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Two C_{21}-steroidal glycosides isolated from *Cynanchum stauntoi*

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Abstract

Studies on the roots of *Cynanchum stauntoi* led to the isolation of two C_{21}-steroidal glycosides, formally named stauntosides A and B. Their structures were elucidated on the basis of spectroscopic evidence, especially that from analysis of 2D-NMR spectra. They were found to possess an unusual skeleton and were identified as stauntogenin 3-*O*-α-L-diginopyranosyl-(1-4)-β-L-cymaropyranosyl-(1-4)-β-D-digitoxopyranosyl-(1-4)-β-D-thevetopyranoside and stauntogenin 3-*O*-α-L-cymaropyranosyl-(1-4)-β-D-digitoxopyranosyl-(1-4)-β-D-3-demethyl-2-deoxy-thevetopyranoside.

Keywords: *Cynanchum stauntoi*; Ascelepiadaceae; C_{21}-steroidal glycosides; Stauntoside A; Stauntoside B; NMR assignments; Two-dimensional NMR techniques

1. Introduction

C_{21}-steroids and their glycosides are of considerable interest because of their bioactivities (Miller, 1973), such as hypolipidemic (Satyavati, 1991) and antitumor activity (Luo, Lin, Cordell, Xue & Johnson, 1993). Many such compounds have been isolated from plants, especially from the Asclepiadaceae family. For example, more than 10 C_{21}-steroidal glycosides with a novel 13,14:14,15-disecopregnane-type skeleton have been isolated from *Cynanchum glaucescens* (Nakagawa, Hayashi & Mitsuhashi, 1982; Nakagawa, Hayashi, Wada & Mitsuhashi, 1983; Tsukamoto, Hayashi & Mitsuhashi, 1985; Nakagawa, Hayashi & Mitsuhashi, 1983a, 1983b). In China, the dried roots of *Cynanchum glaucescens* and a closely related species, *Cynanchum stauntoi* (Decne.) Schltr. ex Levl. (Asclepiadaceae) are known as 'Bai-qian', and are used as antitussives and expectorants in traditional Chinese medicine (Jiangsu New Medical College, 1977). *C. stauntoi* is a perennial herb growing wild in southern China, and no chemical investigation has been reported previously on this species. This paper describes the isolation and structure elucidation of two C_{21}-steroidal glycosides, stauntosides A and B from the roots of *Cynanchum stauntoi*.

2. Results and discussion

Stauntoside A (**1**) showed positive Libermann–Burchard and Keller–Kiliani reactions, suggesting it to be a steroidal glycoside with a 2-deoxysugar moiety (Xu and Chen, 1981). The molecular formula $C_{48}H_{72}O_{19}$ was deduced from its negative APCIMS (m/z: 952 $[M]^-$) and its ^{13}C-NMR spectrum. Its spectral features and physicochemical properties suggested **1** to be a C_{21}-steroidal glycoside. The ^{13}C-NMR spectrum of compound **1** showed a total of 48 carbon signals, of which 21 were assigned to the aglycone part and the remaining 27 (including three OCH_3) were assigned to the oligosaccharide moity.

The ^{13}C-NMR data (see Table 1) obtained for the aglycone of stauntoside A was very similar to those of C_{21}-steroidal glycosides from *Cynanchum glaucescens* (Nakagawa et al., 1983). In the ^{13}C-NMR spectrum of **1**, signals at δ 113.3 (*s*), 119.7 (*d*), 140.1 (*s*), 129.8 (*s*),

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Table 1
NMR spectral data of the aglycone (Pyridine-d^5, δ (ppm)), J = Hz)

