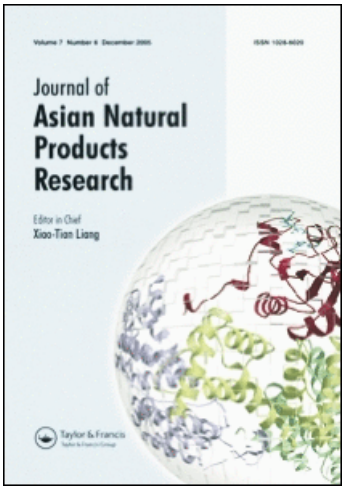

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C_{21} steroidal glycosides from *Cynanchum wallichii* Wight

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Three new C_{21} steroidal glycosides, characterized with rostratamine and qinyangshengenin as aglycone moiety and 2,6-dideoxy-pyranoses as component sugars, have been isolated from the roots of *Cynanchum wallichii* Wight. The structures of the three new C_{21} steroidal glycosides were elucidated as rostratamine-3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside, qinyangshengenin-3-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxo-pyranoside, and qinyangshengenin-3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

Keywords: *Cynanchum wallichii* Wight; C_{21} steroidal glycosides; 2,6-dideoxy-pyranoses

1. Introduction

Cynanchum wallichii Wight, also named Duanjieshen, is a traditional Chinese medicine distributed extensively over southwest China. It is used as the primary drug in the famous Chinese prescription "hulisan" [1], which is used to treat arthrophlogosis and injury from fall or fracture. In our recent study on this herbal medicine, three new and two known C_{21} steroidal glycosides were isolated. In this paper, we report the isolation and the structural elucidation of these pregnane glycosides containing 2,6-dideoxy-pyranoses.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{20} + 18.6$ ($c = 0.89$, MeOH). The molecular formula was determined to be $C_{51}H_{74}O_{16}$ by HR-FAB-MS at *m/z* 965.4873 $[M + Na]^+$. The ^{13}C NMR spectrum of **1** (Table 2) showed three anomeric carbon signals at δ 96.3, 100.5, and 102.2. The carbon signals assignable to the aglycone moiety were similar to those of rostratamine [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 rostratamine-3-*O*-triglycoside (Figure 1).

The ^1H NMR signals of **1** (Table 3) were also assigned to three secondary methyl groups [1.39 (d, 6.0), 1.41 (d, 6.0), and 1.57 (d, 6.0)], three methoxyl groups [3.56 (s), 3.61 (s), and 3.45 (s)], and three anomeric protons [5.27 (dd, 9.6, 1.8), 5.15 (dd, 9.6, 1.8), and 4.76 (dd, 9.6, 1.8)], whose multiplicities showed three 2,6-dideoxy-sugar units and β-configuration of the three units. The ^{13}C shifts of each sugar units were assigned unambiguously by HMBC, HMQC, and ^1H–^1H COSY analyses. The existence of one D-oleandropyranosyl and two D-cymaropyranosyl units

