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Steroidal saponins from *Paris polyphylla* var. *yunnanensis*

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ABSTRACT

Eleven steroidal saponins, along with seven known steroidal saponins, were isolated from rhizomes of *Paris polyphylla* var. *yunnanensis*. Their chemical structures were elucidated on the basis of spectroscopic analyses and acid hydrolysis. Two of these compounds contained a spirostanol saponin aglycone, hitherto unknown in Nature. The isolated compounds were tested for their cytotoxic effects on human nasopharyngeal carcinoma epithelial (CNE) cells, and seven compounds displayed more potent inhibitory effects than cisplatin (the positive control). One compound with diosgenin and tetrasaccharide moieties possessed the strongest inhibitory effect on CNE cells through the induction of apoptosis and cell cycle arrest.

1. Introduction

The genus *Paris* (family Liliaceae) consists of more than 24 species worldwide, among which *Paris polyphylla* var. *yunnanensis* is a member. This species is mainly distributed in southwestern China, especially in the Yunnan and Sichuan provinces (Wu et al., 2004; Huang et al., 2007; Zhang, 2007). The rhizome of *P. polyphylla* var. *yunnanensis* is a Chinese medicine traditionally used for the treatment of traumatic injuries, abscess, mastitis, chronic bronchitis, and hepatopathy (Wu et al., 2004; Chinese Pharmacopoeia Commission, 2005; Huang et al., 2007). Pharmacological studies established that *P. polyphylla* var. *yunnanensis* possessed anti-tumor (Cheng et al., 2008; Man et al., 2009), hemostatic (Fu et al., 2007; Guo et al., 2008), antifungal (Deng et al., 2008), sedative (Wang et al., 1990), immunoregulatory (Chiang et al., 1992), and gastro-protective (Hisashi et al., 2003) effects. The steroidal saponins are regarded as the chief active constituents of *P. polyphylla* var. *yunnanensis* (Cheng et al., 2008; Man et al., 2009).

Paris polyphylla var. *yunnanensis* is also an important ingredient of some Chinese patent medicines, such as "Biyan Qingdu Keli", which is widely used in southern China to treat chronic rhinitis and nasopharyngeal cancer (Guo et al., 2006; Han et al., 2009). During the course of our anti-cancer screening of the medicinal herbs in "Biyan Qingdu Keli", the ethanol extract of the rhizomes of *P. polyphylla* var. *yunnanensis* was found to have remarkable cytotoxicity towards nasopharyngeal carcinoma epithelial (CNE) cells, with an IC_{50} value of 32 μg/ml (Wu et al., 2010). The objective of the present study was to elucidate the active compounds from the ethanol extract of *P. polyphylla* var. *yunnanensis*. As a result, 18 steroidal saponins (**1**–**18**) were obtained, 11 of which were previously unknown. In addition, the cytotoxic effect of the isolated compounds against CNE cells was evaluated. This article reports the isolation, structural elucidation, and cytotoxicity of these compounds.

2. Results and discussion

2.1. Structural elucidation

The ethanol extract of the rhizomes of *P. polyphylla* var. *yunnanensis* was subjected to repeated column chromatography over Diaion HP-20, silica gel, ODS silica gel, Sephadex LH-20 and preparative HPLC to afford eighteen compounds (Fig. 1), including 11 previously unreported ones (**1**–**11**).

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{44}H_{70}O_{16}$ by the quasi-molecular ion $[M+Na]^+$ at *m*/*z* 877.4596 in HR-ESI-MS. The ^1H NMR spectrum of **1** showed signals of two tertiary methyl groups at δ_H 1.05 and 0.83 (each s), three secondary methyl groups at δ_H 1.76 (d, J = 6.0 Hz), 1.14 (d, J = 6.5 Hz) and 0.69 (d, J = 4.0 Hz), one olefinic proton at δ_H 5.32 (m), and three anomeric proton signals at δ_H 6.04 (br s), 5.84 (d, J = 3.2 Hz) and 4.91 (d, J = 7.5 Hz). Acid hydrolysis of **1** afforded D-apiose, L-rhamnose and D-glucose, which were identified by gas chromatographic analysis. Therefore, the resonance at δ_H 1.76 was due to the methyl group of rhamnose.

