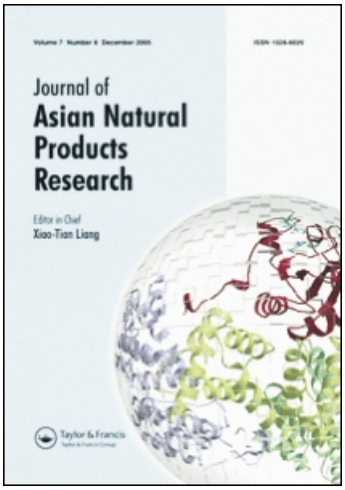

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NOTE

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A new bidesmoside triterpenoid saponin, named stauntoside C1 (**1**), along with three known saponins (**2**–**4**) was isolated from *Stauntonia chinensis* DC. (Lardizabalaceae). Their structures were established by means of spectral and chemical methods as 3-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**1**), scabiosaponin E (**2**), sieboldianoside B (**3**), and kizutasaponin K_{12} (**4**).

Keywords: Lardizabalaceae; bidesmoside triterpenoid saponins; *Stauntonia chinensis*; stauntoside C1

1. Introduction

The stems of *Stauntonia chinensis* DC. (Lardizabalaceae) are used as a Chinese herbal medicine for the treatment of analgesia and sedation [1]. Wang *et al.* [2–7] and Gao *et al.* [8,9] reported many new triterpenoid glycosides from this plant. We now report the isolation and structure elucidation of four bidesmoside triterpenoid glycosides (**1**–**4**) from the extract of the stem of the title plant. Their structures were established by means of spectral and chemical methods as 3-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named stauntoside C1 (**1**), scabiosaponin E (**2**) [11], sieboldianoside B (**3**) [15], and kizutasaponin K_{12} (**4**) [15]. The structures of saponins **2**–**4** were identified on the basis of spectral data (^{1}H, ^{13}C, DEPT, HMQC, and HMBC) and by comparison of their spectral data with those in the literatures [10,12–14].

2. Results and discussion

Compound **1** was obtained as a white amorphous powder with mp 238–240°C, and gave a positive result to the Liebermann–Burchard test. In the (−)- and (+)-ESI-MS of **1**, quasi-molecular ion peaks were observed at *m/z* 1467 $[M-H]^-$ and 1491 $[M+Na]^+$, respectively. Analysis of HR-ESI-MS (*m/z* 1491.6990 $[M+Na]^+$) revealed the molecular formula of **1** to be

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$C_{69}H_{112}O_{33}Na$. Five fragmentary ions at 1021 [M−470+Na], 865 [M−470−132 − H], 733 [M−470−132−132−H], 587 [M−470−132−132−146−H], and 455 [M−470−132−132−146−132−H] indicated the sequential losses of seven sugar moieties (four hexoses and three pentoses).

The aglycone of **1** was determined to be oleanic acid based on the presence of seven methyl signals at δ_H 0.87 (Me-25), 0.88 (Me-29), 0.88 (Me-30), 1.07 (Me-26), 1.14 (Me-24), 1.24 (Me-27), and 1.27 (Me-23), an olefinic proton at δ_H 5.34 (s, 1H, H-12), the signals at δ_C 122.3 and 143.9 assigned to be 12 (13)-en carbons, and a detailed comparison with the literature [10].

Of the 69 carbon signals observed in the ^{13}C NMR spectrum of **1**, 30 were assigned to the aglycone moiety and 39 to the oligosaccharide part. The ^{1}H and ^{13}C NMR spectra of **1** further exhibited seven anomeric sugar protons at δ 4.72 (3-Ara-1), 6.05 (3-Rha-1), 5.10 (3-xyl I-1), 4.74 (3-xyl II-1), 6.21 (28-Glc I-1), 4.85 (28-Glc II-1), and 5.62 (28-Rha-1) and carbons at δ 103.2, 101.4, 106.4, 104.8, 95.6, 104.3, and 102.4 (Tables 1–3); the methyl proton signals of two rhamnoses at δ 1.46 (d, 3H, 6.0 Hz) and 1.55 (d, 3H, 6.0 Hz). Acid hydrolysis of **1** with 2 N HCl–1,4-dioxane (1:1 v/v) furnished L-arabinose, D-xylose, L-rhamnose, and D-glucose in the ratio of 1:2:2:2, which were identified by the HPLC analysis of the thiazolidine derivatives [16].

