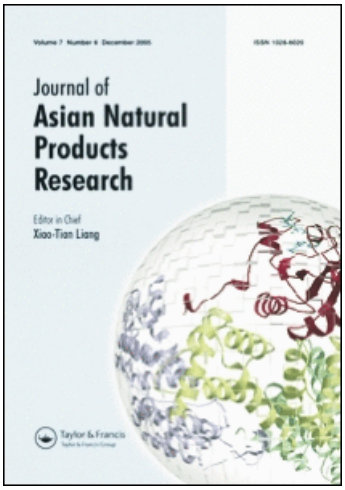

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Triterpenoid saponins from *Stauntonia chinensis*

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A new triterpenoid saponin, named stauntoside A (**1**) along with four known saponins (**2**,**3**,**4**,**5**) was isolated from *Stauntonia chinensis* DC., (Lardizabalaceae). Their structures were elucidated by spectroscopic analysis and chemical methods as 3-*O*-α-L-arabinopyranosyl-30-norhederagenin -28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**1**), 3-*O*-α-L-arabinopyranosyl-30- norhederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**2**), 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-30-norhederagenin-28-*O*-α-L- rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**3**), 3-*O*-α-L- arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D- glucopyranosyl ester (**4**), 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-hederagenin -28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**5**). The ^{1}H and ^{13}C NMR data for Glycoside L-G1 or Sinofoside A are paradox in the reported before. Thus, the structure elucidation of saponin **2**, known as Glycoside L-G1 or Sinofoside A, was discussed and the unambiguous assignments were given.

Keywords: *Stauntonia chinensis*; Lardizabalaceae; Stauntoside A; Triterpenoid saponins

1. Introduction

Stauntonia chinensis DC. (Lardizabalaceae), an evergreen herb growing in southern China, commonly known as "Ye Mu Gua", has been used as a traditional Chinese medicine, especially for analgesic and sedative purposes [1]. The anti-nociceptive and anti-inflammatory effects of the preparation of the plant, Injection Stauntonia, were evaluated [2]. Phytochemical studies on the plant resulted in the isolation of several nortriterpenoid saponins [3–6]. In this paper, we report the isolation and structural elucidation of five triterpenoid saponins (**1**–**5**) from the n-BuOH soluble fraction of the 60% EtOH extract of the rattan of the plant. Their structures were elucidated by spectroscopic analysis and chemical methods as 3-*O*-α-L-arabinopyranosyl-30-norhederagenin-28-*O*-β-D-glucopyranosyl- (1 → 6)-β-D-glucopyranosyl ester (**1**), 3-*O*-α-L-arabinopyranosyl-30-norhederagenin-28-*O*-α-L- rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-gluco-

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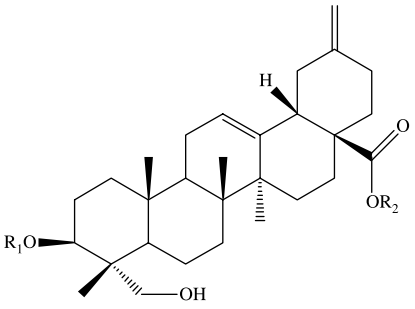
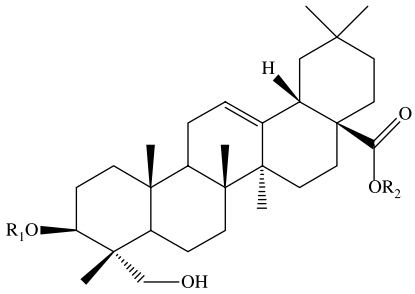


30-norhederagenin R_1=H R_2=H

1 R_1=¦Á-L-arabinopyranosyl
R_2=¦Â-D-glucopyranosyl-(1-6)-¦Â-D-glucopyranosyl

2 R_1=¦Á-L-arabinopyranosyl
R_2=¦Á-L-rhamnopyranosyl-(1-4)- O-¦Â-D-glucopyranosyl-(1-6)-¦Â-D-glucopyranosyl

3 R_1=¦Á-L-rhamnopyranosyl -(1-2)-O-¦Á-L-arabinopyranosyl
R_2=¦Á-L-rhamnopyranosyl-(1-4)- O-¦Â-D-glucopyranosyl-(1-6)-¦Â-D-glucopyranosyl