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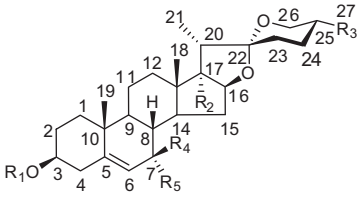
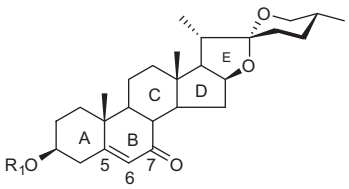


1 R_1 = S1, R_2 = R_4 = R_5 = H, R_3 = CH_3
2 R_1 = S1, R_2 = OH, R_3 = CH_3, R_4 = R_5 = H
3 R_1 = S2, R_2 = OH, R_3 = CH_3, R_4 = R_5 = H
4 R_1 = S3, R_2 = OH, R_3 = CH_3, R_4 = R_5 = H
5 R_1 = S4, R_2 = R_4 = R_5 = H, R_3 = CH_2OH
6 R_1 = S5, R_2 = OH, R_3 = CH_2OH, R_4 = R_5 = H
7 R_1 = S6, R_2 = R_5 = H, R_3 = CH_3, R_4 = OH
8 R_1 = S5, R_2 = R_4 = H, R_3 = CH_3, R_5 = OH
12 R_1 = S8, R_2 = R_4 = R_5 = H, R_3 = CH_3
13 R_1 = S9, R_2 = OH, R_3 = CH_3, R_4 = R_5 = H
14 R_1 = S10, R_2 = R_4 = R_5 = H, R_3 = CH_3
15 R_1 = S6, R_2 = R_4 = R_5 = H, R_3 = CH_3
16 R_1 = S5, R_2 = OH, R_3 = CH_3, R_4 = R_5 = H
17 R_1 = S4, R_2 = OH, R_3 = CH_3, R_4 = R_5 = H



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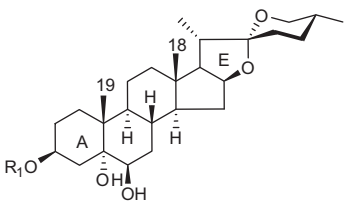

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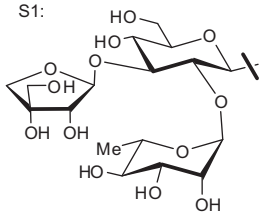

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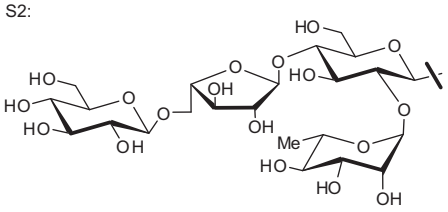

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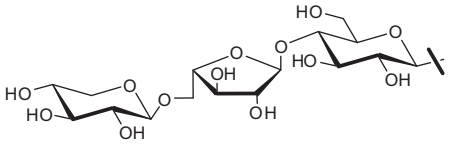

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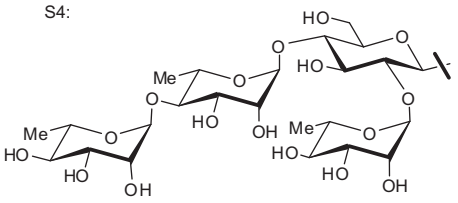

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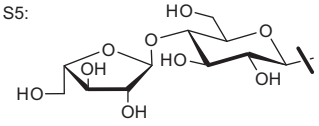

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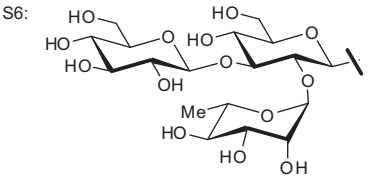

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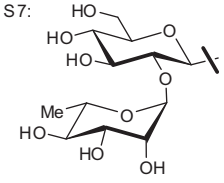

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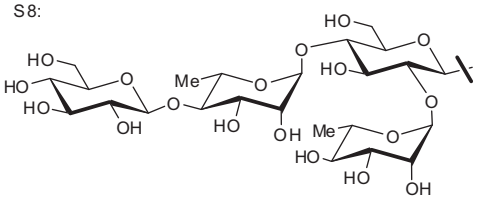

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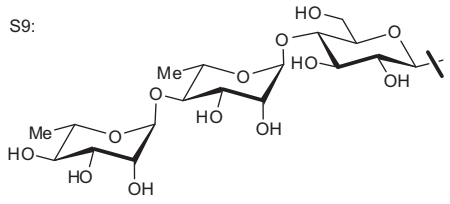

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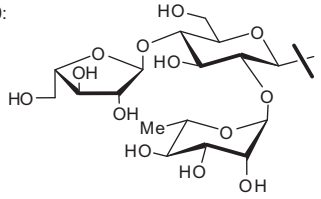

Fig. 1. Chemical structures of saponins **1**–**18**, isolated from *P. polyphylla* var. *yunnanensis*.