Table 1. ^{1}H and ^{13}C NMR spectral data for the aglycone moiety of compound **1** (in pyridine-d_5).

	Aglycone of compound **1**			
No.	Prosapogenin 1c [11] δ_C	δ_C	δ_H	HMBC
1	38.6	38.7		
2	26.6	26.4		
3	88.5	88.6	3.22, d, J = 8.0 Hz	3-Ara-C-1
4	39.4	39.7		
5	55.7	55.7		
6	18.4	18.2		
7	33.1	32.2		
8	39.6	39.3		
9	47.9	47.8		
10	36.9	36.8		
11	23.6	23.5		
12	122.4	122.3	5.34, 1H, s	
13	144.7	143.9		
14	42.0	41.9		
15	28.2	27.9		
16	23.7	23.1		
17	46.5	46.0		
18	41.9	41.4		
19	46.3	46.8		
20	30.8	30.5		
21	34.1	33.7		
22	33.1	33.9		
23	28.1	27.9	1.27, 3H, s	
24	16.8	16.9	1.14, 3H, s	
25	15.4	15.4	0.87, 3H, s	
26	17.3	17.2	1.07, 3H, s	
27	26.0	25.8	1.24, 3H, s	
28	180.0	176.5	–	28-Glc I-C-1
29	33.1	32.9	0.88, 3H, s	
30	23.6	23.5	0.88, 3H, s	

Table 2. ^{1}H and ^{13}C NMR spectral data for the 3-*O*-sugar chain of compound **1** (in pyridine-d_5).

	Sieboldianoside B [9]		**1**		
No	δ_C	δ_H	δ_C	δ_H	HMBC
3-Ara-1	104.7	5.11	103.2	4.72, 1H, d, $J = 7.0$ Hz	Aglycone C-3
2	76.9	4.60	75.2	4.50 s	
3	75.8	4.11	73.5	4.22 m	
4	69.5	4.29	69.4	4.22 m	
5	65.9	4.27/3.67	66.9	4.27/3.70, d, $J = 12.0$ Hz	
3-Rha-1	101.4	6.30 br s	101.4	6.05, 1H, s	3-Ara-C-3
2	71.8	4.92	71.4	4.71	
3	82.6	4.71	82.2	4.45 m	3-Xyl I-C-1
4	73.8	4.50	73.8	4.20 s	
5	69.8	4.73	69.4	4.73	
6	18.6	1.59	18.2	1.46, 3H, d, $J = 6.0$ Hz	
3-Xyl I-1	106.8	5.29	106.4	5.10, 1H, d, $J = 7.5$ Hz	3-Rha-C-3
2	75.3	4.07	75.4	4.07 m	
3	75.2	3.94	74.9	3.88	
4	74.6	3.98	73.5	4.10 m	
5	64.8	3.73/4.30	64.4	3.44, $J = 11.5$ Hz/4.30	
3-Xyl II-1	104.6	5.01	104.8	4.74, 1H, d, $J = 6.0$ Hz	3-Xyl I-C-2
2	75.5	3.95	75.2	3.95 m	
3	75.7	4.12	75.5	4.15 m	
4	70.9	4.30	70.2	4.27 m	
5	67.3	4.28/3.68	66.9	4.29 m/3.68, $J = 11.5$ Hz/4.25	

Table 3. ^{1}H and ^{13}C NMR spectral data for the 28-*O*-sugar chain of compound **1** (in pyridine-d_5).

