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Cytotoxicity of pregnane glycosides of *Cynanchum otophyllum*

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ABSTRACT

Fourteen new pregnane glycosides, including nine caudatin glycosides (**1**–**9**), three qinyangshengenin glycosides (**10**–**12**), one kidjoranin glycosides (**13**) and one gagaminin glycosides (**14**), along with twelve known analogs (**15**–**26**) were isolated from roots of *Cynanchum otophyllum* Schneid. Their structures were deduced by detailed analysis of 1D and 2D NMR spectra, as well as HRESIMS. In this study, all pregnane glycosides obtained (**1**–**26**) were evaluated for their cytotoxic activities using three cancer cell lines (HepG2, Hela, U251). As results, except **6** and **10**, other twenty-four pregnane glycosides showed cytotoxicities at different degrees against three cell lines.

1. Introduction

Pregnane glycosides, also known as C_{21} steroidal glycosides, are the characteristically chemical and bioactive constituents in the genus *Cynanchum* (subfamily Asclepiadoideae within Apocynaceae) [1,2]. Their basic skeletons are the pregnane derivative, whose C-8, C-13, C-15 and C-17 are easy to be substituted by oxygen-containing groups, and C- or D-rings of aglycones are opening occasionally. The sugar moieties in pregnane glycosides consist of 2,6-dideoxysugars mainly, which are usually connected to C-3 of aglycones as a chain [3]. Previous pharmacological experiments have displayed that pregnanes and their glycosides from *Cynanchum* have a variety of bioactivities, such as cytotoxicity, immunoregulation, multidrug-resistance modulation, and antifungus, etc. [4–7].

The perennial herbaceous plant *Cynanchum otophyllum* Schneid., is mainly distributed in southwest of China. Its roots have been used as folk medicine for treatment of rheumatism, lumbago, abdominal pain and distension, etc. [8–12]. In previously chemical studies, our group and other researchers have found this plant is rich in pregnane glycosides [13–23]. As a part of our ongoing research program to isolate novel pregnane glycosides from *Cynanchum* plants, fourteen new pregnane glycosides (**1**–**14**) and twelve known analogs (**15**–**26**) were isolated from roots of *C. otophyllum* (Fig. 1). All steroidal glycosides obtained (**1**–**26**) were evaluated for their cytotoxicities using three cancer cell lines (HepG2, Hela, U251). The results showed all compounds, except **6** and **10**, displayed cytotoxicities against several cell lines at different degrees.

2. Experimental

2.1. General methods

Optical rotations were obtained on a JASCO P-1020 polarimeter. IR spectra were measured on Bruker Tensor 27 spectrometer in KBr pellets. NMR spectra were performed in $CDCl_3$ or C_5D_5N, recorded on Bruker DRX-500 instrument with TMS as internal standard. UV spectra were recorded on Shimadzu UV-2450 spectropolarimeter. HRESIMS were measured on Agilent LC-MSD TOF spectrometer. Silica gel GF_{254} prepared for TLC and silica gel (100–200 mesh) for column chromatography (CC) were obtained from Qingdao Marine Chemical Company, Qingdao, China. Compounds were purified by semi-preparative HPLC (Beijing Tong Heng Innovation Technology Co., Ltd. LC3000), equipped with a column Zorbax SB-C_{18} (9.4 mm × 250 mm, 5 μM).

2.2. Plant material

The roots of *C. otophyllum* were collected from Traditional Chinese Medicine market in Kunming, China, and identified by Prof. Shiming Guo (Yunan Institute of Traditional Chinese Medicine and Material Medica, China). A voucher specimen (No. Co-2012-05) has been deposited in the laboratory of Faculty of Life Science and Technology, Kunming University of Science and Technology.

