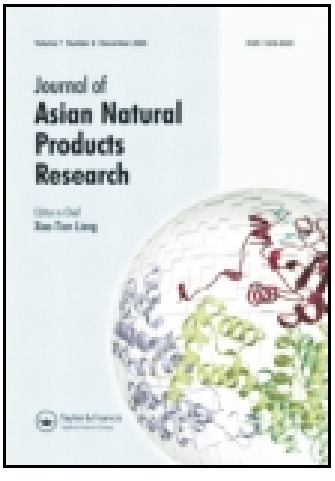

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Two new steroidal glycosides from *Cynanchum wallichii*

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Two new C21 steroidal glycosides were isolated from *Cynanchum wallichii* Wight. Their structures were elucidated as caudatin-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**1**) and 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-digitoxopyranoside (**2**) by spectroscopic methods including 1D and 2D NMR experiments.

Keywords: *Cynanchum wallichii*; C21 steroidal glycosides; 2,6-dideoxy-pyranose

1. Introduction

Cynanchum wallichii Wight, also named Duanjieshen, is a traditional Chinese medicine distributed extensively over southwest China. In the famous Chinese prescription "hulisan", *C. wallichii* has been used as the primary drug to treat arthrophlogosis and injury from fall or fracture. Several C21 steroidal glycosides with 2,6-dideoxysugars were isolated from the title plant [1–3]. In order to find out more C21 steroidal glycosides in this folk medicine, systematic research on the roots of *C. wallichii* was carried out and two new steroidal glycosides were found. In this paper, we report isolation and structural elucidation of two new glycosides **1** and **2** (Figure 1).

2. Results and discussion

Compound **1** was obtained as white amorphous powder, with $[\alpha]_D^{20} + 15.5$ ($c = 0.61$, MeOH). The molecular formula was determined to be $C_{54}H_{86}O_{21}$ by HR-FAB-MS at *m/z* 1093.5552 $[M + Na]^+$. The ^{13}C NMR spectrum of **1** (Table 1) showed four anomeric carbon signals at δ_C 96.4, 99.7, 101.9, and 104.5. The carbon signals assignable to the aglycone moiety were similar to those of caudatin moiety of otophylloside B [4,5]. Thus, compound **1** was considered to be a caudatin-3-*O*-glycoside.

Besides signals assignable to the aglycon, signals assignable to sugars were also observed. Proton signals of the sugar moiety were assigned to three secondary methyl groups at δ_H 1.30 (3H, d, $J = 6.0$ Hz), 1.42 (3H, d, $J = 6.0$ Hz), and 1.69 (3H, d, $J = 6.0$ Hz); two methoxyl groups at δ_H 3.52 (s) and 3.56(s); four anomeric protons at δ_H 4.65 (1H, br d, $J = 9.0$ Hz), 5.12 (1H, br d, $J = 8.0$ Hz), 5.16 (1H, br d, $J = 9.0$ Hz), and 5.45 (1H, br d, $J = 9.0$ Hz), whose multiplicities showed the presence of three 2,6-dideoxysugars with β configuration at the anomeric carbon. Finally, one glucopyranosyl, one oleandropyranosyl, one cymaropyranosyl, and one digitoxopyranosyl were elucidated by comparing NMR data with those in the literatures [6,7] and the

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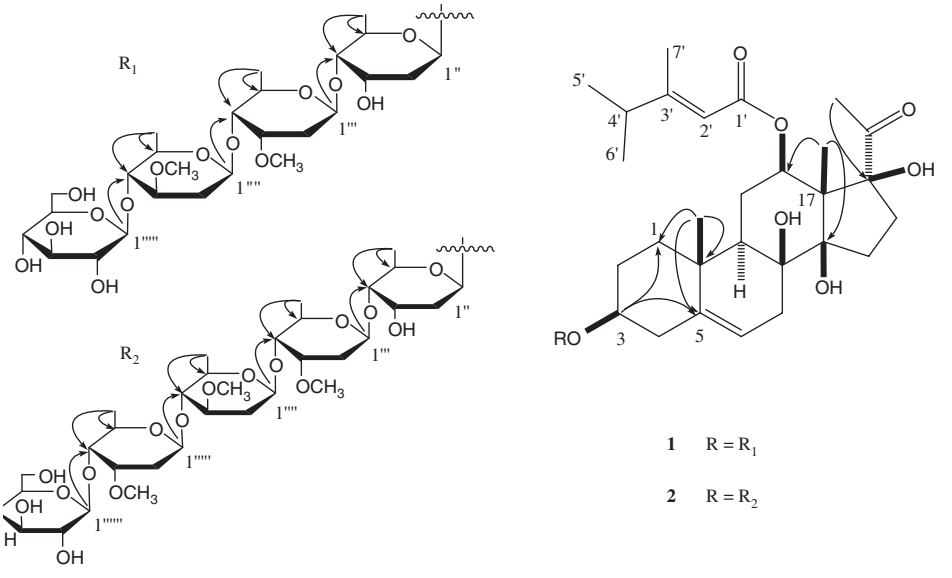

