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Pregnane glycosides from *Cynanchum atratum*

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ABSTRACT

Five new pregnane glycosides, cynanosides K–O (**1–5**) with a 14,15-seco-pregnane-type skeleton as the aglycon, together with five known compounds, cynascyroside C, sublanceoside E_1, sublanceoside I_1, atratoside A and atratoside B, were isolated from the roots of *Cynanchum atratum*. Their structures were determined on the basis of spectroscopic analysis and chemical evidence.

1. Introduction

The roots of *Cynanchum atratum* (Asclepiadaceae), commonly called "Bai Wei" in Chinese, have been used in traditional medicine as an antifebrile, diuretic and antidote in China [1]. Pharmacological studies on this plant have demonstrated cytotoxic, anti-inflammatory and acetylcholinesterase-inhibitory activities [2,3]. The potential medicinal importance and our interest in the chemistry of structurally unique natural products prompted us to investigate the roots of *C. atratum*. Previously, we have reported the isolation of 11 pregnane glycosides [4]. In our continuing phytochemical study on this species, 10 steroidal glycosides were isolated, including five new compounds, named cynanosides K–O (**1–5**) with a 14,15-seco-pregnane-type skeleton as the aglycon (Fig. 1), and five known compounds, cynascyroside C [5], sublanceoside E_1 [6], sublanceoside I_1 [6], atratoside A and atratoside B [7]. This paper describes the isolation and structural elucidation of the new compounds on the basis of spectroscopic analysis and chemical evidence.

2. Experimental

2.1. General

The IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5 dm length cell. The FABMS and HRFABMS were taken on a JEOL JMS-700 Mstation spectrometer. The ^1H and ^{13}C NMR spectra were measured with a JEOL ECP-500 spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). HPLC separations were carried out with a JASCO PU-2080 HPLC system, equipped with a Shodex RI-101 Differential Refractometer detector and a Senshu Pak RP-C_{18} column (150 mm × 20 mm i.d.). Silica gel (silica Gel 60N, Kanto Chemical Co., Inc., Tokyo, Japan), ODS (100–200 mesh, Chromatorex DM1020T ODS, Fuji Silysia Chemical Ltd., Aichi, Japan), and Diaion HP-20 (Mitusbishi Chemical Corporation, Tokyo, Japan) were used for column chromatography (CC).

