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New pregnane glycosides from the roots of *Cynanchum otophyllum*

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ABSTRACT

Six new pregnane glycosides with an acyl at C-12 and a straight sugar chain at C-3, namely otophyllosides H–M (**1–6**), were isolated from the roots of *Cynanchum otophyllum* (Asclepiadaceae) collected from Eryuan County in Yunnan province of China. Their structures were characterized to be qingyangshengenin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**1**), qingyangshengenin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**2**), qingyangshengenin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**3**), qingyangshengenin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-thevetopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**4**), caudatin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranoside (**5**), caudatin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside (**6**), respectively, on the basis of detailed spectroscopic analysis and chemical method.

1. Introduction

Cynanchum otophyllum Schneid (Chinese name Qingyangshen) is a folk medicinal plant endemic to Yunnan province of China, whose root was used for the treatment of epilepsy, rheumatic pain, kidney weakness, and muscle injures by the local people of its growing area. Based on the chemical and pharmacological experiments, it has been developed as an anti-epilepsy remedy and put into industrial production for many years in China [1–7]. The pregnane glycosides were determined as effective ingredients and otophyllosides A–G as main constituents were previously reported [2,8,9]. In a continuation of our phytochemical investigation of traditional Chinese medicinal plants to search for novel biologically active compounds, six new pregnane glycosides were isolated from the roots of this plant. Their structures were determined by detailed spectroscopic analysis, including 2D NMR techniques, and acidic hydrolysis. This paper describes the structure elucidation of these new compounds.

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2. Experimental

2.1. General methods

Melting points were measured on an XRC-I micromelting point apparatus and were uncorrected. Optical rotations were obtained on a SEPA-300 automatic digital polarimeter. IR spectra were determined on a Bruker Tensor 27 spectrometer in KBr pellets. NMR spectra were performed in CD_3OD unless otherwise noted and recorded on Bruker DRX-400 and -500 instruments with TMS as internal standard. MS data were detected on a VG Auto Spec-3000 spectrometer. Silica gel HF_{254} prepared for TLC and silica gel (200–300 mesh) for column chromatography (CC) were obtained from Qingdao Marine Chemical Company, Qingdao, China. Reversed phase silica gel Rp-18 and Rp-8 for CC were purchased from Merck & Co. Inc. L-Glucose, D-glucose, L-cysteine methyl ester hydrochloride, and 1-(trimethylsilyl)imidazole were purchased from Sigma (USA), Supelco (USA), Aldrich (USA), and Fluka (Switzerland), respectively.

2.2. Plant material

The roots of *C. otophyllum* Schneid were collected at Eryuan County, in the northwest of Yunnan province, China, and identified by Prof. C.R. Yang (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen is deposited in the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried roots of *C. otophyllum* (10 kg) were extracted with 90% EtOH at room temperature for three times (72, 72, and 48 h, 15 L × 3). After removal of the organic solvent *in vacuo*, the residue was suspended in water (2 L) and partitioned with $CHCl_3$ for three times (1.5 L × 3) to give a $CHCl_3$ extract (155 g), part of which (150 g) was subjected to a silica gel CC (11 cm × 120 cm) and eluted with $CHCl_3$–MeOH (10:1.5, 7.6 L) to give five fractions (Frs. 1–5). Fr. 3 (16.1 g) was repeatedly chromatographed over silica gel [$CHCl_3$–MeOH (10:0.8) and EtOAc–EtOH–H_2O (12:1:0.5)] and Rp-8 (MeOH–H_2O, 68% → 85%) to afford **1** (370 mg) and **4** (46 mg). Fr. 4 (6.4 g) was chromatographed on silica gel [$CHCl_3$–MeOH (9:1) and EtOAc–EtOH–H_2O (8:1:0.5)], Rp-8 and Rp-18 (MeOH–H_2O, 65% → 100%) columns to give **2** (87 mg), **3** (92 mg), and **5** (59 mg). Fr. 5 (20.0 g) was subjected repeatedly to silica gel ($CHCl_3$–MeOH, 9:1.5) and Rp-18 (MeOH–H_2O, 60% → 100%) CC to yield **6** (210 mg).

Another part of the $CHCl_3$ extract (5 g) was dissolved in MeOH (50 ml) and treated with 5% HCl (10 ml) at 90 °C for 30 min. After cooled down to room temperature and neutralized with NaOH (4 mol/L) to pH 6–7, the reaction mixture was evaporated under reduced pressure to remove of MeOH. The condensation was diluted with water and extracted by $CHCl_3$ for three times (50 ml × 3). The $CHCl_3$ phase (1.8 g) was chromatographed repeatedly on silica gel column to give **7** (76 mg) and **8** (93 mg).