Figure 1. Structures and key HMBC correlations of compounds **1** and **2**.

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1** in C_5D_5N (600 MHz).

No.	δ_C	δ_H (*J* in Hz)		No.	δ_H (*J* in Hz)	δ_C
1	39.0	2.43 (m) and 2.52 (m)	β-D-Dgt	1″	5.45 (br d, 9.0)	96.4
2	29.9	1.78 (m) and 2.06 (m)		2″	[a]	38.9
3	77.7	3.86 (m)		3″	4.60 (m)	67.6
4	39.3	[a]		4″	3.51 (br d, 9.0)	83.3
5	139.4	–		5″	4.27 (m)	68.6
6	119.2	5.30 (br t)		6″	1.42 (d, 6.0)	18.5
7	34.8	2.30 (m) and 2.41 (m)	β-D-Cym	1‴	5.16 (br d, 9.0)	99.7
8	74.3	–		2‴	[a]	36.7
9	44.6	1.70 (m)		3‴	3.87 (m)	77.6
10	37.4	–		4‴	3.38 (br d, 9.0)	83.1
11	25.1	2.12 (m) and 2.25 (m)		5‴	4.17 (m)	69.0
12	72.6	5.03 (dd, 11.6, 3.9)		6‴	1.30 (d, 6.0)	18.5
13	58.0	–		3‴-OMe	3.56 (s)	58.9
14	89.5	–	β-D-Ole	1⁗	4.65 (br d, 9.0)	101.9
15	33.9	2.07 (m, 2H)		2⁗	[a]	37.6
16	32.9	2.48 (m, 2H)		3⁗	3.68[b]	79.3
17	92.4	–		4⁗	3.72 (m)	83.2
18	10.7	2.02 (s, 3H)		5⁗	3.60 (m)	72.0
19	18.2	1.30 (s, 3H)		6⁗	1.69 (d, 6.0)	18.7
20	209.4	–		3⁗-OMe	3.52 (s)	57.2
21	27.6	2.50 (s, 3H)	β-D-Glu	1⁗′	5.12 (br d, 8.0)	104.5
1′	166.0	–		2⁗′	4.01[b]	75.7
2′	114.2	–		3⁗′	4.24 (m)	78.7
3′	165.4	–		4⁗′	3.63	71.9
4′	38.2	1.10 (m)		5⁗′	3.97 (m)	78.0
5′	20.8	0.93 (d, 6.0, 3H)		6⁗′	4.35 (dd, 11.4, 5.4)	63.1
6′	20.9	0.95 (d, 6.0, 3H)			4.53 (br d 11.4)	
7′	16.5	2.26 (s, 3H)				

[a] Overlapped with other sugar signals.
[b] Overlapped with other signals.

hydrolysis experiment. The NMR data for the sugar moiety were assigned by HMBC and HSQC spectra and are shown in Table 1.

The sequence of the sugar moiety was determined by the HMBC spectrum, in which correlations from H-1‴″ at δ_H 5.12 to C-4‴″ at δ_C 83.2, from H-1‴″ at δ_H 4.65 to C-4‴ at δ_C 83.1, from H-1‴ at δ_H 5.16 to C-4″ at δ_C 83.3, and from H-1″ at δ_H 5.45 to C-3 at δ_C 77.7 of the aglycone were observed. Thus, compound **1** was finally determined as caudatin-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

Compound **2** was obtained as white amorphous powder, with $[\alpha]_D^{20} + 9.3$ ($c = 0.32$, MeOH). The molecular formula was determined to be $C_{61}H_{98}O_{24}$ by HR-ESI-MS at m/z 1237.6342 $[M + Na]^+$. The ^{13}C NMR spectrum of **2** (Table 2) assignable to the aglycone moiety was identical to that of compound **1**. Hence, compound **2** was also considered to be a caudatin-3-*O*-glycoside.

Table 2. ^1H and ^{13}C NMR spectral data of compound **2** in C_5D_5N (600 MHz).

No.	δ_C	δ_H (J in Hz)		No.	δ_H (J in Hz)	δ_C
1	39.0	2.43 (m) and 2.52 (m)	β-D-Dgt	1″	5.45 (br d, 9.0)	96.4
2	29.9	1.78 (m) and 2.06 (m)		2″	[a]	38.9
3	77.7	3.86 (m)		3″	4.60 (m)	67.6
4	39.3	[a]		4″	3.51 (br d, 9.0)	83.2
5	139.4	–		5″	4.27 (m)	68.6
6	119.2	5.30 (br t)		6″	1.42 (d, 6.0)	18.5
7	34.8	2.30 (m) and 2.41 (m)	β-D-Cym	1‴	5.16[b]	99.7
8	74.3	–		2‴	[a]	36.7
9	44.6	1.70 (m)		3‴	3.87 (m)	77.6
10	37.4	–		4‴	3.38 (br d, 9.0)	83.4
11	25.1	2.12 (m) and 2.25 (m)		5″	4.17 (m)	69.0
12	72.6	5.03 (dd, 11.6, 3.9)		6‴	1.30 (d, 6.0)	18.5
13	58.0	–		3‴-OMe	3.56 (s)	58.9
14	89.5	–	β-D-Ole	1⁗	4.66 (br d, 9.0)	101.9
15	33.9	2.07 (m, 2H)		2⁗	[a]	37.8
16	32.9	2.48 (m, 2H)		3⁗	3.51 (m)	78.7
17	92.4	–		4⁗	3.46 (m)	82.6
18	10.7	2.02 (s, 3H)		5⁗	3.49[b]	71.7
19	18.2	1.30 (s, 3H)		6⁗	1.40 (m)	18.7
20	209.4	–		3⁗-OMe	3.50 (s)	58.0
21	27.6	2.50 (s, 3H)	β-D-Cym	1⁗″	5.26[b]	98.4
1′	166.0	–		2⁗″	[a]	36.8
2′	114.2	–		3⁗″	4.14 (m)	78.3
3′	165.4	–		4⁗″	3.40 (m)	83.2
4′	38.2	1.10 (m)		5⁗″	4.26 (m)	69.7
5′	20.8	0.93 (d, 6.0, 3H)		6⁗″	1.61 (d, 6.0)	18.7
6′	20.9	0.95 (d, 6.0, 3H)		3⁗-OMe	3.52 (s)	58.9
7′	16.5	2.26 (s, 3H)	β-D-Glu	1⁗‴	4.91 (br d, 8.0)	106.5
				2⁗‴	3.98 (m)	75.3
				3⁗‴	3.96 (m)	78.4
				4⁗‴	4.17 (m)	71.8
				5⁗‴	4.23 (m)	78.4
				6⁗‴	4.37 (dd, 11.4, 5.4) 4.57 (br d, 11.4)	63.1

[a] Overlapped with other sugar signals.
[b] Overlapped with other signals.

Proton signals of the sugar moiety were also assigned to four secondary methyl groups at δ 1.30 (d, $J = 6.0$ Hz), 1.40 (m), 1.42 (d, $J = 6.0$ Hz), and 1.61 (d, $J = 6.0$ Hz); three methoxyl groups at δ 3.50 (s), 3.52 (s), and 3.56 (s); and five anomeric protons at δ 4.66 (br d, $J = 9.0$ Hz), 4.91 (br d, $J = 8.0$ Hz), 5.16, 5.26, and 5.45 (br d, $J = 9.0$ Hz), whose multiplicities showed the presence of four 2,6-dideoxy-sugar units. The ^{13}C shifts of each sugar unit were assigned unambiguously by HMBC and HMQC spectra and are shown in Table 2. The NMR data for the sugar moiety of compound **2** were almost identical to those of otophylloside M [7] except that the inner cymaropyranosyl unit disappeared and one digitoxopyranosyl moiety [2] appeared. The linkage of the sugar units was finally further confirmed by the HMBC correlations from H-1$''''''$ at δ_H 4.91 to C-4$''''''$ at δ_C 83.2, from H-1$''''$ at δ_H 5.26 to C-4$''''$ at δ_C 82.6, from H-1$'''$ at δ_H 4.66 to C-4$'''$ at δ_C 83.4, from H-1$'''$ at δ_H 5.16 to C-4$''$ at δ_C 83.2, and from H-1$''$ at δ_H 5.45 to C-3 at δ_C 77.7 of the aglycone. Hence, compound **2** was elucidated as 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-digitoxopyranoside.

The cytotoxic activities of compounds **1** and **2** were bioassayed against HL-60 cell lines with the same procedures described in the literature [3] and their IC_{50} values were 47.2 and 26.4 μM, respectively

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a P-E 241 MC (Perkin-Elmer Co., Jena, Germany). IR spectra were recorded on a Bruker IFS-55 infrared spectrophotometer with KBr disks (Bruker Co., Zurich, Switzerland). The NMR spectral data were recorded on Bruker AV-600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) with TMS as internal standard (Bruker Co.). HR-FAB-MS data were measured on Micross Mass Autospec-Ultima-TOF spectrometer (Bruker Co.). Silica gel GF254 for TLC and silica gel (200–300 mesh) for column chromatography were obtained from Qingdao Marine Chemical Company, Qingdao, China. HPLC was carried on Shimadzu LC-8A (Shimadzu, Kyoto, Japan) and the detector was Shimadzu SPD-10A. An analytical reversed phase C18 column (Diamonsil C18 ∅ 4.6 mm × 250 mm, Zuanshi Company, Shanghai, China) and a preparative reversed phase C18 column (Inertsil Prep-ODS ∅ 10 mm × 250 mm, Zuanshi Company) were employed.

3.2 Plant material

The roots of *C. wallichii* were collected in August 2011 at Xinxiang City, Henan Province, China and was identified by Prof. Yanjun Zhai. A voucher specimen was deposited in the Liaoning University of Traditional Chinese Medicine (No. 20110510).

3.3 Extraction and isolation

Roots of *C. wallichii* were extracted three times by means of reflux with hot 95% EtOH for 2 h each, and the combined solution was concentrated *in vacuo* to give a syrup (1100 g), followed by suspension in water. The suspension was then extracted with petroleum ether, chloroform, acetic ether, and *n*-butanol, successively. The acetic ether (90 g) was further fractionated by silica gel column chromatography eluted with chloroform–CH_3OH (100:1 → 1:1, v/v) to obtain 10 fractions. Fraction six (632 mg) was further separated by HPLC eluted with MeOH (68%, v/v, 4 ml/min, 210 nm, RT = 18 min for **1** and 25 min for **2**) to give compounds **1** (20 mg) and **2** (15 mg).

*3.3.1 Caudatin-3-O-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**1**)*

White amorphous powder; $[\alpha]_D^{20} + 15.5$ ($c = 0.61$, MeOH); IR (KBr) ν_{max}: 3444, 2930, 1731, 1640, 1451, 1164, 1104, 1062, and 988 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 1093.5552 $[M + Na]^+$ (calcd for $C_{54}H_{86}O_{21}Na$, 1093.5554).

*3.3.2 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-digitoxopyranoside (**2**)*

White amorphous powder; $[\alpha]_D^{20} + 9.3$ ($c = 0.32$, MeOH); IR (KBr) ν_{max}: 3444, 2930, 1731, 1640, 1451, 1164, 1104, 1062, and 988 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 2; HR-ESI-MS: *m/z* 1237.6342 $[M + Na]^+$ (calcd for $C_{61}H_{98}O_{24}Na$, 1237.6340).

3.4 Acid hydrolysis of compounds 1 and 2

A solution of **1** or **2** (10 mg) in MeOH (5 ml) was treated separately with 0.1 N H_2SO_4 (5 ml) at 50°C for 15 min. After H_2O (5 ml) was added, the mixture was evaporated to 10 ml under reduced pressure to remove MeOH and then kept in 60°C for another 30 min. The hydrolyzed mixture was neutralized to pH 7 with Ba $(OH)_2$ and condensed to dryness under reduced pressure. The monosaccharide was isolated and determined to be cymarose and digitoxose by means of preparative TLC developed by $CHCl_3$–MeOH–H_2O (20:3:1, R_f 0.55, 0.50, 0.26 for cymarose, oleandrose, digitoxse, respectively), compared with authentic sample. The absolute configurations of cymarose, digitoxose, and oleandrose were considered to be D-form by their optical values: cymarose: $[\alpha]_D^{20} + 49.9$ ($c = 0.01$, H_2O), oleandrose: $[\alpha]_D^{20} - 15.2$ ($c = 0.01$, H_2O), digitxose: $[\alpha]_D^{20} + 47.3°$ ($c = 0.02$, H_2O).

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