	Huzhangoside D [15]			**1**	
No.	δ_C	δ_H	δ_C	δ_H	HMBC
28-Glc I-1	95.5	6.26	95.6	6.21, 1H, d, $J = 8.5$ Hz	Aglycone C-28
2	73.8	4.09	73.8	4.08 m	
3	78.6	4.18	78.1	4.15 m	
4	70.7	4.28	70.1	4.28 m	
5	78.0	4.07	78.1	4.11 m	
6	69.1	4.63/4.30	69.4	4.64/4.30 m	28-Glc II-C-1
28-Glc II-1	104.7	4.96	104.3	4.85, 1H, d, $J = 8.0$ Hz	28-Glc I-C-6
2	75.3	3.91	73.8	3.925 m	
3	76.4	4.12	76.7	4.11 m	
4	78.1	4.38	78.3	4.28 m	28-Rha-C-1
5	77.0	3.62	76.7	3.64, $J = 11.5$ Hz	
6	61.2	4.17/4.06	60.9	4.18/4.06 m	
28-Rha-1	102.6	5.82	102.4	5.62, 1H, s	28-Glc II-C-4
2	72.5	4.65	72.1	4.69 s	
3	72.7	4.54	72.4	4.54 s	
4	73.9	4.30	72.2	4.28 m	
5	70.2	4.93	70.1	4.72, d, 1H, $J = 8.0$ Hz	
6	18.4	1.66	18.2	1.55, d, 1H, $J = 6.0$ Hz	

The chemical shifts of C-3 (δ_C 88.6) and C-28 (δ_C 176.5) revealed that **1** was a bisdesmosidic glycoside. The ^{13}C NMR spectral data of the sugar moieties indicated that all the monosaccharides were in pyranose forms. The β-anomeric configurations for the glucose and xylose units were determined from $^3J_{H1,H2}$ coupling constants (7.5–8.5 Hz); the arabinose unit was determined to have an α-configuration on the basis of the $^3J_{H1,H2}$ (6.0 Hz) values observed in the $^4C_1$ forms; the anomeric proton of the rhamnose unit was observed as a singlet and indicated an α-configuration.

The oligosaccharide sequence and the glycosidic site of **1** were determined by HMQC, TOCSY, and HMBC spectra. The H-1 of arabinose at δ_H 4.72 correlated with C-3 of aglycone at δ_C 88.6, the H-1 of rhamnose I at δ_H 6.05 correlated with C-2 of arabinose at δ_C 75.2, the H-1 of inner xylose at δ_H 5.10 correlated with C-3 of rhamnose I at δ_C 82.2, the H-1 of terminal xylose at δ_H 4.74 correlated with C-2 of inner xylose at δ_C 75.5, which indicated that the C-3 sugar chain was β-D-xylo-pyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl. The trisaccharide part at C-28 was established by the HMBC experiment (Figure 1): the H-1 of glucose I at δ_H 6.21 correlated with C-28 (δ_C 176.5) of aglycone, the H-1 at δ_H 4.85 of glucose II correlated with C-6 (δ_C 69.4) of glucose I (inner), the H-1 (δ_H 5.62) of rhamnose II correlated with C-4 (δ_C 78.3) of glucose II, as α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl. The sugar linkages of the oligosaccharide chains are shown in Figure 2. Thus, the structure of **1** was elucidated as 3-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 3)-*O*-