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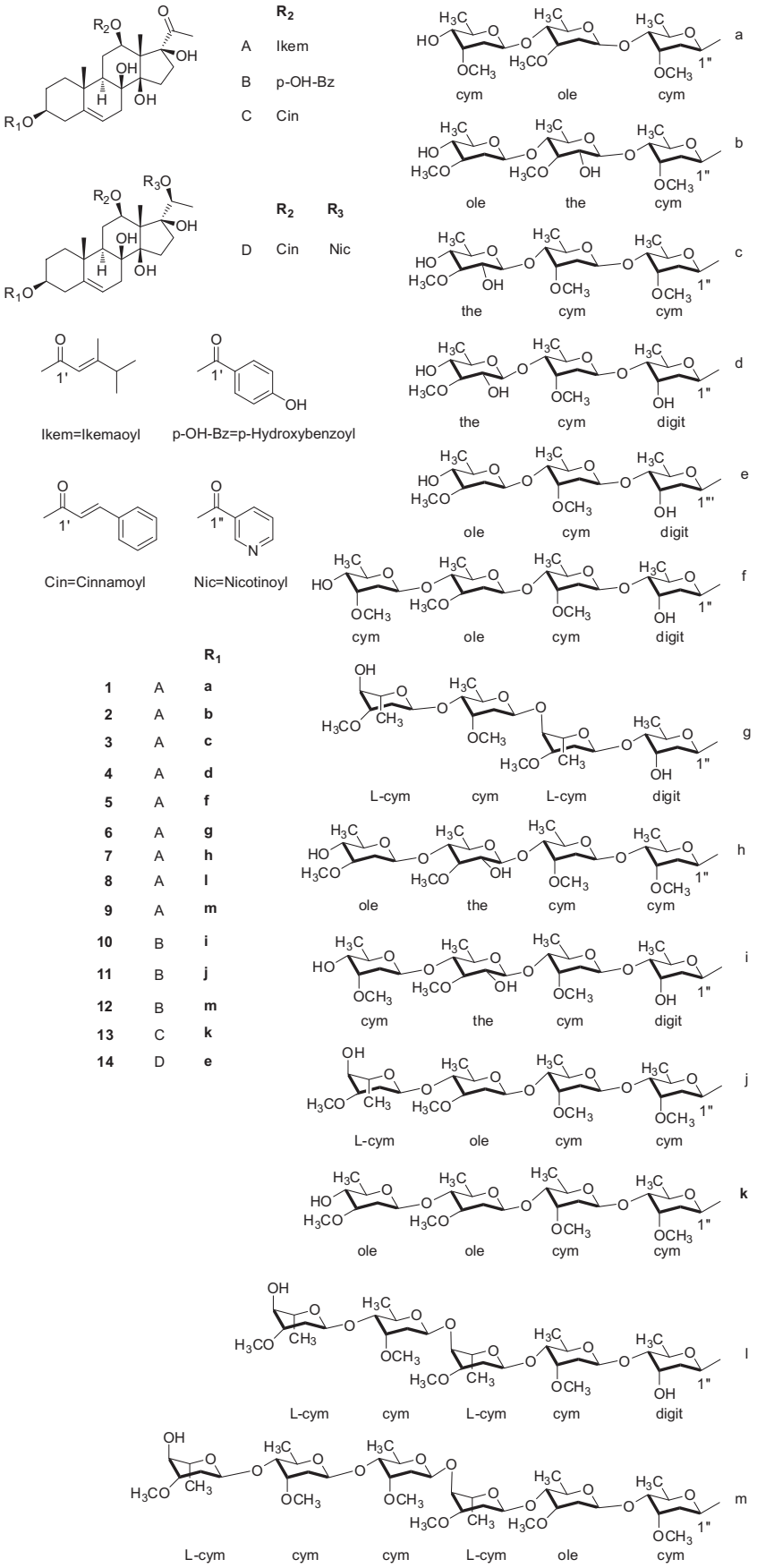

Fig. 1. Pregnane glycosides (**1**–**14**) isolated from the roots of *C. otophyllum*.