No.	Stauntoside A (**1**)		Stauntoside B (**2**)		HMBC(H → C)
	^{1}H	^{13}C	^{1}H	^{13}C	
1α	1.09 (*ddd*, 3.9, 13.6,13.6)	37.2	1.09 (*ddd*, 3.7, 13.6, 13.6)	37.2	19
1β	1.74 (*ddd*, 3.7, 3.7, 13.6)		1.74 (*ddd*, 3.7, 3.7, 13.6		
2α	2.05 (*m*)	30.1	2.05 (*m*)	30.1	
2β	1.62 (*m*)		1.64 (*m*)		
3	3.80 (*m*)	77.4	3.75 (*m*)	76.9	1′
4α	2.63 (*dddd*, 2.2, 2.2, 2.2, 13.4)	38.8	2.52 (*dddd*, 2.2, 2.2, 2.2, 13.4)	38.8	2, 3, 5, 10
4β	2.31 (*m*)		2.30 (*m*)		
5		140.1		140.1	
6	5.22 (*m*)	119.7	5.28 (*m*)	119.8	7, 8, 10
7α	2.36 (*m*)	29.9	2.37 (*m*)	29.9	5, 6, 8, 9, 14
7β	2.14 (*m*)		2.19 (*dddd*, 2.9, 5.9, 11.0, 11.0)		9
8	2.48 (*m*)	40.9	2.51 (*m*)	40.9	14
9	2.05 (*m*)	52.1	2.05 (*m*)	52.1	7, 8, 10, 11, 19
10		38.0		38.0	
11α	2.35 (*m*)	27.1	2.35 (*m*)	27.1	9, 12, 13
11β	4.10 (*ddd*, 12.0, 12.0, 12.0)		4.10 (*ddd*, 12.0, 12.0, 12.0)		9, 10, 12, 13
12	6.14 (*ddd*, 1.7, 4.6, 12.0)	146.9	6.14 (*ddd*, 1.7, 4.6, 12.0)	146.8	9, 10, 17, 18
13		129.9		129.8	
14		179.3		179.3	
15α	4.42 (*dd*, 7.3, 10.5)	71.2	4.42 (*dd*, 7.3, 10.5)	71.2	17, 20
15β	4.02 (*d*, 4.9, 10.5)		4.02 (*dd*, 4.9, 10.5)		16
16	5.74 (*dd*, 4.9, 7.3, 7.8)	77.3	5.74 (*ddd*, 4.9, 7.3, 7.8)	77.3	14, 20
17	3.54 (*br d*, 7.8)	54.4	3.54 (*br d*, 7.8)	54.4	12, 13, 18, 21
18		167.4		167.5	
19	1.02 (*s*)	20.0	1.09 (*s*)	20.0	5, 9, 10,
20		113.3		113.3	
21	1.63 (*s*)	23.4	1.63 (*s*)	23.4	17, 20

146.9 (*d*), 167.5 (*s*) and 179.3 ppm (*s*) suggested the presence of two trisubstituted double bonds, two carbonyls and a ketal function. Like glaucogenin C (Nakagawa et al., 1983) (the main aglycone of C_{21}-steroidal glycosides from *Cynanchum glaucescens*), the ^{1}H-NMR spectrum of **1** showed signals corresponding to an angular methyl at δ 1.02 (*s*, 3H) and an olefinic proton at δ 5.22 (*m*, 1H), indicating that **1** is a Δ5-steroid possessing ordinary A and B rings. In addition, the signal δ 6.14 (*ddd*, J = 1.7, 4.6, 12.0 Hz, 1H) suggest the presence of another trisubstituted double bond. However, as compared to glaucogenin C, the ^{1}H-NMR spectrum of the aglycone of stauntoside A showed the absence of a proton at δ 6.27 (*d*, $J = 2$ Hz, ^{1}H), and the presence of a new proton at δ 6.14 (*ddd*, J = 1.7, 4.6, 12.0 Hz, 1H). In the ^{13}C-NMR spectrum, major differences include the absence of two carbons at δ 118.5 and 143.8 and one carbon in the region δ 20–30, with the appearance of three carbons at δ 146.8, 129.8, and 167.5. Taken together, the NMR evidence suggests differences in the nine-membered lactone ring (C) and in one five-membered ring (D). The structures of rings C and D were confirmed based on the following results: the HMBC spectrum of **1** (Table 1) showed that H-12 was correlated with C-18, C-17, C-9 and C-10; C-12 was correlated with H-17 and H-11; C-13 was correlated with H-17 and H-11; H-17 was correlated with C-12, C-13, C-18 and C-21; C-17 was correlated with H-12, H-15 and H-21, and C-14 was correlated with H-7, H-8 and H-16. Therefore, together with the ^{1}H- and ^{13}C-NMR spectra, the structure of the aglycone of stauntoside A was assigned as shown in Fig. 1.

