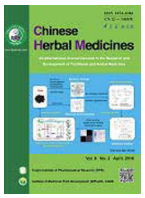

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Letter

Three New C_{21} Steroidal Glycosides from Roots of *Cynanchum otophyllum*

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ABSTRACT

Objective To investigate the chemical structures of glycosides in the roots of *Cynanchum otophyllum* (Asclepiadaceae) and to find new glycosides. **Methods** The total glycosides in the roots of *C. olophyllum* were separated by silica gel column chromatography. The structures of the resulting compounds were determined by NMR and FAB-MS spectra. **Results** Three C_{21} steroidal glycosides were separated. Their structures were determined as caudatin 3-*O*-(4-*O*-methyl-β-*D*-cymaropyranosyl)-(1→4)-α-*D*-oleandropyranosyl-(1→4)-β-*D*-glucopyranosyl-(1→4)-α-*L*-rhamnopyranoside (**1**), caudatin 3-*O*-β-*D*-digitoxopyranosyl-(1→4)-α-*D*-oleandropyranosyl-(1→4)-β-*D*-diginopyranosyl-(1→4)-β-*D*-glucopyranoside (**2**), and caudatin 3-*O*-β-*D*-diginopyranosyl-(1→4)-α-*D*-oleandropyranosyl-(1→4)-α-*D*-oleandropyranosyl-(1→4)-β-*D*-glucopyranoside (**3**), respectively. **Conclusion** Glycosides **1**−**3** are new compounds.

Key words

Asclepiadaceae; *Cynanchum otophyllum*; C_{21} steroidal glycoside

1. Introduction

Cynanchum otophyllum Schneid., a plant of genus *Cynanchum* L. (Asclepiadaceae), is distributed extensively over southwestern China. The roots of *C. otophyllum*, called Qingyangshen in Chinese, are important for the treatment of epilepsy by local people in China. Pharmacodynamic and clinical experiments showed that the chloroform and ethyl acetate extracts of the roots were particularly effective against epilepsy and chronic hepatitis (Zhou, 1991). Since 1984, Qingyangshen Tablets (total glycosides from the roots of *C. otophyllum*) have been manufactured by Lijiang Pharmaceutical Co., Yunnan Baiyao Group, China. From the roots of *C. otophyllum*, Prof. Mu et al isolated nine constituents including two C_{21} steroidal glycosides (Mu et al, 1986). Then, Mu and co-workers developed *C. otophyllum* into three novel medicines (Patents of China: ZL 98 1 18938.5, ZL 98 1 18173.2, and ZL 96 1 11270.0). From the total glycosides (ethyl acetate extract of the roots) and their acidic hydrolysis part, we isolated seven new carbohydrates (Zhao et al, 2004; 2008), and nine new C_{21} steroidal glycosides (Zhao et al, 2005; 2006; 2014; 2015). Seven constituents including two novel compounds were also isolated from the roots (Zhao et al, 2007). In addition, this article reports three new C_{21} steroidal glycosides obtained from the total glycosides in the roots of *C. otophyllum*.

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2. Materials and methods

2.1 General

Melting points were determined on a WC−1 Micromelting Point Apparatus (uncorrected, Instrument Plant of Sichuan University, China). Optical rotations were measured on a Horiba Sepa−300 Digital Polarimeter (Japan). The IR spectra were determined on a Perkin-Elmer 577 Spectrophotometer (USA). The UV spectra were determined on a Shimadzu Double-beam 210A Spectrophotometer (Japan). FABMS was measured on a VG AutoSpec−3000 Spectrometer (UK). Bruker Am−400 and DRX−500 Instruments (USA) were used to record ^{1}H-NMR, 2D NMR (400 MHz), and ^{13}C-NMR. C_5D_5N was the NMR solvent and internal standard at room temperature. Silica gel (200−300 mesh) for column chromatography (CC) and silica gel plate (GF−254) for thin-layer chromatography (TLC) were the products of Qingdao Haiyang Chemical Group Co., China. RP-18 reversed-phase silica gel was from Merck Corporation (Germany).

2.2 Plant materials

The roots of *Cynanchum otophyllum* Schneid. were bought from a drug market in Kunming, China. It was identified by Dr. Yue-mao Shen and a voucher specimen (KUN No. 0776933) was deposited in Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

2.3 Extraction and separation

The powder of dried roots of *C. otophyllum* (40 kg) was extracted with EtOH (120 L × 3). The extracts were evaporated, then extracted with EtOAc (6 L), and defatted with petroleum ether (1.4 L). The resulted extract was called the total glycosides (0.70 kg). Above procedure was completed at the processing factory of the institute. A part of total glycosides (300 g) were separated into 23 fractions (Frs. 1−23) through CC over silica gel by elution with a gradient mixture of $CHCl_3$-MeOH (100:0→100:25). Fr. 10 (1.25 g, 100:12 needed) was subjected to silica gel CC (71 g) eluted with $CHCl_3$-MeOH (9:1), and then to silica gel CC (20 g) eluted with $CHCl_3$-acetone (3:7), and finally to RP-18 CC (21 g) eluted with MeOH-H_2O (8:2, 75:25), to afford glycoside **1** (76 mg, yield: 0.000 44%). Fr. 8 (30.5 g, 100:10 needed) was subjected to two silica gel CCs and three RP-18 CCs to yield glycoside **2** (44 mg, yield: 0.000 26%). Fr. 9 (0.735 g, 100:12) was subjected to silica gel CC (71 g) eluted with $CHCl_3$-MeOH (9:1) and to silica gel CC (12 g) eluted with EtOAc-MeOH (100:5, 100:7, 100:9, and 8:2) to afford glycoside **3** (30 mg, yield: 0.000 18%).

