the new compounds were elucidated on the basis of spectroscopic analysis and acid hydrolysis. All the triterpenoid saponins are obtained for the first time from the genus *Paris*. The isolated compounds were assayed for their cytotoxic activities against human nasopharyngeal carcinoma epithelial (CNE) cells, and compounds **7**, **8**, and **10** exhibited inhibitory effects on CNE cell growth with IC_{50} values of 16.53, 16.77, and 12.69 μm, respectively.

1. Introduction

The rhizome of *Paris polyphylla* var. *yunnanensis* (Fr.) Hand.-Mazz. (Liliaceae) is a Chinese medicine traditionally used for the treatment of various inflammations and injuries.[1–3] Pharmacological studies have demonstrated that the plants of genus *Paris* possessed hemostatic, antitumor, uterine contractile, analgesic, and sedative effects.[4–6] Phytochemical investigations have indicated that the main constituents of the genus *Paris* were steroidal saponins.[7,8] In the previous study, we obtained some new and (or) cytotoxic steroidal saponins and sterol glycosides from *P. polyphylla* var. *yunnanensis*.[9,10] In order to discover more novel biologically active compounds from *P. polyphylla* var. *yunnanensis*, our continuing studies led to fifteen oleanane-type triterpenoid saponins including six new ones isolated and identified from the rhizomes of this plant. It is noteworthy that all the triterpenoid saponins are reported for the first time from the genus *Paris*. The cytotoxic effects of the triterpenoid saponins on human nasopharyngeal carcinoma epithelial (CNE) cells were evaluated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and some of them showed moderate to strong effects. Herein, we report the structure elucidation and cytotoxic activities of these compounds.

2. Results and discussion

Dried rhizomes of *P. polyphylla* var. *yunnanensis* were extracted with EtOH–H_2O (70:30, v/v). The crude extract was successively subjected to column chromatographies (CC) over Diaion HP-20, silica gel, ODS silica gel, Sephadex LH-20, and preparative HPLC to afford fifteen oleanane-type triterpenoid saponins (**1**–**15**, Fig. 1).

Compound **1** was obtained as white amorphous powder. The molecular formula was determined as $C_{41}H_{66}O_{13}$ by the quasimolecular ion $[M+Na]^+$ at *m*/*z* 789.4383 in HR-ESIMS. The IR spectrum of **1** indicated the existence of hydroxyl and γ-lactone moieties (3421, 1743, 1077 cm^{-1}). The ^1H NMR spectrum of **1** displayed seven methyl singlets at δ_H 1.59, 1.28, 1.22, 1.04, 0.90, 0.82, and 0.80 (each 3H, s), as well as two anomeric protons at δ_H 5.18 (1H, d, J = 7.7 Hz) and 4.93 (1H, d, J = 5.8 Hz). Acid hydrolysis of **1** yielded D-glucose and L-arabinose, which were identified by GC analysis of their L-cysteine methyl ester-TMS derivatives. The ^{13}C NMR and DEPT spectra exhibited 41 carbon signals, 30 of which were attributed to the aglycone part, including 7 methyls, 10 methylenes, 5 methines, and 8 quaternary carbons. Among them, a carbon signal of a γ-lactone moiety at δ_C 179.7 and three oxygenated carbon signals at δ_C 91.4, 88.7, and 75.7 were observed. The above data indicated that compound **1** was a triterpenoid disaccharide. The ^1H and ^{13}C NMR data of the aglycone moiety of **1** (Tables 1 and 2) closely resembled those of kochianoside III,[11,12] suggesting the aglycone of **1** to be 3β,12α-dihydroxyolean-28,13β-olide. The relative configuration of the aglycone was confirmed by the NOESY correlations as

* Corresponding authors. Tel.: +86 20 85221728; fax: +86 20 85221559 (Y.-L.L), Tel.: +86 20 85220936, fax: +86 20 85221559 (W.-C.Y).
E-mail addresses: chywc@yahoo.com.cn (W.-C. Ye), tliyl@jnu.edu.cn (Y.-L. Li).

0008-6215/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carres.2012.11.027