With the help of a Dreiding stereo model, the stereochemistry of the aglycone of 1 was determined from the ^{1}H-NMR and NOESY spectra. Observed NOE correlations and deshielding effects of the carbonyl are shown in Fig. 2. Thus, the aglycone of stauntoside A has an unsual skeleton, and was named stauntogenin.

The type of sugar units and the sequence of the oligosaccharide chain of stauntoside A were established by a combination of 2D-NMR experiments and chemical analysis. The presence of L-cymanose, L-diginose, D-digitoxose and D-thevetose in the hydrolysates of this compound was confirmed by TLC comparison with authentic samples.The ^{1}H- and ^{13}C-NMR spectra of 1 showed four sugar anomeric protons at δ 4.79 (*d*,

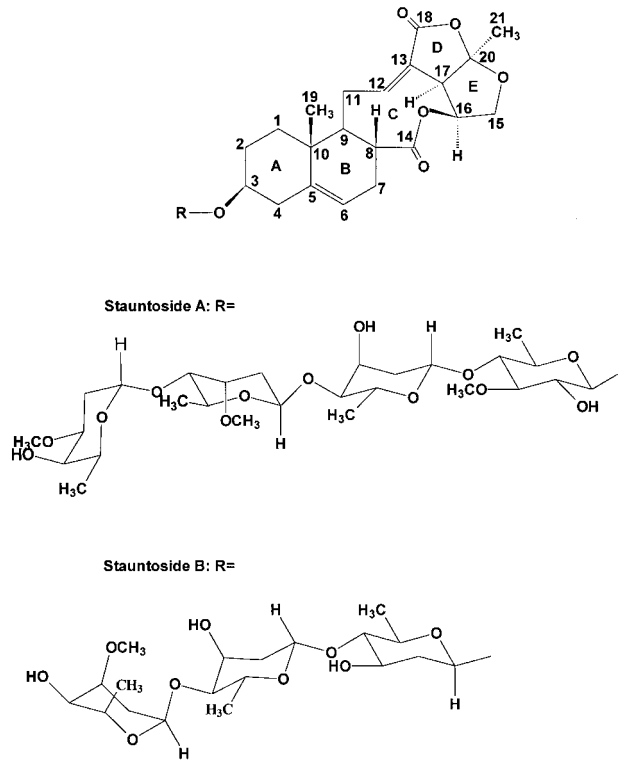

Fig. 1. Structures of stauntoside A (**1**) and stauntoside B (**2**).

$J = 7.6$ Hz), 5.51 (*dd*, $J = 1.7$, 9.5 Hz), 5.12 (*dd*, $J = 1.7$, 9.8 Hz), 5.18 (*br d*, $J = 3.4$ Hz) and carbons at δ 102.0, 98.9, 99.6, 101.7 respectively.

Utilizing the anomeric proton the β-D-thevetopyranosyl unit H-1′ at δ 4.79 (*d*, $J = 7.6$ Hz) as a starting point, H-2′ and H-3′ signals were assigned by the correlations shown in the ^1H–^1H COSY spectrum. Based on the cross peak between H-2′ with H-4′ in the NOESY spectrum, the H-4′ signal was assigned. Then using the HMBC spectrum, the H-5′ (correlations of H-5′ with C-1′ and of H-4′ with C-5′) and –OCH_3 signals (correlated with C-3′) were determined. Finally, H-6′ was assigned by the strong $^1H - ^1H$ Cosy correlation with H-5′. Thus, the first sugar unit displayed a doublet proton (H-1′, δ 4.79, *d*, $J = 7.6$ Hz), two