Table 1 – ^{13}C NMR data for the aglycone moieties of 1–6[a] (δ in ppm, in CD_3OD, 100 MHz)

Position	1	2	3	4[a]	5	6[a]
1	39.8	39.8	39.8	39.2	39.8	39.3
2	30.1	30.1	30.1	29.9	30.2	29.9
3	79.3	79.3	79.3	77.6	79.3	77.7
4	39.8	39.8	39.8	39.1	39.8	39.0
5	140.3	140.3	140.3	139.4	140.3	139.4
6	119.4	119.6	119.6	119.2	119.6	119.2
7	35.2	35.2	35.2	34.8	35.2	34.8
8	75.0	75.0	75.0	74.3	75.0	74.4
9	45.1	45.1	45.1	44.5	45.2	44.6
10	38.1	38.1	38.1	37.4	38.1	37.5
11	25.5	25.5	25.5	25.2	25.4	25.1
12	73.7	73.5	74.0	73.4	73.3	72.6
13	59.1	59.1	59.1	58.4	58.7	57.5
14	90.0	90.0	90.0	89.6	89.9	89.5
15	34.3	34.3	34.3	33.9	34.2	33.9
16	33.4	33.4	33.5	33.2	33.2	33.0
17	93.1	93.0	93.1	92.5	93.0	92.4
18	10.6	10.6	10.6	10.9	10.4	10.7
19	18.6	18.6	18.5	18.2	18.5	18.2
20	211.9	211.8	212.0	209.9	211.5	209.5
21	27.7	27.7	27.8	27.9	27.5	27.6
1′	167.3	167.5	166.9	165.4	167.4	166.0
2′	119.6	118.3	121.5	122.0	114.3	114.2
3′	132.8	132.8	132.8	132.5	167.4	165.5
4′	117.6	118.1	116.6	116.2	39.3	38.2
5′	168.3	169.9	164.9	163.6	21.3	20.9
6′	117.6	118.1	116.6	116.2	21.2	21.0
7′	132.8	132.8	132.8	132.5	16.7	16.5

[a] The spectral data were obtained in C_5D_5N.

2.3.1. Otophylloside H (1)

White amorphous powder, mp 213–216 °C, $[\alpha]_D^{25}$ + 8.0° (*c* = 0.83, MeOH), IR (KBr) ν_{max} 3442, 2934, 1709, 1610, 1591, 1504, 1452, 1384, 1309, 1276, 1163, 1072, 911, 854, 773 cm^{-1}. FAB-MS (negative ion mode): *m/z* 1242 $[M]^-$, 1105 $[M-137]^-$, 943 $[M-137-162]^-$. HRFAB-MS (negative ion mode): *m/z* 1241.5628 $[M(C_{60}H_{90}O_{27})-H]^-$ (calcd. 1241.5591). ^1H and ^{13}C NMR: see Tables 1–3 .

2.3.2. Otophylloside I (2)

White amorphous powder, mp 201–203 °C, $[\alpha]_D^{27}$ + 1.5° (*c* = 0.55, MeOH), IR (KBr) ν_{max} 3447, 2934, 1708, 1609, 1590, 1504, 1452, 1383, 1369, 1309, 1276, 1163, 1093, 1004, 912, 854, 773 cm^{-1}. FAB-MS (negative ion mode): *m/z* 1080 $[M]^-$, 942 $[M-1-137]^-$, HRFAB-MS (negative ion mode): *m/z* 1079.5030 $[M(C_{54}H_{80}O_{22})-H]^-$ (calcd. 1079.5063). ^1H and ^{13}C NMR: see Tables 1–3.

2.3.3. Otophylloside J (3)

White amorphous powder, mp 179–182 °C, $[\alpha]_D^{27}$ + 12.1° (*c* = 0.46, MeOH), IR (KBr) ν_{max} 3449, 2934, 1711, 1610, 1594, 1515, 1452, 1382, 1276, 1165, 1094, 1005, 912, 866, 853, 772 cm^{-1}. ESI-MS (negative ion mode): *m/z* 1224 $[M]^-$, 1062 $[M-162]^-$. HRESI-MS (negative ion mode): *m/z* 1223.5840 $[M(C_{61}H_{92}O_{25})-H]^-$ (calcd. 1223.5849). ^1H and ^{13}C NMR: see Tables 1–3.