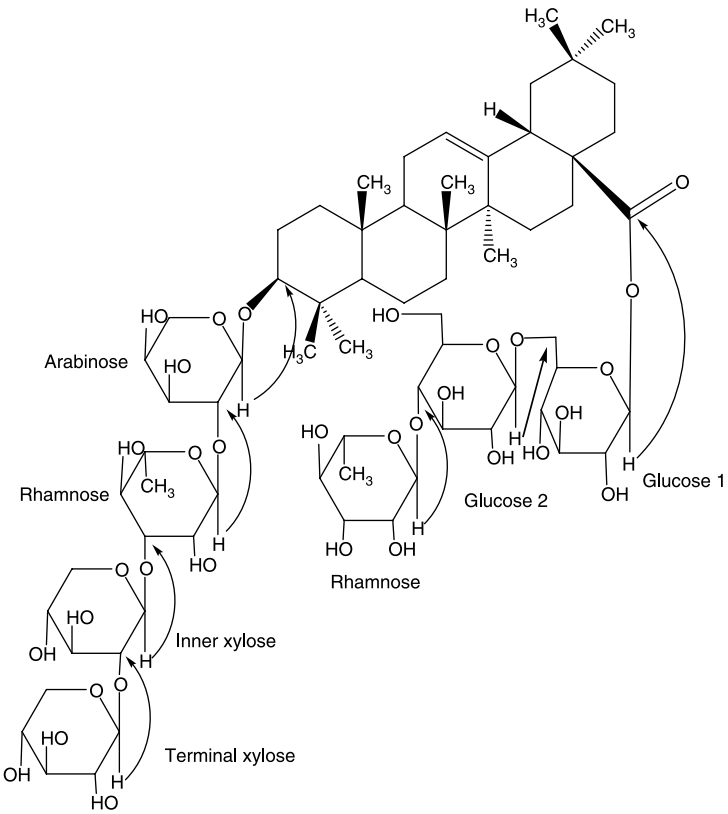
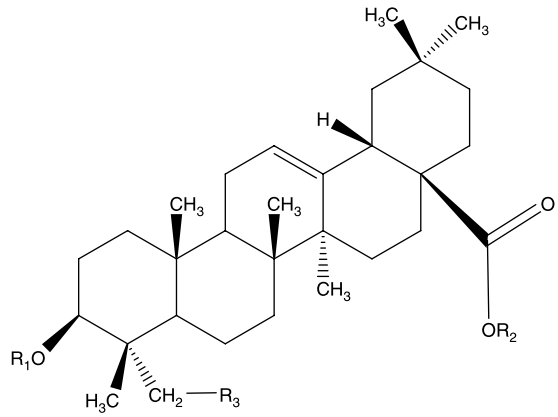


Figure 1. Key HMBC correlations of compound **1**.



1 $\mathbf{R_1}$ = β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl;
$\mathbf{R_2}$ = α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl; $\mathbf{R_3}$ = H
2 $\mathbf{R_1}$ = β-D-xylopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl;
$\mathbf{R_2}$ = β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl; $\mathbf{R_3}$ = H
3 $\mathbf{R_1}$ = β-D-xylopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl;
$\mathbf{R_2}$ = α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl; $\mathbf{R_3}$ = H
4 $\mathbf{R_1}$ = α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl;
$\mathbf{R_2}$ = α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl; $\mathbf{R_3}$ = OH

Figure 2. Structures of compounds **1**–**4**.

α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named stauntoside C1.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT_4-100 micromelting apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. IR spectra were obtained on a Nicolet 5700 IR spectrometer. NMR spectra were recorded on an Inova 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer. ESI-MS was performed with Agilent 1100 LC/MSD. For column chromatography, silica gel (200–300 mesh; Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), ODS (40–60 μm; Daisogel, DAISO Co., Ltd, Osaka, Japan), and Sephadex™ LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used.

The analytical HPLC was performed on an Agilent 1200 LC with DAD (Agilent Technologies, Waldbronn, Germany) and the preparative HPLC was performed on a Shimadzu LC-20A (Shimadzu Corporation, Kyoto, Japan) with a YMC-Pack ODS column (20 × 250 mm, 10 μm; YMC Co., Ltd, Kyoto, Japan).