4 R_1=¦Á-L-arabinopyranosyl
R_2=¦Á-L-rhamnopyranosyl-(1-4)-O-¦Â-D-glucopyranosyl-(1-6)-¦Â-D-glucopyranosyl

5 R_1=¦Á-L-rhamnopyranosyl-(1-2)-O-¦Á-L-arabinopyranosyl
R_2=¦Á-L-rhamnopyranosyl-(1-4)-O-¦Â-D-glucopyranosyl-(1-6)-¦Â-D-glucopyranosyl

Figure 1. Structures of **1**–**5**.

pyranosyl ester (**2**) [7–9], 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-30-norhederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**3**) [10], 3-*O*-α-L-arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl- (1 → 6)-β-D-glucopyranosyl ester (**4**) [11–14], 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L- arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**5**) [12–14] (figure 1). The structures of saponin **3**, **4**, **5** were identified by comparisons of their physical and spectral properties with references [10–14]. However, the ^{1}H and ^{13}C NMR data for Glycoside L-G1 or Sinofoside A are paradox in the reported before [7–9] (table 1). Thus, the structure elucidation of saponin **2**, known as Glycoside L-G1 or Sinofoside A, was discussed and the unambiguous assignments were given. The data in this paper are consistent with those in reference [7], differential from those in reference [8,9].

2. Results and discussion

Saponin **1** was obtained as white amorphous powder, which gave positive results for the Liebermann–Burchard reaction and Molish reagent. Its molecular formula was determined

Table 1. ^{13}C NMR data of **1**–**5** in pyridine-d_5 at 100 MHz.