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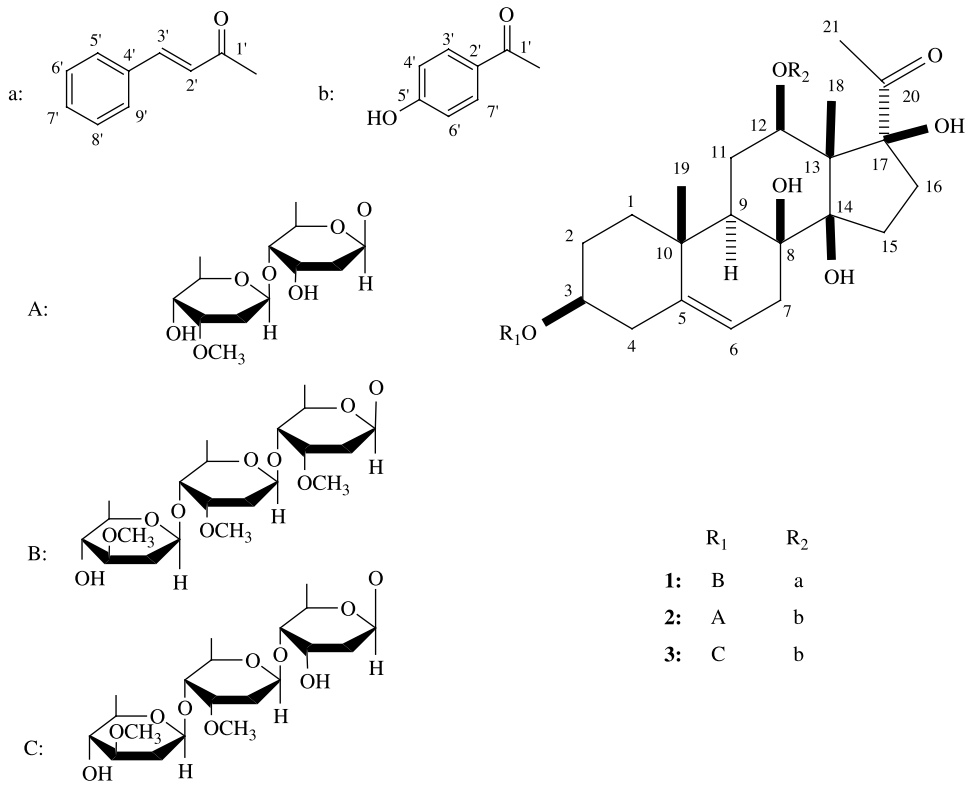

Figure 1. Structures of compounds **1**–**3**.

were confirmed by their comparison with spectroscopic data in the literature [2,3]. The sequence of these three sugar units were demonstrated by HMBC spectrum (Figure 2), in which distinct correlations from H-1″ (5.27) to C-3 (77.6) of the aglycone and from H-1‴ (5.15) to C-4″ (83.4) and from H-1⁗ (4.76) to C-4‴ (83.1) were observed. Thus, the structure of compound **1** was established as rostratamine-3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside.

Compound **2** was obtained as a white amorphous powder, $[\alpha]_D^{20} + 20.6$ ($c = 0.20$, MeOH). The molecular formula was determined to be $C_{41}H_{58}O_{14}$ by HR-FAB-MS at *m/z* 797.3726 $[M + Na]^+$. Compared to compound **1**, the ^{13}C NMR spectrum of **2** (Table 2) showed only two anomeric carbon signals at δ 96.4 and 99.7, and the carbon signals assignable to the aglycone moiety were different. Further, compared with literature [4], the aglycone moiety was finally determined as qinyangshengenin, with glycosylation shifts at C-3 (+5.2), C-2 (−2.1), and C-4 (−4.0). Hence, compound **2** was considered to be qinyangshengenin-3-*O*-diglycoside.

Proton signals were also assigned to two secondary methyl groups [1.45 (d, 6.1) and 1.44 (d, 6.1)], one methoxyl group [3.45 (s)], and two anomeric protons [5.46 (dd, 9.6, 1.3) and 5.11 (dd, 9.6, 1.3)], whose multiplicities showed two 2,6-dideoxy-sugar units and β-configuration of the two units. The ^{13}C shifts of each sugar units were assigned unambiguously by HMBC, HMQC, and ^1H–^1H COSY analyses. The existence of one D-digitoxopyranosyl and one D-cymaropyranosyl units were

