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Five new steroidal glycosides from the roots of *Cynanchum stauntonii*

Kaiming Wang[a], Ang Li[a], Ruiling Zhang[b], Siying Li[a], Fang Zhang[a], Lei Zhao[c,*], Zhongxi Zhao[a,d,**], Shanzhong Li[d], Jianhua Cai[d], Jimin Cao[d]

[a] School of Pharmaceutical Sciences, Shandong University, 44 West Wenhua Road, Jinan, Shandong 250012, China
[b] Shandong Analysis and Test Center, Jinan, Shandong 250014, China
[c] Shandong Reyoung Pharmaceutical Co., Ltd., Yiyuan, Shandong 256100, China
[d] Jiangsu Shengshi Kangde Biotech Corporation, Lianyungang, Jiangsu 222006, China

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ABSTRACT

Five new steroidal glycosides, named as stauntosides D_1 (**1**), D_2 (**2**), D_3 (**3**), I_1 (**4**), and I_2 (**5**), were isolated from the 95% ethanol extract of the roots of *Cynanchum stauntonii*. Their structures were elucidated by extensive spectroscopic analyses, including 1D, 2D NMR, and HRESI–MS, and qualitative chemical methods. All compounds were tested for cytotoxicity against five human tumor cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) where they were found to be inactive. Their significance in terms of the chemotaxonomy of *C. stauntonii* is discussed.

1. Introduction

Cynanchum stauntonii (Decne.) Schltr. ex Levl., a perennial medicinal herb belonging to the family of Asclepiadaceae, is widely distributed in south-central region of China. It is given the Chinese name of 'Bai Qian', which has been used as antitussives and expectorants in traditional Chinese medicine (TCM) (Anon., 1995). Previous chemical study had reported the occurrences of steroidal saponins, especially pregnane glycosides in the roots of *C stauntonii*, including 14,15-secopregnane-type skeleton glycosides and 13,14:14,15-disecopregnane-type skeleton (Shibano et al., 2012; Wang et al., 2004; Yu et al., 2013; Yu and Zhao, 2015). This class of compound has been accepted as the most important and characteristic chemical constituents in *Cynanchum* species. As a result of the chemical constituents of this plant, herein we describe the isolation and structural determination of five new C_{21} steroidal glycosides (**1**–**5**) from the roots of *C. stauntonii*. The isolated compounds containing steroid aglycones with either the 13,14:14,15-disecopregnane-type skeleton or the 14,15-secopregnane-type skeleton were given the trivial names stauntosides D_1 (**1**), D_2 (**2**), D_3 (**3**), I_1 (**4**), and I_2 (**5**). In this paper, we report the evaluation of their cytotoxic activities against five human tumor cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780). Unfortunately, none showed activity ($IC_{50} > 10\ \mu M$).

2. Experimental

2.1. General methods

Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter at 20 °C. IR spectra were recorded on a Nicolet 5700 spectrometer. NMR spectra were obtained using a Varian INOVA-500 spectrometer operating at 500 (^1H) and 125 (^{13}C) MHz with TMS as an internal standard. ESI–MS and HRESI–MS were carried out on an LTQ-Orbitrap XL. GC analyses were conducted on an Agilent 7890A instrument. Preparative HPLC was performed on an Agilent 1260 system equipped with a G1311B quaternary pump, a G1311B degasser, and a G4212B DAD detector, using a ZORBAX SB-C_{18} column (9.4 mm × 250 mm, 5 μm). Silica gel (200–

* *Corresponding author at: Shandong Reyoung Pharmaceutical Co., Ltd. Yiyuan, No. 6 Erlangshan Road, Yi Yuan County Yuan County 256100, China.
** Corresponding author at: School of Pharmaceutical Sciences, Shandong University, 44 West Wenhua Road, Jinan 250012, China.
E-mail addresses: zhaolei2008boy@163.com (L. Zhao), zxzhao@sdu.edu.cn (Z. Zhao).

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300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China) and C_{18} reversed-phase silica gel (150–200 mesh, Merck) were used for column chromatography (CC). Pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co., Ltd.) were used for TLC. Spots were visualized under UV light or by spraying with H_2SO_4-EtOH (1:9, v/v) followed by heating. Acetonitrile used in preparative HPLC procedure was in HPLC grade, and other solvents were of analytical grade.