C	*30-norhederagenin*	***1***	***2***	***3***	***4***	***5***	*Sinofoside A*
1	38.8	38.8	38.8	39.0	38.8	39.0	38.8
2	27.7	26.0	26.0	26.1	26.0	26.1	28.2
3	73.5	81.8	81.9	81.0	81.9	81.0	81.7
4	42.9	43.4	43.4	43.4	43.4	43.5	43.4
5	48.7	47.4	47.5	47.6[a]	47.6	47.7	48.1
6	18.6	18.1	18.1	18.1	18.1	18.1	18.1
7	33.0	32.7	32.7	32.7	32.7	32.8	32.7
8	39.8	39.9	39.9	39.8	39.9	39.9	39.9
9	48.1	48.1	48.1	48.1	48.1	48.1	47.4
10	37.2	36.9	36.9	36.8	36.9	36.8	36.6
11	23.8	23.8	23.8	23.7	23.8	23.8	23.4
12	122.6	123.3	123.3	123.3	122.9	122.9	123.2
13	144.9	143.4	143.4	143.3	144.0	144.0	143.4
14	42.0	42.0	42.0	42.0	42.1	42.1	43.0
15	28.3	28.2	28.2	28.2	28.2	28.3	30.0
16	23.8	23.4	23.4	23.4	23.3	23.3	23.7
17	47.1	47.3	47.3	47.2	47.0	47.0	47.2
18	48.0	47.5	47.5	47.4[a]	41.6	41.6	47.4
19	42.0	41.6	41.6	41.6	46.1	46.1	46.0
20	148.5	148.3	148.3	148.3	30.7	30.7	148.3
21	30.4	30.0	30.0	30.0	33.9	33.9	37.6
22	38.4	37.6	37.6	37.6	32.5	32.5	30.0
23	68.1	64.4	64.4	63.9	64.5	63.9	64.4
24	13.1	13.5	13.6	13.9	13.5	13.9	13.5
25	16.0	16.2	16.2	16.1	16.1	16.1	16.1
26	17.5	17.5	17.5	17.5	17.5	17.5	17.5
27	26.2	26.0	26.0	25.9	26.0	26.0	26.0
28	179.4	175.8	175.8	175.7	176.4	176.5	175.7
29	107.0	107.2	107.3	107.3	33.0	33.0	107.3
30					23.6	23.6	
28-Glc-1		95.7	95.7	95.7	95.6	95.6	95.6
2		73.8	73.8	73.8	73.8	73.8	73.9
3		78.6	78.6	78.6	78.7	78.7	78.3
4		70.9	70.8	70.8	70.8	70.8	70.8
5		77.8	77.9	77.9	78.0	78.0	77.1
6		69.5	69.3	69.3	69.2	69.2	69.2
28-Glc-1′		105.3	104.9	104.9	104.8	104.8	104.8
2′		75.1	75.3	75.3	75.3	75.3	75.2
3′		78.3	76.5	76.4	76.5	76.5	76.4
4′		71.5	78.2	78.2	78.2	78.2	78.7
5′		78.4	77.1	77.1	77.1	77.1	77.6
6′		62.6	61.3	61.3	61.2	61.2	61.3
28-Rha-1			102.7	102.7	102.7	102.7	102.8
2			72.5	72.5	72.5	72.5	72.5
3			72.7	72.7	72.7	72.7	72.7
4			74.0	73.9	73.9	73.9	73.7
5			70.3	70.2	70.2	70.3	70.7
6			18.5	18.5	18.5	18.5	18.4
3-Ara-1		106.6	106.6	104.3	106.6	104.3	106.6
2		73.1	73.1	75.7	73.1	75.8	73.0
3		74.7	74.7	74.1[b]	74.7	74.1[a]	74.8
4		69.5	69.6	69.3	69.6	69.3	69.5
5		66.9	66.9	65.6	66.9	65.6	66.8
3- Rha − 1				101.6		101.6	
2				72.3		72.3	
3				72.5		72.5	
4				74.6[b]		74.6[a]	
5				69.6		69.7	
6				18.4		18.5	

[a,b]These assignments may be interchanged in each column.
30-norhederagenin: ^{13}C NMR data are taken from reference [16].
Sinofoside A: ^{13}C NMR data are taken from reference [8–9].

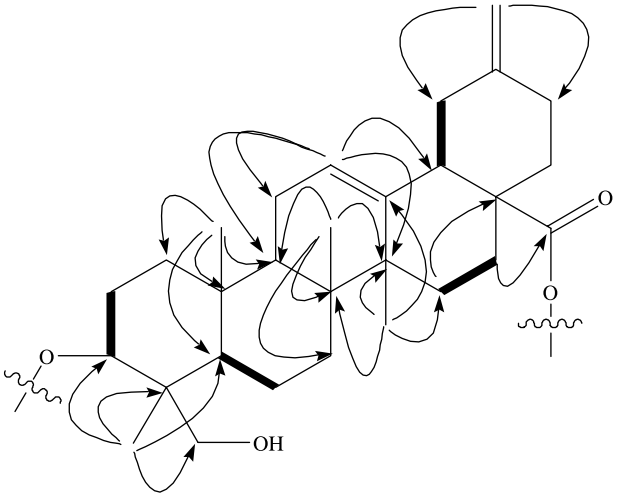


Figure 2. Key HMBC and COSY correlations of the Aglycone moity of **1**.