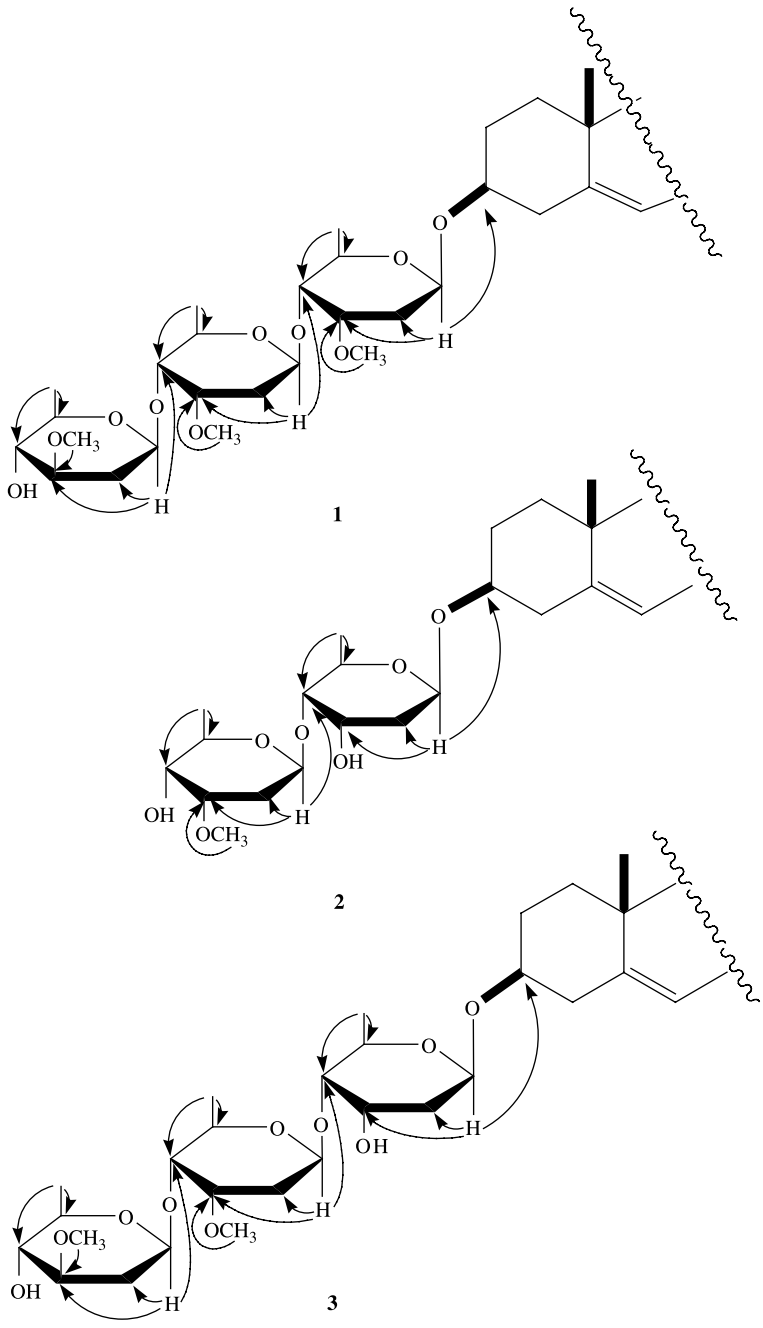

Figure 2. Key HMBC correlations of compounds **1**–**3**.

confirmed by their comparison with the spectroscopic data in the literature [2,5]. The sequence of these two sugar units were demonstrated by HMBC spectrum (Figure 2), in which distinct correlations from H-1″ (5.46) to C-3 (77.6) of the aglycone and from H-1‴ (5.11) to C-4″ (83.5) were observed. Thus, the structure of compound **2** was established as qinyangshengenin-3-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

Compound **3** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ + 15.1 ($c = 0.41$, MeOH). The molecular formula was determined to be $C_{48}H_{70}O_{17}$ by HR-FAB-MS at *m/z* 941.4512 $[M + Na]^+$. Compared with **1** and **2**, the ^{13}C NMR spectrum of **3** (Table 2) showed three anomeric carbon signals at δ 96.4, 99.8, and 102.2. The carbon signals assignable to the aglycone moiety were similar to those of compound **2**. So compound **3** was considered to be qinyangshengenin-3-*O*-triglycoside.

