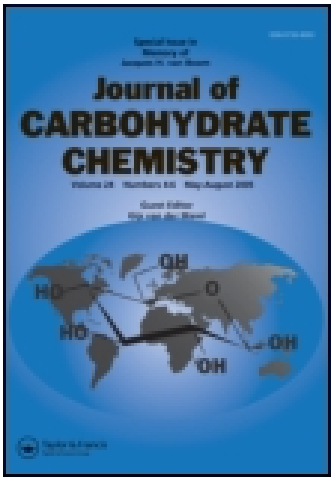

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C_{21} Steroidal Glycosides from Prunella vulgaris

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C_{21} Steroidal Glycosides from *Prunella vulgaris*

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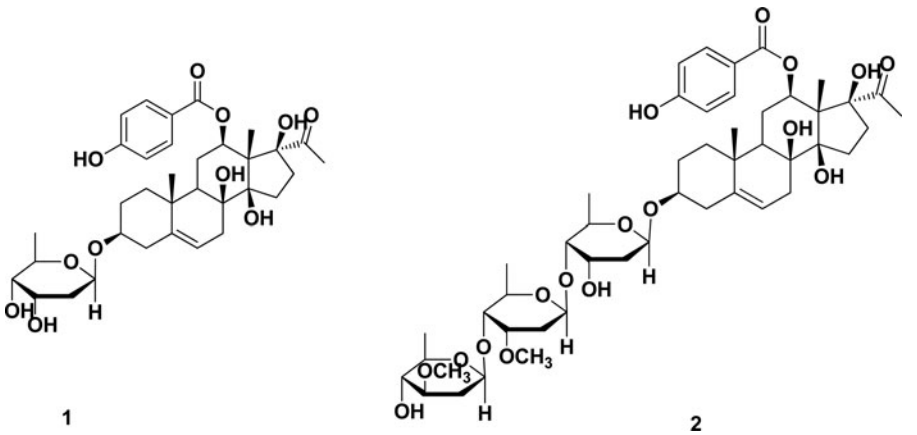
GRAPHICAL ABSTRACT



A new C_{21} steroidal glycoside, qinyangshengenin-3-*O*-β-D-digitoxopyranoside (**1**), together with a known steroidal glycoside, qinyangshengenin-3-*O*-β-D-oleandropyranosyl-(1→4)-β-D- cymaropyranosyl-(1→4)-β-D-digitoxopyranoside (**2**), was isolated from the medicinal plant *Prunella vulgaris* Linn. The structure of **1** was elucidated on the basis of extensive spectroscopic analysis, including HR-MS, 1D and 2D NMR experiments, and chemical evidence.

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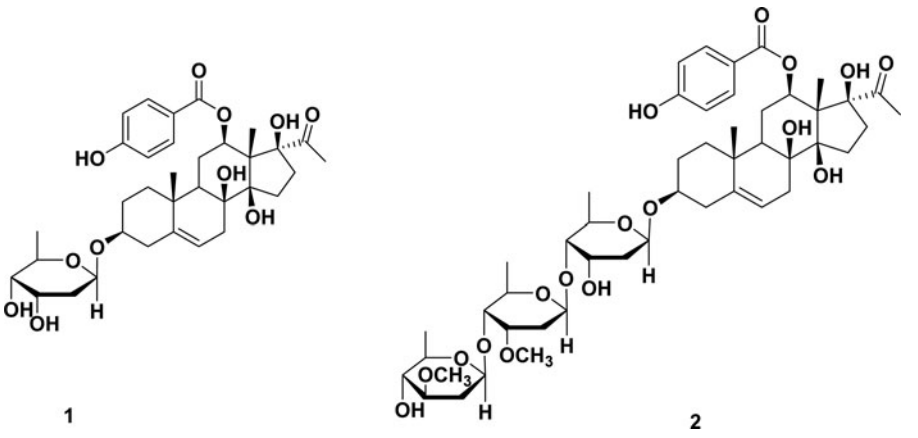

Figure 1: The structures of compounds **1** and **2.**

Keywords *Prunella vulgaris* Linn; C_{21} steroidal glycoside; Isolation; Qingyangshengenin

INTRODUCTION

Prunella vulgaris Linn., a traditional Chinese medicine belonging to the family of Labiatae, is widely distributed in southern China. Modern pharmacological studies on this herb medicine showed that it had extensive pharmacological activities, such as hypoglycemic,[1] antimicrobial,[2] anticancer,[3] and other biological activities.[4] Extensive studies on the chemical constituents in this plant have resulted in the isolation of coumarins,[5] triterpenoids,[5] phenylpropanoids,[6] saccharide,[7] diterpenes,[8] and others.[9] In our recent study on this plant collected in Guizhou Province, a new and a known C_{21} steroidal glycoside were isolated from the whole herb of *P. vulgaris* Linn. In this article, we report the isolation and structural elucidation of these two compounds (Fig. 1). The structure of the new compound **1** was elucidated on the basis of extensive spectroscopic analysis, including HR-MS, 1D and 2D NMR experiments, and chemical evidence, and the known compound **2** was determined by comparing its spectroscopic data with that of the literature.

RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous power, and its molecular formula was determined to be $C_{34}H_{46}O_{11}$ by HR-ESI-MS at m/z 631.3115 $[M+H]^+$ (Calcd. 631.3118), indicating 12 degrees of unsaturation. The ^1H NMR spectrum of **1** (Table 1) showed two obvious steroidal methyl signals at 1.64 (3H, s) and 1.15 (3H, s) ppm. The ^{13}C NMR and DEPT spectra of **1** exhibited 34 carbon signals, including 4 methyl groups, 8 methylene groups,

Table 1: ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of **1** and **2** in CD_3OD

	1		**2**	
No.	δ_H, *J*/Hz	δ_C	δ_H, *J*/Hz	δ_C
1	1.15 (m)1.90 (m)	39.8	1.15 (m)1.89 (m)	39.8
2	1.60 (m)	30.1	1.60 (m)	30.2
3	3.55 (m)	79.3	3.55 (m)	79.3
4	2.42 (m)	39.8	2.39 (m)	39.0
5		140.2		140.2
6	5.36 (d, 4.8)	119.6	5.36 (m)	119.7
7	2.18 (m)	35.2	2.19 (m)	35.2
8		75.0		75.0
9	1.59 (m)	45.1	1.58 (m)	45.1
10		38.1		38.1
11	1.76 (m)1.92 (m)	25.5	1.76 (m)1.92 (m)	25.5
12	4.75 (dd, 11.6, 4.4)	74.0	4.78 (dd, 9.6, 3.2)	74.1
13		59.1		59.1
14		90.0		90.0
15	1.89 (m)2.05 (m)	34.3	1.89 (m)2.08 (m)	34.3
16	1.75 (m)	33.5	1.73 (m)	33.5
17		93.1		93.1
18	1.64 (s)	10.6	1.65 (s)	10.6
19	1.15 (s)	18.5	1.15 (s)	18.5
20		212.1		212.1
21	2.07 (s)	27.8	2.08 (s)	27.8
1′		166.8		166.8
2′		122.3		122.3
3′	7.80 (d, 8.8)	132.8	7.80 (d, 6.8)	132.8
4′	6.82 (d, 8.8)	116.2	6.82 (d, 7.2)	116.2
5′		163.6		163.6
6′	6.82 (d, 8.8)	116.2	6.82 (d, 6.8)	116.2
7′	7.81 (d, 8.8)	132.8	7.80 (d, 7.2)	132.8
1″	4.97 (dd, 9.6, 1.6)	97.1	4.76 (dd, 9.2, 2.8)	97.0
2″	1.59 (m)	39.8	1.58 (m)	39.8
3″	4.00 (m)	69.2	3.85 (m)	69.5
4″	3.13 (dd, 3.2, 9.6)	74.4	3.18 (m)	83.8
5″	3.75 (m)	70.8	4.23 (m)	70.0
6″	1.23 (d, 6.4)	18.6	1.22 (d, 6.4)	18.6
1‴			4.83 (dd, 9.2, 2.8)	100.7
2‴			1.65 (m)	36.4
3‴			3.83 (m)	76.9
4‴			3.20 (m)	83.6
5‴			4.23 (m)	64.7
6‴			1.13 (d, 6.4)	19.4
-OCH_3			3.44 (s)	58.6
1⁗			4.59 (dd, 9.6, 1.6)	102.9
2⁗			1.60 (m)	37.4
3⁗			3.31 (m)	81.6
4⁗			3.20 (m)	76.9
5⁗			3.61 (m)	73.3
6⁗			1.32 (d, 6.4)	18.5
-OCH_3			3.35 (s)	57.4

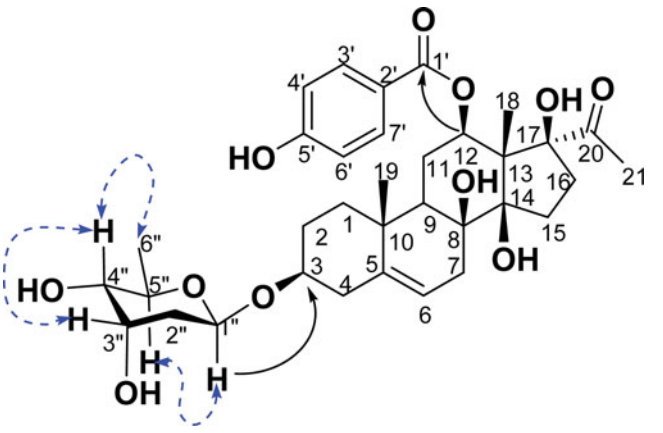

Figure 2: The key HMBC correlations (⌒) and selected NOESY correlations (⌒) of compound **1**.

12 methane groups, and 10 quaternary carbons. The characteristic chemical shift of a carbon signal at δ_C 97.1 was typical for anomeric carbon of saccharides, and the aglycone moiety signals showed the presence of one typical carbonyl carbon (δ_C 212.1), one double bond (δ_C 119.7, 140.2), and one benzoyl group (δ_C 166.8, 163.6, 122.3, 132.8×2, 116.2×2). All the signals assignable to the aglycone moiety were similar to those of a C_{21} steroid, qingyangshengenin, with only a tiny difference for the chemical shifts of C-5 (−0.1), C-6 (+0.1), C-20 (+0.1), and C-1' (-0.1) as compared to that of the reference.[10] The co-TLC results of the acidic hydrolyzate of **1** with an authentic sample of qingyangshengenin further supported this conclusion. In addition, proton signals assigned to one methyl group [δ_H 1.23 (3H, d, J = 6.4 Hz)], one methylene group [δ_H 1.59 (2H, m)], and one anomeric proton [δ_H 4.97 (dd, J = 9.6, 1.6 Hz)] in the ^{1}H NMR spectrum indicated the presence of a β-digitoxopyranose unit in **1** on the basis of the coupling constant.[11] From the 2D NMR spectra, the HMBC correlation from H-1″ (4.97) to C-3 (79.3) indicated that the monosaccharide unit was connected to C_3-OH of qingyangshengenin. In the NOESY experiment, the NOE correlations of H-3″/H-4″ and H-4″/6″-CH_3 showed that they were cofacial, and NOE correlation of H-1″/H-5″ indicated that they were α-oriented (Fig. 2). Therefore, compound **1** was considered to be qingyangshengenin-3-*O*-β-digitoxopyranoside. The absolute configuration (D or L) of the digitoxopyranose unit was further confirmed by comparing its optical rotation value ($[\alpha]_D^{20}$+41.6 (*c* 0.2, H_2O) with that of the references ($[\alpha]_D^{20}$+48.4 (c 0.9, H_2O) for D-digitoxopyranose) after acidic hydrolysis of **1**.[11,12] Therefore, the structure of **1** was unambiguously established as qingyangshengenin-3-*O*-β-D-digitoxopyranoside (Fig. 1).

Compound **2** was isolated as a white powder. The molecular formula was established as $C_{48}H_{70}O_{17}$ by ESI-MS (*m*/*z* 919 $[M+H]^+$, 941 $[M+Na]^+$),

which was supported by 1D NMR spectroscopic data (Table 1). Comparison of the NMR and MS data of **2** with those of the reference[13,14] indicated that compound **2** was qinyangshengenin-3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D- digitoxopyranoside (Fig. 1).

Compounds **1** and **2** were tested in vitro for cytotoxic activity against the human leukemia cancer cell line HL60 and the human lung cancer cell line A549.[15] The results, however, showed that the two compounds had no clear inhibition on these cancer cells.

CONCLUSION

A new C_{21} steroidal glycoside named qingyangshengenin-3-*O*-β-D-digitoxopyranoside (**1**), along with a known steroidal glycoside (**2**), was isolated from the whole herb of *P. vulgaris* Linn. The structure of compound **1** was elucidated on the basis of extensive spectroscopic analysis, including HRMS, 1D and 2D NMR experiments, and chemical evidence. The known compound **2** was determined by comparison of its spectroscopic data with that reported in the literature.

EXPERIMENTAL SECTION

General Methods

Optical rotation was measured at 20°C using a Rudolph-IV polarimeter equipped with a 2.5-cm pathlength cell. All NMR data were collected on a Varian INOVA 400-MHz spectrometer in $CDCl_3$ or CD_3OD (TMS as internal standard). MS data were recorded on Agilent 1100 LC/MS and STAR Pulsar I system spectrometers. The semipreparative HPLC was performed on a Waters-600 machine with a W2489 UV detector, column: ODS (5 μm, 10 × 150 mm, Waters Co, Ltd., USA). Silica gel (200–300 mesh and 300–400 mesh, Qingdao Ocean Chemical Factory, PR China) was used for TLC and column chromatography, respectively. Sephadex LH-20 (25–100 μm, Amersham Biosciences, Sweden) was also used for chromatography.

Plant Material

The whole herb of *P. vulgaris* Linn. was collected in Longli, Guizhou Province, of P.R. China, in June 2008, which was authenticated by Prof. Deyuan Chen from Guiyang College of Traditional Chinese Medicine. The voucher specimen (No. 20080624) was deposited at the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences.

Extraction and Isolation

The air-dried and powdered whole herb of *P. vulgaris* Linn. (10 kg) was extracted with 75% EtOH under reflux for 5 h three times. Then the combined EtOH extracts were concentrated under reduced pressure to yield a residue, which was suspended in H_2O and extracted successively with petroleum ether and ethyl acetate. The ethyl acetate soluble extract (180 g) was subjected to column chromatography on silica gel and eluted with $CHCl_3$/MeOH (100:0-0:100) to afford seven subfractions 1-7. Fr. 1 was further isolated on an MCI column and purified repeatedly by silica gel to provide compound **2** (13 mg). Fr. 4 was purified by reverse-phase RP-18 column (MeOH-H_2O) and semipreparative HPLC to yield compound **1** (29 mg).

Acidic hydrolysis: A solution of compound **1** (15.0 mg) in MeOH (5 mL) and 2N HCl (10 mL) was heated at 60°C for 3 h. After hydrolysis, the solvent was filtered off and evaporated, and the residue (the mixture of aglycone and sugar) was subjected to CC (column chromatography, $CHCl_3$:MeOH = 50:1–0:1) to yield fractions of sugar and aglycone. The aglucone fraction of **1** showed the same TLC behavior as qingyangshengenin (R_f 0.4, $CHCl_3$:MeOH = 10:1). Sugar fraction was identified by TLC (R_f 0.4; $CHCl_3$:MeOH = 8:1) as well as optical rotation $[\alpha]_D^{20}$+41.6; *c* 0.2, H_2O), which indicated the presence of a D-digitoxopyranoside unit ($[\alpha]_D^{20}$+48.4; c 0.9, H_2O) according to the reference.

Qingyangshengenin-3-*O*-β-D-digitoxopyranoside (**1**), white amorphous power ($[\alpha]_D^{20}$+1.7; *c* 0.7, CH_3OH); ^1H and ^{13}C NMR, see Table 1; HR-ESI-MS *m*/*z* 631.3115 $[M+H]^+$ (Calcd 631.3118, $C_{34}H_{46}O_{11}$). Qinyangshengenin-3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-digitoxopyranoside (**2**), white amorphous powder; ^1H and ^{13}C NMR, see Table 1; ESI-MS *m*/*z* 919 $[M+H]^+$, 941 $[M+Na]^+$.

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SUPPLEMENTAL MATERIAL

Supplemental data for this article can be accessed on the publisher's website.

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