as $C_{46}H_{72}O_{18}$ according to the positive HR-ESI-Q-TOF-MS. After acid hydrolysis of saponin **1** the gas chromatography analysis of acetylated aldononitriles revealed the presence of L-arabinose and D-glucose in a ratio of 1:2, comparing with standard aldononitrile peracetates of sugars. The IR spectrum of **1** showed absorption band at 1742 cm^{-1}, indicating the presence of ester carbonyl group in the molecule. In the ESI-IT-MSn experiments, the MS2 experiment of the ion at *m/z* 935 $[M + Na]^+$ gave positive fragments at *m/z* 611 $[M + Na - 324]^+$, 567 $[M + Na - 324 - 44]^+$ and 347 $[324 + Na]^+$, suggesting that a sugar chain comprising two glucoses was connected to saponin **1** by ester bond, based on the fact that the cleavage readily occurs at the ester linkage of glycosides in CID experiments and the charge reserves either in the sugar moiety or in the other moiety [15]. The MS3 experiment of the ion at *m/z* 587 $[M - H - 324]^-$ gave negative fragments at *m/z* 455 $[(M - H - 324) - 132]^-$, suggesting that the arabinose was a terminal sugar. The aglycone was determined to be 30-norhederagenin based on the detailed analysis of the information of the COSY and HMBC experiments (figure 2), and the relative configuration of the aglycone was confirmed by analysis of the correlations in the NOESY experiment (figure 3). The signals of the sugar moiety of saponin **1** were assigned based on the analysis of the

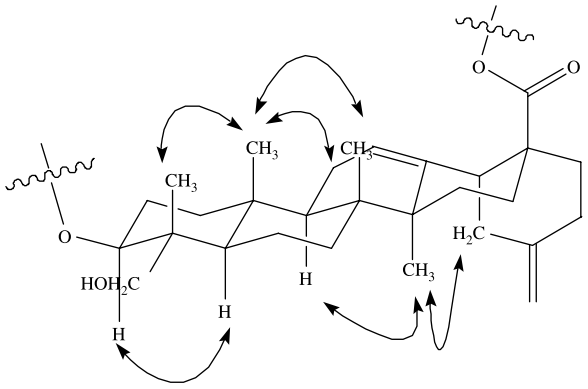


Figure 3. Key NOESY correlations of the Aglycone moity of **1**.

information of the COSY and TOCSY experiments. The HMBC correlation signals found for the carbon at δ 106.6 and the proton at δ 4.26; for the carbon at δ 81.8 and the proton at δ 4.97 and for the carbon at δ 175.8 and the proton at δ 6.20 indicated that the the arabinose was attached at C-3 of the aglycone and the glucose was attached at C-28 of the aglycone by ester bond. The comparison of ^{13}C NMR data of **1** with those of 30-norhederagenin showed the evident glycosidation shift [16], which confirmed the above-mentioned conclusion. The HMBC correlation signals found for the carbon at δ 69.5 and the proton at δ 4.99 indicated that the C-28 sugar chain was glucopyranosyl-(1 → 6)-glucopyranosyl. Judged by three anomeric proton signals at δ 6.20 (1H, d, $J = 8.1$ Hz, H28-Glc-1), 4.99 (1H, d, $J = 7.8$ Hz, H28-Glc-1′) and 4.97 (1H, d, $J = 7.2$ Hz, H3-Ara-1), the relative configuration of the sugar units were determined as α-arabinose and β-glucose. Therefore saponin **1** was determined as 3-*O*-α-L-arabinopyranosyl-30-norhederagenin-28-*O*-β-D-glucopyranosyl -(1 → 6)-β-D-glucopyranosyl ester. Saponin **1** is a new compound, named stauntoside A.