The NMR spectral data for the sugar moiety of compound **3** was a little different from those of compound **1**. Proton signals were also assigned to three secondary methyl groups [1.43 (d, 6.0), 1.35 (d, 6.0), and 1.55 (d, 6.0)], two methoxyl groups [3.56 (s) and 3.45 (s)], and three anomeric protons [5.47 (dd, 9.6, 1.8), 5.18 (dd, 9.6, 1.8), and 4.75 (dd, 9.6, 1.8)], whose multiplicities showed three 2,6-dideoxy-sugar units and β-configuration of the three units. The ^{13}C shifts of each sugar units were assigned unambiguously by HMBC, HMQC, and ^1H–^1H COSY analyses. The existence of one D-digitoxopyranosyl, one D-oleandropyranosyl, and one D-cymaropyranosyl units were confirmed by their comparison with the spectroscopic data in the literature [2,5,6]. The sequence of these three sugar units were demonstrated by HMBC spectrum (Figure 2), in which distinct correlations from H-1″ (5.47) to C-3 (77.6) of the aglycone and from H-1‴ (5.18) to C-4″ (83.4) and from H-1⁗ (4.75) to C-4‴ (83.1) were observed. Thus, the structure of compound **3** was established as qinyangshengenin-3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

3. Experimental

3.1 General experimental procedures

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

3.2 Plant material

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

3.3 Extraction and isolation

The dried roots of *C. wallichii* Wight (10 kg) were extracted with EtOH (95%) for thrice, 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 5 (30 g) was purified by means of Sephadex LH-20 (CH_3OH) to yield two fractions. About 4 g of the second fraction (15 g) was subjected to HPLC, eluted with CH_3OH (70%), and afforded compounds **1** (50 mg), **2** (15 mg), and **3** (35 mg).

Table 1. ^{13}C NMR spectral data for the aglycone moieties of **1–3**.

Position	1	2	3
1	39.9	39.2	39.2
2	29.8	29.9	29.9
3	77.6	77.6	77.6
4	38.9	39.1	39.1
5	139.4	139.4	139.4
6	119.2	119.1	119.1
7	34.7	34.8	34.8
8	74.2	74.3	74.3
9	44.5	44.5	44.6
10	37.4	37.4	37.4
11	25.0	25.2	25.2
12	73.6	73.4	73.4
13	58.1	58.4	58.4
14	89.5	89.6	89.6
15	34.7	33.9	33.9
16	33.8	33.2	33.2
17	92.4	92.5	92.5
18	10.7	10.9	10.9
19	18.1	18.2	18.2
20	209.9	209.8	209.8
21	27.7	27.8	27.8
1′	165.8	165.4	165.4
2′	119.2	122.0	122.1
3′	144.9	132.4	132.4
4′	135.0	116.2	116.2
5′ (9′)	128.6	163.6	163.6
6′ (8′)	129.3	116.2	116.2
7′	130.6	132.4	132.4

Measured at 150 MHz in pyridine-d_5 at 35°C.

*3.3.1 Rostratamine-3-O-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside (**1**)*

White amorphous powder; $[\alpha]_D^{20}$ − 18.6 (c = 0.89, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3443, 1715, 1905, 1660, and 1225; ^{1}H and ^{13}C NMR spectral data, see Tables 1–3; HR-FAB-MS *m/z*: 965.4873 $[M + Na]^+$ (calcd for $C_{51}H_{74}O_{16}Na$, 965.4875).

*3.3.2 Qinyangshengenin-3-O-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**2**)*

White amorphous powder; $[\alpha]_D^{20}$ + 20.6 (c = 0.20, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3443, 1710, 1640, and 1225; ^{1}H and ^{13}C NMR spectral data, see Tables 1–3; HR-FAB-MS *m/z*: 797.3726 $[M + Na]^+$ (calcd for $C_{41}H_{58}O_{14}Na$, 797.3724).

*3.3.3 Qinyangshengenin-3-O-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**3**)*

White amorphous powder; $[\alpha]_D^{20}$ + 15.1 (c = 0.41, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3443, 1710, 1640, and 1225; ^{1}H and ^{13}C NMR spectral data, see Tables 1–3; HR-FAB-MS *m/z*: 941.4512 $[M + Na]^+$ (calcd for $C_{48}H_{70}O_{17}Na$, 941.4511).