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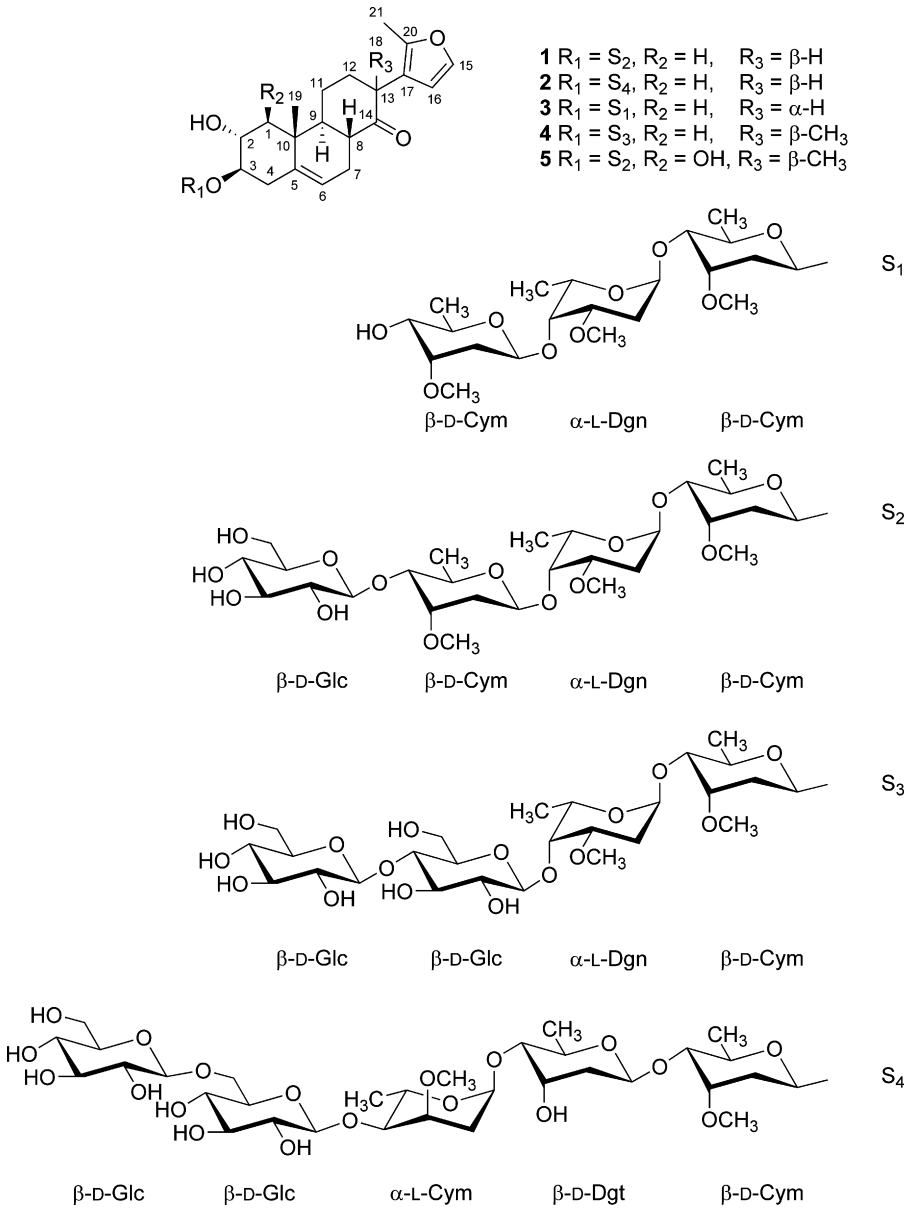

Fig. 1 – Structures of 1–5.

TLC was conducted in Kieselgel 60 F_{254} plates (E. Merck, U.S.A.).

2.2. *Plant material*

Plant material used in this research was collected from Hebei province of P. R. China in November 2003 and identified as *C. atratum* Bge. by Prof. Qishi Sun (Shenyang Pharmaceutical University). Voucher specimens are deposited in the Faculty of Pharmaceutical Sciences, Toho University.

2.3. *Extraction and isolation*

The roots of *C. atratum* (1.8 kg) were extracted with MeOH, and then partitioned between EtOAc and H_2O by the same procedure as our previous report [4]. The EtOAc extract (162 g) was subjected to silica gel CC with a gradient of $CHCl_3$/MeOH to give four fractions (1–4). Fraction 2 (82.9 g) was loaded on a Diaion HP-20 column and eluted with 50%, 70%, 90% and 100% MeOH. The 90% MeOH fraction (25.6 g) was separated by chromatography on a silica gel column using $CHCl_3$/MeOH (99:1, 97:3, 95:5 and 9:1) to give five fractions (A–E). Fraction B (6.17 g) was subjected to Sephadex LH-20 CC with MeOH to give three fractions (B_1–B_3). Fraction B_2 (1.72 g) was purified by HPLC (50% CH_3CN) to give **3** (6 mg), sublanceoside E_1 (8 mg), sublanceoside I_1 (108 mg) and atratoside A (242 mg). Further separation of fraction 3 (19.1 g) was achieved by ODS CC with 50%, 80% and 100% MeOH. The fraction eluted with 80% MeOH (7.90 g) was purified by HPLC (45% CH_3CN) to give **1** (253 mg), cynascyroside C (200 mg) and atratoside B (943 mg). In addition, the H_2O fraction was evaporated under reduced pressure below 40 °C to remove EtOAc, and then subjected to a Diaion HP-20 column with 30%, 50% and 100% MeOH. The 100% MeOH fraction (21.0 g) was then chromatographed on a silica gel