Saponin **2** was obtained as white amorphous powder, which gave positive results for the Liebermann–Burchard reaction and Molish reagent. Its molecular formula was determined as $C_{52}H_{82}O_{22}$ according the positive HR-ESI-Q-TOF-MS. After acid hydrolysis of saponin **2**, the gas chromatography analysis of acetylated aldononitriles revealed the presence of L-arabinose, L-rhamnose and D-glucose in a ratio of 1:1:2. The IR spectrum of **2** showed absorption band at 1740 cm^{-1}, indicating the presence of ester carbonyl group in the molecule. The ESI-IT-MSn experiments suggested that a sugar chain comprising two glucoses and one rhamnose was connected to saponin **2** by ester bond. The aglycone was also determined to be 30-norhederagenin based on the analysis of the information of the COSY, HMBC and NOESY experiments. The signals of the sugar moiety were assigned based on the analysis of the information of the COSY and TOCSY experiments. The HMBC correlation signals found for the carbon at δ 106.6 and the proton at δ 4.26; for the carbon at δ 81.9 and the proton at δ 4.96 and for the carbon at δ 175.8 and the proton at δ 6.17 indicated that the arabinose was attached at C-3 of the aglycone and the glucose was attached at C-28 of the aglycone by ester bond. The HMBC correlation signals found for the carbon at δ 104.9 and the proton at δ 4.63; for the carbon at δ 69.3 and the proton at δ 4.94; for the carbon at δ 102.7 and the proton at δ 4.39 and for the carbon at δ 78.2 and the proton at δ 5.83 indicated that the C-28 sugar chain was rhamnopyranosyl-(1 → 4)-glucopyranosyl-(1 → 6)-glucopyranosyl. Judged by anomeric proton signals, the relative configuration of the sugar units were determined as α-arabinose, α-rhamnose and β-glucose. Therefore saponin **2** was determined as 3-*O*-α-L-arabinopyranosyl-30-norhederagenin-28-*O*-α-L- rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, known as Glycoside L-G1 or Sinofoside A. The ^{1}H and ^{13}C NMR data for Glycoside L-G1 or Sinofoside A are paradox in the reported before [7–9] (table 1). Thus, the structure elucidation of saponin **2** was discussed and the unambiguous assignments were given. The data in this paper are consistent with those in reference [7], differential from those in reference [8,9].

3. Experimental

3.1 General experimental procedures

NMR spectra were recorded on a Bruker AVANCE 400 NMR spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). ESI-IT-MS spectra were performed on a Bruker esquire 2000 mass spectrometer and HR- ESI-Q-TOP-MS were obtained on a Micromass Q-TOF mass

spectrometer. IR spectra were recorded on a Shimadzu FTIR8400 spectrophotometer. Optical rotations were measured using a JASCO P-1020 polarimeter. The analytical HPLC was performed on a Shimadzu class-vp system with refractive index detector (RID-10A) using a Shim-pack VP-ODS column (4.6 × 250 mm) and the preparative HPLC was performed on a Shim-pack PRC-ODS column (10 × 250 mm) with refractive index detector (RID-10A). Column chromatography was carried out on silica gel (200–300 mesh) (Qingdao Haiyang Chemical Group Corporation, Qingdao, China), Sephadex LH-20 (Amersham Biosciences AB) and ODS (60–80 μm, Merck). Silica gel GF_{254} (Qingdao Haiyang Chemical Group Corporation, Qingdao, China) and RP-18 F_{254} (Merck) were used for analytical TLC.

3.2 Plant material

The fresh plant of *Stauntonia chinensis* DC. was collected by LifeTech Pharmaceuticals Ltd, Guangzhou in Jiangxi Province in November 2004, and identified by Traditional Chinese Medicine Department, Shenzhen Institute of Drug Control. A voucher specimen is deposited at Research Center of Traditional Chinese Medicine and Natural Products, Shenzhen, China.

3.3 Extraction and isolation

The air-dried rattan of *Stauntonia chinensis* (20 kg) was refluxed with 60% EtOH for two times. After evaporation of EtOH *in vacuo*, the aqueous residue was extracted with *n*-BuOH for three times. A portion of the *n*-BuOH (150 g) extract was first subjected to column chromatography on silica gel, eluted with $CHCl_3$–MeOH–H_2O (C-M-W) gradiently (100:0:0, 98:2:0, 95:5:0, 90:10:0, 80:20:2, 70:30:5, 60:40:8, 0:100:0) to yield 8 fractions. Fraction 6 (C-M-W 70:30:5) was further applied to a ODS column chromatography, eluted with MeOH–H_2O (M-W) gradiently (0:100 → 100:0) to give 7 subfractions. Subfraction 4 (M-W 50:50) was purified on a Sephadex LH-20 column with M-W (1:1) as eluent and finally repeated preparative HPLC on a Shim-pack PRC-ODS column with M-W (60:40) resulted in saponin **1** (13 mg), saponin **2** (68.1 mg), saponin **3** (10.2 mg), saponin **4** (13.6 mg), saponin **5** (25.4 mg).