2.2. Plant material

The roots of *C. stauntonii* were collected in Henan Province, China, in August 2011 and identified by associate Prof. Huning Chen (a savant in plant systematics, School of Pharmaceutical Sciences, Shandong University). A voucher specimen (No. 20130821) was deposited at the Department of Natural Products Chemistry, School of Pharmaceutical Sciences, Shandong University, P. R. China.

2.3. Extraction and isolation

The dried-up and pulverized roots (10 kg) of *C. stauntonii* were extracted with 95% EtOH at room temperature (3 × 15 L, each for one week). Evaporation of the solvent in vacuo provided a dark residue (3500 g). The residue was suspended in 80% aqueous ethanol (ca. 10000 mL) and then extracted with, firstly, petroleum ether, and secondly, EtOAc, respectively and successively, in separatory funnel (3–5 × 6000 mL). After removal of the organic solvent in vacuo at 40 °C, 150 g of EtOAc brown residue was obtained. This resulting residue was fractionated by CC over silica gel (6 × 120 cm) eluted with gradient solvents of CH_2Cl_2-MeOH (100:0 to 1:1, 2–3 L each of the solvent mixture) to yield 13 fractions (designated as fractions A to M) according to their TLC profiles. Fraction F (6.0 g) was then further separated by Flash C_{18} column chromatography (3 × 120 cm) eluted with CH_3OH/H_2O (2:3 to 0:1, 0.5 L each of the solvent mixture) to give six subfractions (F1 to F6). Fraction F6 (0.7 g) was applied to preparative HPLC system [mobile phase: CH_3CN/H_2O (48:52, v/v); flow rate: 5 mL/min; UV detection at 210 nm] resulting in the isolation of compound **3** (60 mg, purity > 90% by HPLC). Fraction G (6.0 g) was subjected to Flash C_{18} column chromatography (3 × 120 cm) eluted with CH_3OH/H_2O (2:3 to 0:1) to give seven subfractions (G1 to G7), resulting in the isolation of compound **2** (60 mg, purity >91% by HPLC). Fraction G5 (0.65 g) was purified by preparative HPLC system [mobile phase: CH_3CN/H_2O (40:60, v/v); flow rate: 5 mL/min; UV detection at 210 nm and 280 nm] to obtain compound **5** (90 mg, purity >90% by HPLC). Fraction H (10.0 g) was further separated by Flash C_{18} column chromatography (3 × 120 cm) eluted with CH_3OH/H_2O (2:3 to 0:1, 0.5 L each of the solvent mixture) to give six subfractions (H1 to H6). Fraction H3 (0.15 g) was subjected to preparative HPLC system [mobile phase: CH_3OH/H_2O (60:40, v/v); flow rate: 5 mL/min; UV detection at 210 nm and 280 nm] to yield compound **4** (80 mg, purity >92% by HPLC). Fraction H4 (0.50 g) was separated using preparative HPLC system [mobile phase: CH_3CN/H_2O (40:60, v/v); flow rate: 5 mL/min; UV detection at 210 nm] to yield compound **1** (20 mg, purity >91% by HPLC).

2.3.1. Stauntoside D_1 (**1**)

White lamellae (CH_2Cl_2—CH_3OH), $[\alpha]_D^{20}$ −10.3 (c = 0.12, $CHCl_3$, 20 °C). IR (KBr) ν_{max}: 3440, 2933, 1732, 1648, 1450, 1381, 1163, 1070, 874 cm^{-1}. ESI–MS (positive mode) *m/z*: 513.3 $[M + Na]^+$. HRESI–MS (positive mode) *m/z*: 513.2460 $[M + Na]^+$, calcd for $C_{27}H_{38}O_8Na$, 513.2459. ^1H NMR (500 MHz, pyridine-d_5) data for aglycone and sugar moiety: see Tables 1 and 2. ^{13}C NMR (125 MHz, pyridine-d_5): see Tables 2 and 3.