3. Results and discussion

Glycoside **1**: colorless gum; mp 157.0−158.5 °C; $[\alpha]_D^{20.0}$ +19.9° (*c* 0.22, EtOH); UV (EtOH) λ_{max} (log ε): 224.6 (4.07) nm; IR (KBr) υ_{max}: 3443, 2967, 2933, 2364, 2339, 1713, 1640, 1456, 1384, 1316, 1277, 1224, 1195, 1165, 1084, 1005, 946, 912, 895, 864, 823, 668, 562, 533, 417 cm^{-1}; ^{1}H-NMR, ^{13}C-NMR, and 2D NMR data were presented in Table 1. FAB-MS *m/z*: 1099 $[M - 2H]^-$ (100), 1055 (1.5), 971 (18), 159 (2.5); HRFAB-MS *m/z*: 1100.5733 $[M]^-$ (calcd. for $C_{55}H_{88}O_{22}$, 1100.5767).

The molecular formula of glycoside **1** was determined by HRFAB-MS as $C_{55}H_{88}O_{22}$. The ^{13}C-NMR and DEPT spectra showed two carbonyls, two pairs of double bond, 12 methyls, 10 methylenes, and numerous methines and quaternary carbons. The spectra were compared with the ^{13}C-NMR and DEPT data (Zhang et al, 2000) of known C_{21} steroidal aglycones, and the aglycone was determined as caudatin. In compound **1**, C-3 of the aglycone corresponded to the proton at δ 4.03 (m) in the HMQC, and it had a long-range correlation with the resonance at δ 104.9. The signal for C-3 was at δ 78.0, so the compound was 3-*O*-glycoside of caudatin. The anomeric carbon resonances at δ_C 104.9, 106.0, 96.4, and 100.5 revealed the presence of four sugar residues. In Table 1, the proton at δ 5.12 (m) correlated with the signal at δ 104.9 in the HMQC, and had a correlation with H-2^I in the ^{1}H-^{1}H COSY. The assignment for C-2^I (δ 75.9) was obtained from the correlation with H-2^I (δ 4.03) in the HMQC. The proton at δ 4.03 (H-2^I, m) had correlations with H-1^I and H-3^I in the ^{1}H-^{1}H COSY, from which C-3^I (δ 72.0) was obtained. In this case, the carbons at δ 104.9, 75.9, 72.0, 83.0, 69.3, and 18.7, were determined to be the carbons of the sugar residuse I by ^{1}H-^{1}H COSY and HMQC. The ^{13}C-NMR data of carbons of the sugar were compared with the literature (Zhang et al, 2000) and the sugar was determined to be α-*L*-rhamnopyranose. C-4^I was found to be at δ 83.0, and its corresponding proton in the HMQC, 3.85 (m), had a long-range correlation with δ 106.0 in HMBC. Consequently, the O-4^I was linked with the sugar unit whose anomeric carbon (C-1^{II}) was at δ 106.0. On the basis of correlations between the protons in ^{1}H-^{1}H COSY and correlations in HMQC in Table 1, all the ^{13}C-NMR data of unit II were determined. The data were compared with the literature (Zhang et al, 2000) and the moiety II was determined to be β-*D*-glucopyranose. C-4^{II} at δ 78.7, and in HMBC, the H-1^{IV} of the sugar at δ 100.5 (C-1^{IV}) had a long-range correlation with the C-4^{III} of the sugar unit whose anomeric carbon (C-1^{III}) was at δ 96.4. Thus, O-4^{II} was linked with the sugar unit III. This sugar was determined to be α-*D*-oleandropyranose (Table 1). The methoxy group (59.0) was located by the correlation of the resonance of δ 3.54 (m), with C-3^{III} in the HMBC spectrum. C-4^{III} was apparent at δ 83.4, and it showed a long-range correlation with the proton at δ 5.08 m, which was correlated with resonance at δ 100.5 in HMQC. Consequently, O-4^{III} was linked with the sugar with the anomeric carbon at δ 100.5. This sugar was determined to be 4-*O*-methyl-β-*D*-cymaropyranose (Table 1). Since C-4^{IV} was linked with the MeO- group, and there was no remaining sugar, so it was the terminal sugar moiety. Therefore, compound **1** was elucidated as caudatin 3-*O*-(4-*O*-methyl-β-*D*-cymaropyranosyl)-