3.3.1 Saponin 1. white amorphous powder; $[\alpha]_D^{25}$ 23.7 (c 0.12, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3388, 2938, 1742, 1652, 1389, 1073; ^1H NMR data δ: 0.92 (3H, s, H24), 0.97 (3H, s, H25), 1.04 (1H, m, H1a), 1.10 (3H, s, H26), 1.10 (1H, m, H15a), 1.14 (3H, s, H27), 1.29 (1H, m, H7a), 1.34 (2H, m, H6), 1.56 (1H, m, H1b), 1.58 (1H, m, H7b), 1.67 (1H, m, H5), 1.72 (1H, m, H9), 1.73 (1H, m, H22a), 1.88 (1H, m, H11a), 1.99 (1H, m, H2a), 2.00 (1H, m, H16a), 2.00 (1H, m, H11b), 2.02 (1H, m, H22b), 2.05 (1H, m, H21a), 2.09 (1H, m, H16b), 2.15 (1H, m, H19a), 2.16 (1H, m, H21b), 2.23 (1H, m, H2b), 2.29 (1H, m, H15b), 2.53 (1H, t, $J = 13.6$ Hz, H19b), 3.10 (1H, dd, $J_1 = 13.4$ Hz, $J_2 = 5.0$ Hz, H18), 3.68 (1H, m, H23a), 3.71 (1H, m, H3-Ara-5a), 3.87 (1H, m, H28-Glc-5′), 3.99 (1H, m, H28-Glc-2′), 4.05 (1H, m, H3-Ara-3), 4.06 (1H, m, H28-Glc-5), 4.09 (1H, m, H28-Glc-2), 4.18 (1H, m, H28-Glc-3), 4.19 (1H, m, H28-Glc-4′), 4.24 (1H, m, H3-Ara-4), 4.26 (1H, m, H3), 4.27 (1H, m, H3-Ara-5b), 4.27 (1H, m, H23b), 4.29 (1H, m, H28-Glc-4), 4.32 (1H, m, H28-Glc-6a), 4.35 (1H, m, H28-Glc-6′a), 4.40 (1H, m, H28-Glc-3′), 4.41 (1H, m, H3-Ara-2), 4.47 (1H, m, H28-Glc-6′b), 4.63 (1H, brs, H29a), 4.67 (1H, m, H28-Glc-6b), 4.70 (1H, brs, H29b),

4.97 (1H, d, $J = 7.2$ Hz, H3-Ara-1), 4.99 (1H, d, $J = 7.8$ Hz, H28-Glc-1′), 5.41 (1H, brs, H12), 6.20 (1H, d, $J = 8.1$ Hz, H28-Glc-1); ^{13}C NMR data, see table 1; + ESI-IT-MS *m/z*: 935 $[M + Na]^+$, 951 $[M + K]^+$, + ESI-IT-MS2 (935) *m/z*: 611 $[M + Na - 324]^+$, 567 $[M + Na - 324 - 44]^+$, 347 $[324 + Na]^+$, + ESI-IT-MS3 (935–611) *m/z*: 567 $[(M + Na - 324) - 44]^+$, − ESI-IT-MS *m/z*: 911 $[M - H]^-$, 947 $[M + Cl]^-$, − ESI-IT-MS2 (911) *m/z*: 587 $[M - H - 324]^-$, − ESI-IT-MS3 (911–587) *m/z*: 455 $[(M - H - 324) - 132]^-$; + HR-ESI-Q-TOF-MS *m/z:* 930.5002 $[M + NH_4]^+$ (calcd for $C_{46}H_{76}NO_{18}$, 930.5062).