2.3.2. Stauntoside D_2 (**2**)

White amorphous powder (CH_2Cl_2—CH_3OH), $[\alpha]_D^{20}$ +4.6 (*c* = 0.13, CH_3OH, 20 °C). IR (KBr) ν_{max}: 3409, 2930, 1739, 1658, 1434, 1370, 1301, 1160, 1064, 869, 811 cm^{-1}. ESI–MS (positive mode) *m/z* 643.3 $[M + Na]^+$. HRESI–MS (positive mode) *m/z*: 643.3111 $[M + Na]^+$, calcd for $C_{33}H_{48}O_{11}Na$, 643.3089. ^1H NMR (500 MHz, pyridine-d_5) data for aglycone and sugar moiety: see Tables 1 and 2. ^{13}C NMR (125 MHz, pyridine-d_5): see Tables 2 and 3.

2.3.3. Stauntoside D_3 (**3**)

White amorphous powder (CH_2Cl_2—CH_3OH), $[\alpha]_D^{20}$ +21.1 (*c* = 0.16, CH_3OH, 20 °C). IR (KBr) ν_{max}: 3452, 2934, 1732, 1651, 1450, 1381, 1308, 1191, 1069, 913, 873 cm^{-1}. ESI–MS (positive mode)

Table 1
The ^1H NMR chemical shifts of the aglycone moieties of compounds **1-5** (500 MHz in pyridine-d_5; δ in ppm, *J* values in Hz).

Position	1	2	3	4	5
1α	0.95 td (12.5, 4.0)	0.94 td (13.5, 4.0)	0.94 td (13.5, 3.5)	1.44 td (13.5, 3.0)	1.13 td (14.0, 3.0)
1β	1.83 m	1.81 dt (13.5, 4.0)	1.83 dt (13.5, 3.5)	1.58 m	1.58 m
2α	2.06 m	2.11 m	2.03 m	2.15 m	2.10 m
2β	1.40 m	1.39 m	1.40 m	1.67 m	1.65 m
3	3.84 m	3.77 m	3.77 m	3.86 m	3.79 ov
4α	2.59 dd (11.5, 2.0)	2.55 m	2.45 m	2.62 brd (14.0)	2.61 brd (13.5)
4β	2.30 brt (11.5)	2.28 brt (11.0)	2.30 brt (11.0)	2.32 brd (14.0)	2.32 brd (13.5)
6	5.42 m	5.40 m	5.41 m	5.38 m	5.38 m
7α	2.15 m	2.15 m	2.13 m	2.72 brd (20.5)	2.74 brd (21.0)
7β	2.64 dt (12.5, 2.5)	2.63 dt (12.5, 2.5)	2.64 dt (11.5, 3.0)	3.16 td (20.5, 3.5)	3.18 td (21.0,4.0)
8	2.50 td (11.5, 4.5)	2.50 m	2.51 m	–	–
9	1.23 br t (10.0)	1.22 br t (10.5)	1.23 br t (10.0)	2.12 m	2.12 m
11α	2.60 m	2.59 m	2.61 m	1.57 m	1.57 m
11β	1.39 m	1.38 m	1.39 m	1.27 m	1.26 m
12α	1.73 m	1.71 m	1.71 m	1.43 m	1.44 m
12β	2.10 m	2.02 m	2.10 m	1.95 dt (12.5, 3.0)	1.95 dt (12.5, 3.5)
15α	4.24 dd (7.0, 8.5)	4.24 dd (7.5, 8.0)	4.25 m	4.25 d (11.0)	4.26 d (10.5)
15β	3.96 d (8.5)	3.95 dd (8.5, 9.5)	3.95 m	3.78 dd (4.5, 11.0)	3.80 dd (4.5, 10.5)
16	5.45 dd (9.5, 7.5)	5.45 dd (9.5, 7.5)	5.45 dd (9.5, 7.5)	4.76 m	4.77 m
17	3.56 ov	3.56 ov	3.57 ov	2.81 d (8.0)	2.81 d (8.0)
18	6.48 br s	6.48 br s	6.48 br s	a4.02 d (8.5)	a4.00 d (9.0)
				b4.09 dd (1.5, 8.5)	b4.07 ov
19	0.84 s	0.83 s	0.85 s	0.77 s	0.77 s
21	1.54 s	1.54 s	1.54 s	1.57 s	1.57 s

ov, overlapped.