3.2 Plant materials

The stems of *S. chinensis* DC. (Lardizabalaceae) were collected by Prof. Guo-Ping Zhou in Jiangxi Province on May 2008, and identified by Prof. Gui-Ping Yuan of Jiangxi Provincial Institute for Drug and Food Control. A voucher specimen (No. 350-B40-20-1) has been deposited at Jiangxi Provincial Institute for Drug and Food Control.

3.3 Extraction and isolation

The stems of *S. chinensis* (10.0 kg) were powdered and extracted with H_2O (10 times × 3), and then the aqueous residue (1600 g) was extracted with *n*-BuOH saturated with water three times to give the *n*-BuOH-soluble fraction (530 g), part of which (100 g) was subjected to column chromatography on macroporous resin

HP-20 with gradient H_2O, 30, 50, 70, and 95% EtOH to give five fractions (I–V). Fraction IV (eluted with 70% EtOH) was separated on a C-18 medium pressure column, eluted with MeOH–H_2O gradiently (30:70 → 100:0) to give 16 fractions (IV-1–16). Fraction IV-5 was purified with preparative HPLC eluted with 27% CH_3CN (flow rate, 4 ml/min) to give compound **1** (210 mg, 210 nm, t_R = 19.10 min). Fraction IV-8 was separated on preparative HPLC eluted with 28% CH_3CN (flow rate, 4 ml/min) to give compound **3** (90 mg, 210 nm, t_R = 27.64 min), Fraction IV-11 was eluted with 30% CH_3CN (flow rate, 4 ml/min) to give compound **2** (45 mg, 210 nm, t_R = 35.30 min) and compound **4** (15 mg, 210 nm, t_R = 30.50 min).

*3.3.1 Stauntoside C1 (**1**)*

A white amorphous powder, $[\alpha]_D^{20} - 37.1$ ($c = 0.013$, MeOH); mp 238–240°C; IR ν_{max} (cm^{-1}): 3376, 2939, 1742, 1650, 1434, 1388, 1072; ^1H NMR and ^{13}C NMR spectral data: see Tables 1–3. In the (−)- and (+)-ESI-MS of **1**, quasi-molecular ion peaks were observed at *m/z* 1467 $[M-H]^-$ and 1491 $[M+Na]^+$, +HR-ESI-Q-TOF-MS: *m/z* 1491.6990 $[M+Na]^+$ (calcd for $C_{69}H_{112}O_{33}Na$, 1491.6978).

3.4 Acid hydrolysis of compound 1

Take 80 μl D-glucose, L-rhamnose, D-xylose, and L-arabinose aqueous solution (each 2 mg/ml) with 80 μl of 0.5 mol/l PMP CH_3OH solution and 80 μl of 0.3 mol/l NaOH aqueous solution, and the mixtures were heated at 70°C for 30 min, cooled to room temperature for 10 min, added 80 μl of 0.3 mol/l HCl aqueous solution, and extracted with $CHCl_3$ (0.5 ml, three times). The water fractions were identified by the HPLC analysis (Phenomenex C18, 250 mm × 4.6 mm, 5 μm; flow phase A, CH_3CN–20 mmol/l NH_4OAc aqueous solution (15:85); B, CH_3CN–20 mmol/l NH_4OAc aqueous solution (40:60); flow rate, 1.2 ml/min; gradient elution, 0 → 20 min, volume fraction of B from 0 to 60%; detection wavelengths, 245 nm; sample volume, 20 μl).

Compound **1** (5 mg) was heated in an ampule with 2 ml of aqueous 2 M HCl–1,4-dioxane (1:1) at 80°C for 6 h. The aglycone was extracted with chloroform, and the aqueous layer was evaporated under reduced pressure and was taken as preparations of the normal sugar derivatives. Then, it furnished L-arabinose (t_R = 6.24 min), D-xylose (t_R = 8.24 min), L-rhamnose (t_R = 10.21 min), and D-glucose (t_R = 11.28 min) in the ratio of 1:2:2:2, which were identified by the HPLC analysis of the derivatives [16].

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