3.4 Acid hydrolysis of compounds 1–3

A solution of **1–3** (each 5 mg) in MeOH (5 ml) was treated separately with 0.1 N H_2SO_4 (5 ml) at 50°C for 15 min. After added H_2O (5 ml), the

Table 2. ^{13}C NMR spectral data for the sugar moieties of **1–3**.

Position	1	2	3
	β-D-cym	β-D-dgt	β-D-dgt
1″	96.3	96.4	96.4
2″	37.9	39.1	38.9
3″	77.8	67.5	67.5
4″	83.4	83.5	83.4
5″	68.9	68.6	68.6
6″	18.5	18.6	18.5
—OCH_3	58.8	–	–
	β-D-cym	β-D-cym	β-D-cym
1‴	100.5	99.7	99.8
2‴	37.2	35.6	36.7
3‴	78.0	78.8	77.7
4‴	83.1	74.1	83.1
5‴	69.0	70.9	69.1
6‴	18.6	18.9	18.5
—OCH_3	58.8	58.0	58.8
	β-D-ole	–	β-D-ole
1⁗	102.2	–	102.2
2⁗	37.2	–	37.2
3⁗	81.4	–	81.4
4⁗	76.2	–	76.2
5⁗	72.9	–	72.9
6⁗	18.6	–	18.6
—OCH_3	57.0	–	57.0

Measured at 150 MHz in pyridine-d_5 at 35°C.

Table 3. ^{1}H NMR spectral data for the sugar moieties of **1**–**3**.

Position	**1**	**2**	**3**
	β-D-cym	β-D-dgt	β-D-dgt
1″	5.27 (dd, 9.6, 1.8)	5.46 (dd, 9.5, 1.3)	5.47 (dd, 9.6, 1.8)
2″	–[a]	–[a]	–[a]
3″	4.05 (br s)	4.63 (d, 2.9)	4.64 (br s)
4″	3.52 (dd, 9.6, 3.0)	3.51 (dd, 9.5, 2.9)	3.52 (dd, 9.6, 3.0)
5″	4.20 (m)	4.30 (m)	4.29 (m)
6″	1.39 (d, 6.0)	1.45 (d, 6.1)	1.43 (d, 6.0)
—OCH_3	3.56 (s)	–	–
	β-D-cym	β-D-cym	β-D-cym
1‴	5.15 (dd, 9.6, 1.8)	5.11 (dd, 9.5, 1.3)	5.18 (dd, 9.6, 1.8)
2‴	–[a]	–[a]	–[a]
3‴	4.09 (br s)	3.71 (d, 3.0)	4.05 (br s)
4‴	3.50 (dd, 9.6, 3.0)	3.46 (dd, 9.5, 3.0)	3.45[a]
5‴	4.18 (m)	4.09 (m)	4.29 (m)
6‴	1.41 (d, 6.0)	1.44 (d, 6.1)	1.43 (d, 6.0)
—OCH_3	3.61 (s)	3.45 (s)	3.56 (s)
	β-D-ole	–	β-D-ole
1⁗	4.76 (dd, 9.6, 1.8)	–	4.75 (dd, 9.6, 1.8)
2⁗	–[a]	–	–[a]
3⁗	3.49[a]	–	3.47[a]
4⁗	3.48[a]	–	3.45[a]
5⁗	3.60 (m)	–	3.60 (m)
6⁗	1.57 (d, 6.0)	–	1.55 (d, 6.0)
—OCH_3	3.45 (s)	–	3.45 (s)

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

mixture was evaporated to 10 ml under reduced pressure to remove MeOH. 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 residue was dissolved in MeOH and was subjected to HPLC when compared with the authentic sample of qinyangshengenin and rostratamine, respectively. The retention time of qinyangshengenin and rostratamine was 20.5 and 35.5 min, respectively [pump: Shimadazu LC-10A vp, Japan; detector: SPD-10A vp, Japan; ODS column: Diamonsil, C_{18}, 5 μm 250 × 4.6 nm, USA; flow rate: 1.0 ml/min; solvent: CH_3OH (80%)].

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