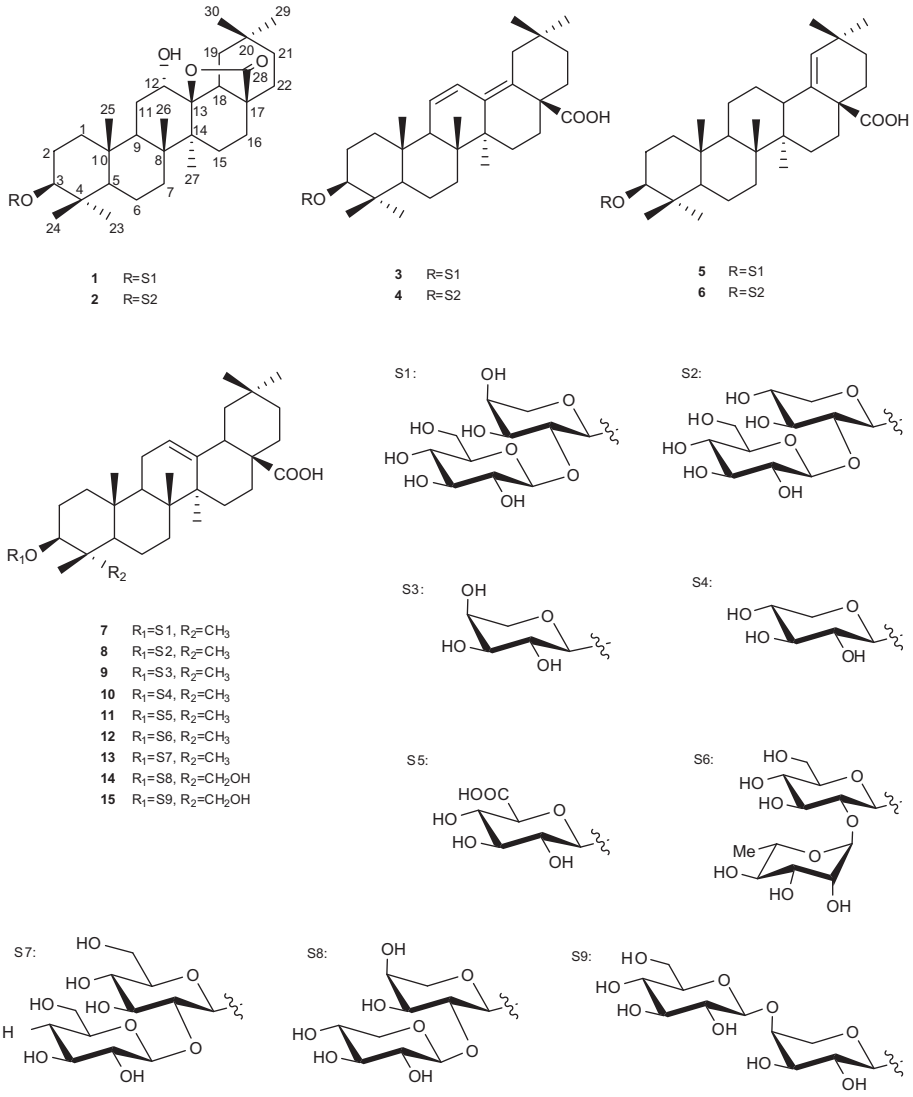

Figure 1. Chemical structures of compounds **1**–**15**.

shown in Figure 2. For the sugar part, the ^{1}H and ^{13}C NMR data of sugars (Tables 1 and 2) indicated they were pyranoses. The β-anomeric configuration of glucopyranose was judged from their $^{3}J_{H1-H2}$ coupling constant (J = 7.7 Hz) and α-anomeric configuration of arabinopyranose was deduced from their $^{3}J_{H1-H2}$ coupling constant (J = 5.8 Hz). The sequence of the sugars and binding site to the aglycone of **1** could be determined by an HMBC experiment. The HMBC spectrum (Fig. 2) of **1** demonstrated correlations of δ_H 4.93 (H-1 of Ara) with δ_C 88.7 (C-3 of the aglycone) and δ_H 5.18 (H-1 of Glc) with δ_C 81.1 (C-2 of Ara), revealing the glucose was connected to C-2 of arabinose, and the disaccharide was linked to C-3 of the aglycone, which was further confirmed in agreement with the ^{1}H and ^{13}C NMR data of the sugar part of **7**.[13] Thus, the structure of **1** was deduced as 3β,12α-dihydroxyolean-28,13β-olide 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside, and generically named as paritriside A.

Compound **2** was also isolated as amorphous powder. It was shown to have the molecular formula of $C_{41}H_{66}O_{13}$ from its HR-ESIMS data (m/z 789.4400 $[M+Na]^+$). The IR spectrum of **2** showed absorption bands at 3402, 1743, and 1077 cm^{-1} due to hydroxyl, carboxyl, and lactone groups. Comparison of the ^{1}H and ^{13}C NMR data of the aglycone of **2** (Tables 1 and 2) with those of **1** revealed that the signals were identical, suggesting that **2** had the same aglycone (3β,12α-dihydroxyolean-28,13β-olide) as **1**. The NMR data for the sugar moiety and gas chromatographic (GC) analysis of the derivatives of the hydrolyzate of **2** indicated the existence of D-glucopyranose and D-xylopyranose. The anomeric proton signals of **2** at δ_H 5.34 (1H, d, J = 7.7 Hz) and 4.80 (1H, d, J = 6.7 Hz), led to the assignment of the anomeric configurations of glucose and xylose units as β. The ascription and sequence of disaccharide were determined by the combination of ^{1}H–^{1}H COSY, HSQC, and HMBC. In the HMBC spectrum, the long-range correlations of δ_H 4.80 (H-1 of xylose) with δ_C 88.7 (C-3 of aglycone) and δ_H 5.34 (H-1 of glucose) with δ_C 83.2 (C-2 of xylose) were observed. The ^{1}H and ^{13}C NMR spectra of the sugar parts of **2** (Tables 1 and 2) were in accordance with those of **8**.[14] Therefore, the structure of **2** was identified as 3β,12α-dihydroxyoleane-28,13β-olide 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside, and named as paritriside B.

The HR-ESIMS of **3** exhibited a quasimolecular ion at m/z 771.4292 ($[M+Na]^+$), corresponding to a molecular formula $C_{41}H_{64}O_{12}$. The presence of a heteroannular conjugated diene system was revealed by its UV spectrum (λ_{max} at 243, 250, 260 nm). The IR spectrum of **3** showed the existence of the hydroxyl (3402 cm^{-1}) and double bond (1641 cm^{-1}). The ^{1}H NMR spectrum exhibited two olefinic protons at δ_H 6.66 (1H, m) and 5.72 (1H, d,