Table 2
Oligosaccharide unit NMR spectral data of stauntoside A (**1**)

No.	^1H	^{13}C	HMBC(H →C)
1′	4.79 (*d*, 7.6)	102.0	3, 5′
2′	3.92 (*m*)	74.6	1′,
3′	3.68 (*dd*, 8.5, 8.5)	85.8	2′, 4′, 3′-OCH_3
4′	3.72 (*dd*, 8.5, 8.5)	82.9	3′, 5′, 6′, 1″
5′	3.65 (*m*)	71.6	1′
6′	1.45 (3H, *d*, 6.3)	18.7	4′, 5′
3′-OCH_3	3.94 (3H, *s*)	60.5	3′
1″	5.51 (*dd*, 1.7, 9.5)	98.9	4′, 2″
2″a	2.01 (*m*)	39.1	1″
2″e	2.42 (*m*)	4″	
3″	4.63 (*m*)	67.7	1″
4′	3.48 (*dd*, 2.7, 9.5)	83.2	5″, 6″, 1‴
5″	4.30 (*m*)	68.8	1″, 4″
6″	1.41(3H, *d*, 6.3)	18.6	4″, 5″
1‴	5.12 (*dd*, 1.7, 9.8)	99.6	4″, 2‴
2‴a	1.67 (*ddd*, 2.0, 9.8, 13.7)	34.8	1‴
2‴e	2.36 (*m*)	3‴, 4‴	
3‴	3.92(*m*)	77.4	1‴
4‴	3.38(*dd*, 2.7, 9.5)	82.2	5‴, 6‴, 1⁗
5‴	4.20(*dq*, 9.5, 6.3)	69.3	1‴, 4‴
6‴	1.29(3H, *d*, 6.3)	18.5	4‴, 5‴
3‴-OCH_3	3.51(3H, *s*)	57.3	3‴
1⁗	5.18(*br d*, 3.4)	101.7	4‴, 3⁗, 5⁗
2⁗a	2.06 (*m*)	30.9	1⁗, 3⁗, 4⁗
2⁗e	2.36 (*m*)		1⁗, 3⁗
3⁗	3.84 (*m*)	75.7	3⁗-OCH3
4⁗	4.06 (*m*)	67.5	2⁗, 3⁗
5⁗	4.29 (*m*)	67.7	1⁗, 4⁗, 6⁗
6⁗	1.55(3H, *d*, 6.9)	17.7	4⁗
3⁗-OCH_3	3.30(3H, *s*)	55.0	3⁗

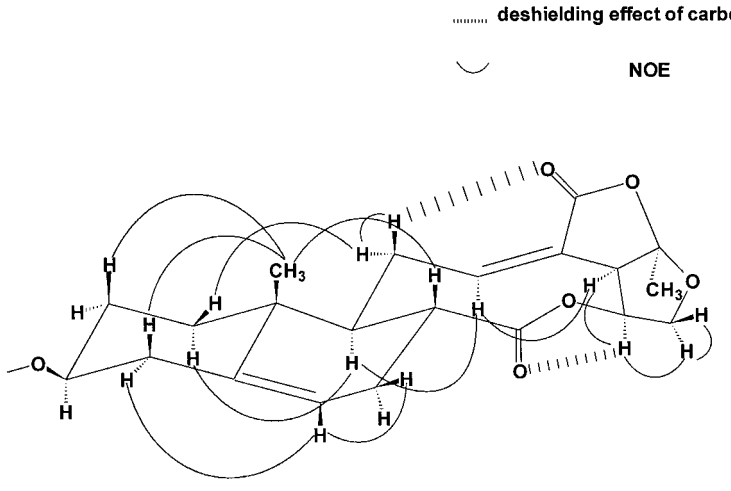

Fig. 2. