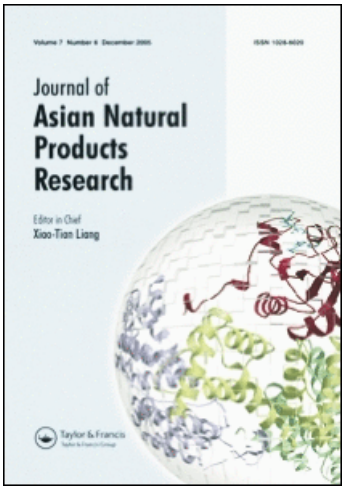

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new C_{21} steroidal glycosides from *Cynanchum wallichii* Wight

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To cite this Article Chen, Gang , Wang, Ding and Pei, Yue-Hu(2008) 'Two new C_{21} steroidal glycosides from *Cynanchum wallichii* Wight', Journal of Asian Natural Products Research, 10: 7, 671 — 676
To link to this Article: DOI: 10.1080/10286020801967243
URL: http://dx.doi.org/10.1080/10286020801967243

Journal of Asian Natural Products Research
Vol. 10, No. 7, July 2008, 671–676

Two new C_{21} steroidal glycosides from *Cynanchum wallichii* Wight

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(*Received 17 January 2007; final version received 30 May 2007*)

Two new C_{21} steroidal glycosides, characterized with caudatin as the aglycone moiety and 2,6-dideoxy-pyranoses as component sugars, have been isolated from the roots of *Cynanchum wallichii* Wight. Their structures were elucidated as caudatin-3-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**1**) and caudatin-3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranoside (**2**) by spectroscopic evidence.

Keywords: *Cynanchum wallichii* Wight; C_{21} steroidal glycosides; 2,6-dideoxy-pyranoses; caudatin-3-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside; caudatin-3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranoside

1. Introduction

Cynanchum wallichii Weight, Duanjieshen, is a traditional Chinese medicine distributed extensively over southwest China. It is used as the primary drug in the famous Chinese prescription 'hulisan,' which is used to treat arthrophlogosis and injury from fall or fracture. A pentaglycoside and wallicoside, has been isolated from its roots [1]. In this paper, we report the isolation and the structural elucidation of two novel pregnane glycosides from the roots of *C. wallichii* Weight.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{20} + 20.6$ (*c* 0.89, MeOH). The molecular formula was determined to be $C_{41}H_{64}O_{13}$ by HRFABMS at *m/z* 764.4373 $[M]^+$. The ^1H NMR spectrum of **1** showed two anomeric proton signals at δ 5.46 ($J = 9.5$ and 1.3 Hz) and 5.11 ($J = 9.5$ and 1.3 Hz). The ^{13}C NMR spectrum of **1** showed two anomeric carbon signals at δ 96.4 and 99.7. The carbon signals assignable to the aglycone moiety were similar to those of caudatin [1], with glycosylation shifts at C-3 (+5.2), C-2 (−2.1), and C-4 (−4.0). Hence, compound **1** was considered to be caudatin 3-*O*-diglycoside.

In $^1H-^1H$ COSY spectrum (Table 1), the signal at δ 4.30 (H-5″) correlated with the signals at δ 3.51 (H-4″) and δ 1.45 (H-6″), the signal at δ 3.51 (H-4″) correlated with the signal at δ 4.63 (H-3″). In HMBC spectrum, the signal at δ 4.30 (H-5″) correlated with the anomeric carbon signal at δ 96.4 (C-1″), the anomeric proton signal at δ 5.46 (H-1″) showed a correlation with the signals at δ 77.6 (C-3) and 39.1 (C-2″) (Figure 2). Thus, the signals at δ 96.4, 39.1, 67.5, 83.5, 68.6, and 18.6 were determined to be those of the sugar which was attached to C-3 of the aglycone by analysis of $^1H-^1H$ COSY, HMBC, and HSQC spectral data. The coupling constants of H-4″ (9.5 and 2.9 Hz) and H-3″ (2.9 Hz) showed that H-4″ and H-5″ were axial and H-3″ was equatorial. In addition, the configuration of H-1″ was

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020801967243
http://www.informaworld.com

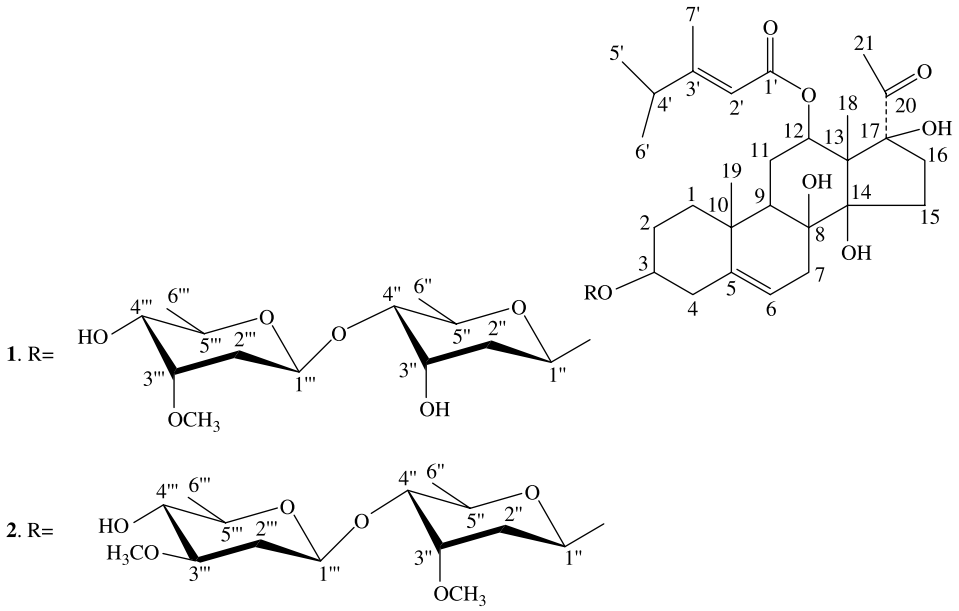

Figure 1. The structures of compounds **1** and **2**.

axial according to the coupling constant of H-1″ (9.5 and 1.3 Hz), the sugar was determined as β-D-digitoxopyranoses by comparing the NMR spectral data with those reported in the literature [2,3].

The terminal sugar was determined in the same way, the correlations between H-6‴ and H-5‴, H-5‴ and H-4‴, and H-4‴ and H-3‴ were observed in ^{1}H–^{1}H COSY spectrum. In HMBC spectrum, the signal at δ 4.09 (H-5‴) showed a long-range correlation with the anomeric carbon signal at δ 99.7 (C-1‴), the anomeric proton signal at δ 5.11 (H-1‴) correlated with the signals at δ 83.5 (C-4″) and 35.6 (C-2‴). In addition, a long-range correlation between the signal at δ 3.45 (—OCH_3) and the signal at δ 78.8 (C-3‴) was observed. The signals at δ 99.7, 35.6, 78.8, 74.1, 70.9, 18.9, and 58.0 were determined to be those of the terminal sugar which was attached to the C-4″ of β-D-digitoxopyranose. According to the coupling constants of H-4‴ (9.5 and 3.0 Hz), H-3‴ (3.0 Hz) and H-1‴ (9.5 and 1.3 Hz), H-1‴, H-4‴, and H-5‴ were considered to be axial, and H-3‴ was considered to be equatorial. Thus, the terminal sugar was determined as β-D-cymaropyranose by comparing the NMR spectral data with those reported in literatures [2,3]. Thus, compound **1** was elucidated as caudatin 3-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (Figure 1).

Compound **2** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ − 15.0 (*c* 0.83, MeOH). The molecular formula was determined to be $C_{42}H_{66}O_{13}$ by HRFABMS at *m/z* 778.4517 $[M]^+$. The ^{1}H NMR spectrum of **2** showed two anomeric proton signals at δ 5.27 (J = 9.5 and 1.3 Hz) and 4.76 (J = 9.5 and 1.3 Hz). The ^{13}C NMR spectrum of **2** showed two anomeric carbon signals at δ 96.4 and 102.2. Comparing its NMR signals with those of compounds **1** and **2** was determined to be a caudatin 3-*O*-diglycoside with different sugar chain.

The signal at δ 1.45 (H-6″) correlated with the signal at δ 4.24 (H-5″), the signal at δ 3.54 (H-4″) correlated with the signals at δ 4.24 (H-5″) and δ 4.08 (H-3″) in ^{1}H–^{1}H COSY spectrum (Table 2). In HMBC spectrum, the

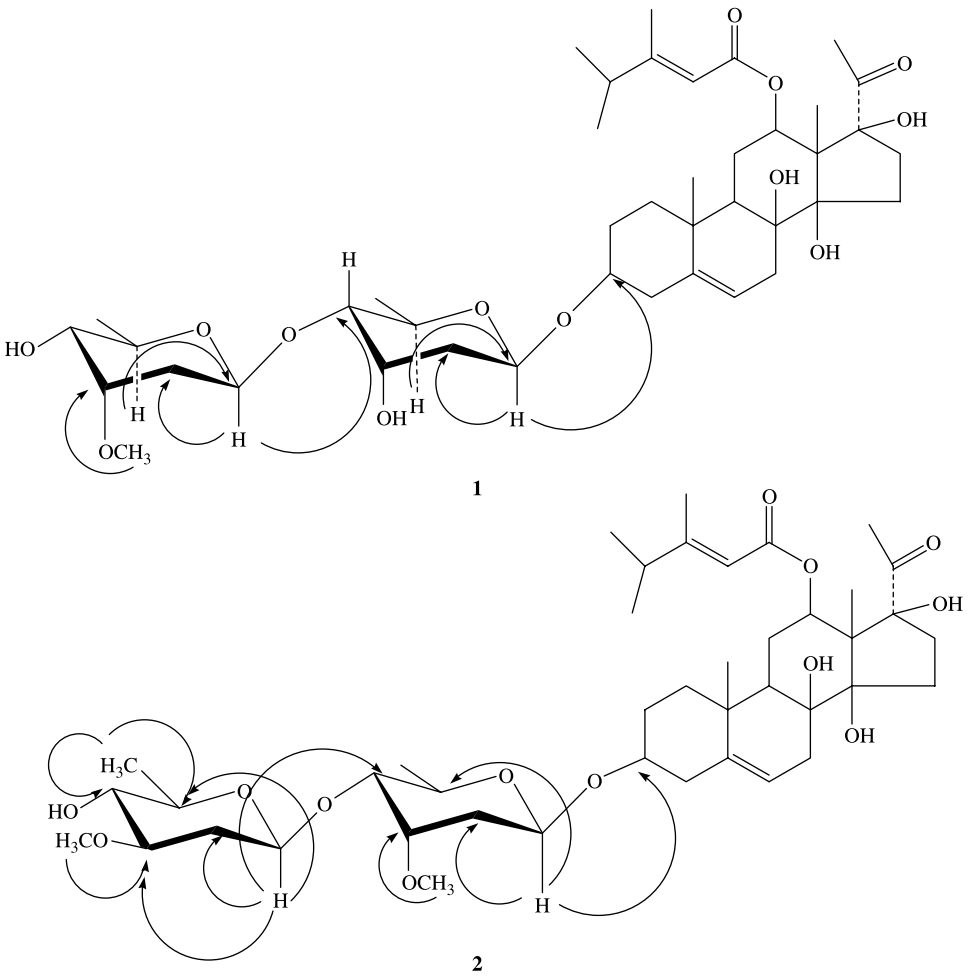

Figure 2. The key HMBC correlations of compounds **1** and **2**.

signal at δ 3.57 (—OCH_3) showed a long-range correlation with the signal at δ 77.9 (C-3″), and the anomeric proton signal at δ 5.27 correlated with the signals at δ 77.7 (C-3), 69.0 (C-5″), and 37.2 (C-2″). Thus, the signals at δ 96.4, 37.2, 77.9, 83.5, 69.0, 18.7, and 58.8 were determined to be the signals of the sugar which was attached to C-3 of aglycone by analysis of the $^1H-^1H$ COSY, HMBC, and HSQC spectral data. Since, the coupling constants of H-4″, H-3″, and H-1″ were 9.5 and 2.6, 2.6, and 9.5 and 1.3 Hz, respectively, the configurations of H-4″, H-1″, and H-5″ were considered to be axial, and H-3″ was considered to be equatorial. Thus, the sugar was determined as β-D-cymaropyranoses by comparing the spectral NMR data with those reported in literatures [2,4].

The terminal sugar was determined by analysis of the HMBC and HSQC spectral data. In HMBC spectrum, the proton signal at δ 1.55 (H-6‴) showed correlation with the signals at δ 72.9 (C-5‴) and 76.2 (C-4‴), the anomeric proton signal at δ 4.76 (H-1‴) correlated with the signals at δ 72.9 (C-5‴), 81.4 (C-3‴), 37.2 (C-2‴), and 83.5 (C-4″), the signal at δ 3.46 (—OCH_3) showed a long-rang

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1**.

Position	δ_C	δ_H	Position	δ_C	δ_H
1	38.9	2.43 (m) and 2.52 (m)	2′	114.2	–
2	29.8	1.78 (m) and 2.06 (m)	3′	165.4	–
3	77.6	3.86 (m)	4′	38.1	1.10 (m)
4	39.2	(a)	5′	20.8	0.93 (d, 6.0, 3H)
5	139.4	–	6′	20.9	0.95 (d, 6.0, 3H)
6	119.2	5.30 (br. t)	7′	16.5	2.26 (s, 3H)
7	34.7	2.30 (m) and 2.41 (m)	β-D-Dgt		
8	74.2	–	1″	96.4	5.46 (dd, 9.5, 1.3)
9	44.6	1.70 (m)	2″	39.1	(a)
10	37.4	–	3″	67.5	4.63 (d, 2.9)
11	25.1	2.12 (m) and 2.25 (m)	4″	83.5	3.51 (dd, 9.5, 2.9)
12	72.6	5.03 (dd, 11.6, 3.9)	5″	68.6	4.30 (m)
13	57.9	–	6″	18.6	1.45 (d, 6.5)
14	89.4	–	β-D-Cym		
15	33.8	2.07 (m, 2H)	1‴	99.7	5.11 (dd, 9.5, 1.3)
16	27.5	2.48 (m, 2H)	2‴	35.6	(a)
17	92.4	–	3‴	78.8	3.71 (d, 3.0)
18	10.7	2.02 (s, 3H)	4‴	74.1	3.46 (dd, 9.5, 3.0)
19	18.2	1.30 (s, 3H)	5‴	70.9	4.09 (m)
20	209.4	–	6‴	18.9	1.44 (d, 6.1)
21	32.9	2.50 (s, 3H)	—OCH_3	58.0	3.45 (s)
1′	165.9	–			

Measured at 600 MHz in pyridine-d_5 at 35°C. (a) Overlapping with other signals.

Table 2. ^{1}H and ^{13}C NMR spectral data of compound **2**.

Position	δ_C	δ_H	Position	δ_C	δ_H
1	39.0	2.43 (m) and 2.52 (m)	2′	114.2	–
2	29.9	1.78 (m) and 2.06 (m)	3′	165.4	–
3	77.7	3.85 (m)	4′	38.2	1.10 (m)
4	39.3	(a)	5′	20.8	0.93 (d, 6.0, 3H)
5	139.4	–	6′	20.9	0.95 (d, 6.0, 3H)
6	119.2	5.30 (br. t)	7′	16.5	2.26 (s, 3H)
7	34.8	2.30 (m) and 2.41 (m)	β-D-Cym		
8	74.3	–	1″	96.4	5.27 (dd, 9.5, 1.3)
9	44.6	1.70 (m)	2″	37.2[a]	(a)
10	37.4	–	3″	77.9	4.08 (d, 2.6)
11	25.1	2.12 (m) and 2.25 (m)	4″	83.5	3.54 (dd, 9.5, 2.6)
12	72.6	5.03 (dd, 11.6, 3.9)	5″	69.0	4.24 (m)
13	58.0	–	6″	18.7[b]	1.45 (d, 6.0)
14	89.5	–	—OCH_3	58.8	3.57 (s)
15	33.9	2.07 (m, 2H)	β-D-Ole		
16	27.6	2.48 (m, 2H)	1‴	102.2	4.76 (dd, 9.5, 1.3)
17	92.4	–	2‴	37.2[a]	(a)
18	10.7	2.02 (s, 3H)	3‴	81.4	3.47 (a)
19	18.2	1.30 (s, 3H)	4‴	76.2	3.48 (a)
20	209.4	–	5‴	72.9	3.58 (m)
21	32.9	2.50 (s, 3H)	6‴	18.7[b]	1.55 (d, 6.0)
1′	166.0	–	—OCH_3	57.1	3.46 (s)

Measured at 600 MHz in pyridine-d_5 at 35°C. (a) Overlapping with other signals.
[a]Interchangeable. [b]Interchangeable.

Table 3. ^{1}H and ^{13}C NMR spectral data of the sugar chain of compound **2**.

Position	δ_C	δ_H
β-D-Cym		
1″	97.1	4.85 (dd, 9.5, 1.3)
2″	36.9	2.04 (a) and 1.56 (a)
3″	78.4	3.84 (br. t)
4″	83.8	3.24 (dd, 9.5, 1.3)
5″	69.9	3.84 (m)
6″	18.6[a]	1.27 (d, 6.0)
—OCH_3	58.5	3.43 s
β-D-Ole		
1‴	102.7	4.58 (dd 9.5, 1.3)
2‴	37.3	2.31 (a) and 1.36 (a)
3‴	81.5	3.20 (ddd, 9.5, 8.7, 2.5)
4‴	76.8	2.96 (t, 9.5)
5‴	73.1	4.49 (m)
6‴	18.5[b]	1.20 (d, 6.0)
—OCH_3	57.4	3.41 (s)

Measured at 600 MHz in CD_3OD at 35°C. (a) Overlapping with other signals.
[a]Interchangeable. [b]Interchangeable.

correlation with the signal at δ 81.4 (C-3‴). So the signals at δ 102.2, 37.2, 81.4, 76.2, 72.9, 18.7, and 57.1 were determined to be the signals of the terminal sugar which was attached to C-4″ of β-cymaropyranoses. Since, the proton signals of H-3‴ and H-4‴ were partly overlapped in C_5D_5N, in order to determine the configurations of H-3‴, H-4‴, and H-5‴, we retested the ^{1}H NMR, ^{13}C NMR, and 2D NMR spectral data of compound **2** in CD_3OD. The signals of sugars in CD_3OD were determined by analysis of $^{1}H-^{1}H$ COSY, HMBC, and HSQC spectral data (Table 3). The signal of H-4‴ in CD_3OD was triplet at δ 2.96 ($J = 9.5$ Hz), and the signal of H-3‴ in CD_3OD was at δ 3.20 (ddd, $J = 9.5$, 8.7, and 2.5 Hz), that could only happen if H-3‴, H-4‴, and H-5‴ were all axial. In addition to the axial configuration of H-1‴ with coupling constant $J = 9.5$ and 1.3 Hz, the terminal sugar was considered to be β-D-oleandropyranose, which was in acquirement by comparing the NMR data with those reported in literature [2]. Thus, compound **2** was determined as caudatin 3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranoside.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin-EImer 241 polarimeter. UV spectra were measured on a Shimadzu UV-1601. IR spectra were taken on a Bruker IFS-55 infrared spectrophotometer. The NMR spectral data were recorded on Bruker AV-600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) in C_5D_5N with TMS as internal standard. The HRFABMS data were obtained on the Micros Mass Autospec-UltimaE TOF mass spectrophotometer. Chromatography was performed on silica gel (200–300 mesh, Qingdao Haiyang Chemical Factory), Sephadex LH-20, reversed-phase HPLC (Shimadzu LC-8A vp).

3.2. Plant material

The roots of *C. wallichii* Wight were bought in October 2005 in Yunnan province, China. A voucher specimen (No. 6040) was identified by Professor Qishi Sun and deposited in the School of Traditional Chinese Materia Medica of Shenyang Pharmaceutical University, Shenyang City, China.

3.3. Extraction and isolation

The dried roots (10 kg) were extracted with EtOH (95%) for three times, 2 h each. The extract (1300 g) was successively partitioned with EtOAc, *n*-BuOH, and H_2O. The EtOAc soluble fraction (150 g) was subjected to silica gel column, eluted with $CHCl_3$–CH_3OH (100:1–0:1), yielding 12 fractions. Fraction 4 (20 g) was purified by Sephadex LH-20 (CH_3OH) to yield two fractions. 4 g of the second fraction (15 g) was subjected to HPLC, eluted with CH_3OH (80%) and afforded compounds **1** (50 mg) and **2** (35 mg).

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

White amorphous powder; $[\alpha]_D^{20} + 20.6$ (*c* 0.89, MeOH); UV (MeOH) λ_{max}: 220 nm;

IR (KBr) ν_{max} (cm^{-1}) 1710, 1640, and 1225; ^1H NMR and ^{13}C NMR spectral data, see Table 1; HRFABMS *m/z*: 764.4373 $[M]^+$ (calcd for $C_{41}H_{64}O_{13}$, 764.4347).

*3.3.2. Caudatin-3-O-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranoside (**2**)*

White amorphous powder; $[\alpha]_D^{20}$ − 15.0 (*c* 0.83, MeOH); UV (MeOH) λ_{max}: 220 nm; IR (KBr) ν_{max} (cm^{-1}) 1710, 1640, and 1225; ^1H NMR and ^{13}C NMR spectral data, see Table 2; HRFABMS *m/z*: 778.4517 $[M]^+$ (calcd for $C_{42}H_{66}O_{13}$, 778.4503).

Acknowledgment

This research work was financially supported by the National Nature Science Foundation of China, Project No. 30672612.

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