3.3.2 Saponin 2. white amorphous powder; $[\alpha]_D^{24}$ 22.3 (c 0.15, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3445, 2939, 1740, 1651, 1389, 1061; ^{1}H NMR data δ: 0.92 (3H, s, H24), 0.96 (3H, s, H25), 1.03 (1H, m, H1a), 1.08 (1H, m, H15a), 1.09 (3H, s, H26), 1.14 (3H, s, H27), 1.27 (1H, m, H7a), 1.32 (2H, m, H6), 1.56 (1H, m, H1b), 1.57 (1H, m, H7b), 1.66 (1H, m, H5), 1.68 (3H, d, $J = 6.2$ Hz, H28-Rha-6), 1.69 (1H, m, H22a), 1.71 (1H, m, H9), 1.87 (1H, m, H11b), 1.98 (1H, m, H16a), 1.98 (1H, m, H11a), 1.99 (1H, m, H2a), 2.00 (1H, m, H22b), 2.05 (1H, m, H21a), 2.08 (1H, m, H16b), 2.14 (1H, m, H21b), 2.14 (1H, m, H19a), 2.24 (1H, m, H2b), 2.27 (1H, m, H15b), 2.52 (1H, t, $J = 13.6$ Hz, H19b), 3.08 (1H, dd, $J_1 = 13.2$ Hz, $J_2 = 4.8$ Hz, H18), 3.64 (1H, m, H28-Glc-5′), 3.67 (1H, m, H23a), 3.70 (1H, m, H3-Ara-5a), 3.92 (1H, m, H28-Glc-2′), 4.04 (1H, m, H28-Glc-5), 4.05 (1H, m, H3-Ara-3), 4.07 (1H, m, H28-Glc-6′a), 4.07 (1H, m, H28-Glc-2), 4.12 (1H, m, H28-Glc-3′), 4.17 (1H, m, H28-Glc-3), 4.19 (1H, m, H28-Glc-6′b), 4.22 (1H, m, H3-Ara-4), 4.26 (1H, m, H3), 4.27 (1H, m, H23b), 4.27 (1H, m, H3-Ara-5b), 4.27 (1H, m, H28-Glc-4), 4.28 (1H, m, H28-Glc-6a), 4.30 (1H, m, H28-Rha-4), 4.39 (1H, m, H28-Glc-4′), 4.41 (1H, m, H3-Ara-2), 4.53 (1H, m, H28-Rha-3), 4.63 (1H, m, H28-Glc-6b), 4.65 (1H, m, H28-Rha-2), 4.67 (1H, brs, H29a), 4.73 (1H, brs, H29b), 4.93 (1H, m, H28-Rha-5), 4.94 (1H, d, $J = 7.8$ Hz, H28-Glc-1′), 4.96 (1H, d, $J = 6.8$ Hz, H3-Ara-1), 5.42 (1H, t, $J = 3.8$ Hz, H12), 5.83 (1H, brs, H28-Rha-1), 6.17 (1H, d, $J = 8.1$ Hz, H28-Glc-1); ^{13}C NMR data, see table 1; + ESI-IT-MS *m/z*: 1081 $[M + Na]^+$, 1097 $[M + K]^+$; + ESI-IT-MS2 (1081) *m/z*: 611 $[M + Na - 470]^+$, 567 $[M + Na - 470 - 44]^+$, 493 $[470 + Na]^+$; + ESI-IT-MS3 (1081–611) *m/z*: 567 $[(M + Na - 470) - 44]^+$; + ESI-IT-MS3 (1081–493) *m/z*: 475, 447, 421, 405, 349, 347, 331, 289, 203; − ESI-IT-MS *m/z*: 1057 $[M - H]^-$, 1093 $[M + Cl]^-$; − ESI-IT-MS2 (1057) *m/z*: 587 $[M - H - 470]^-$, 469 $[470 - H]^-$; + HR-ESI-Q-TOF-MS *m/z*: 1076.5602 $[M + NH_4]^+$ (calcd for $C_{52}H_{86}NO_{22}$, 1076.5641).

3.4 Acid hydrolysis of saponin 1 and saponin 2

Saponin **1** (5 mg) was heated in an ampule with 5 mL of aqueous 12% HCl at 100°C for 2 h. The aglycone was extracted with chloroform, and the aqueous residue was evaporated under reduced pressure. Then, 1 mL of pyridine and 2 mg of $NH_2OH{\cdot}HCl$ were added to the dry residue, and the mixture was heated at 100°C for 1 h. After cooling, Ac_2O (1.5 mL) was added, and the mixture was heated at 100°C for 1 h. The reaction mixture was evaporated under reduced pressure, and the resulting aldononitrile peracetates were analyzed by GC-MS comparing with standard aldononitrile peracetates of sugars, which indicated that the sugar moiety of saponin **1** was constituted with L-arabinose and D-glucose in a ratio of 1:2.

Saponin **2** (5 mg) was treated with the same method with saponin **1**, which indicated that the sugar moiety of saponin **2** was constituted with. L-arabinose, L-rhamnose and D-glucose in a ratio of 1:1:2

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References

[1] Jiangsu New Medical College, *Zhong Yao Da Ci Dian*, **2**, 2185 (1977).
[2] X.L. Peng, X.L. Gao, J. Chen, X. Huang, H.S. Chen. *Sheng Li Xue Bao*, **55**, 516 (2003).
[3] H.B. Wang, D.Q. Yu, X.T. Liang, N. Watanabe, M. Tamai, S. Omura. *Planta Med.*, **55**, 303 (1989).
[4] H.B. Wang, D.Q. Yu, X.T. Liang, N. Watanabe, M. Tamai, S. Omura. *Yao Xue Xue Bao*, **24**, 444 (1989).
[5] H.B. Wang, D.Q. Yu, X.T. Liang, N. Watanabe, M. Tamai, S. Omura. *J. Nat. Prod.*, **53**, 313 (1990).
[6] H.B. Wang, D.Q. Yu, X.T. Liang. *J. Nat. Prod.*, **54**, 1097 (1991).
[7] L.A. Yakovishin, V.I. Grishkovets, A.S. Shashkov, V.Ya. Chirva. *Chem. Nat. Comp.*, **35**, 543 (1999).
[8] X.H. Yang, R.J. Ma, R.H. Lu, X.N. Wei, Y.P. Wang, H.Q. Wang, J. Kong. *Chin. Chem. Lett.*, **12**, 55 (2001).
[9] X.H. Yang, R.J. Ma, Q. Xia, R.H. Lu, X.N. Wei, H. Wang, J. Kong. *Indian J. Chem. Sec. B: Org. Chem. Includ. Med. Chem.*, **41B**, 677 (2002).
[10] A.S. Sbashkov, V.I. Grishkovets, L.A. Yakovishin, I.N. Shchipanova, V.Y. Chirva. *Chem. Nat. Comp.*, **34**, 690 (1998).
[11] H.B. Wang, R. Mayer, G. Ruecker. *Phytochemistry*, **34**, 1389 (1993).
[12] H. Kizu, M. Koshijima, M. Hayashi, T. Tomimori. *Chem. Pharm. Bull.*, **33**, 1400 (1985).
[13] H. Kizu, S. Kitayama, F. Nakatani, T. Tomimori, T. Namba. *Chem. Pharm. Bull.*, **33**, 3324 (1985).
[14] H. Kizu, S. Hirabayashi, M. Suzuki, T. Tomimori. *Chem. Pharm. Bull.*, **33**, 3473 (1985).
[15] M. Cui, W.X. Sun, F.R. Song, Z.Q. Liu, S.Y. Liu. *Mass Spectrom.*, **13**, 873 (1999).
[16] A. Ikuta, H. Itokawa. *Phytochemistry*, **27**, 2813 (1988).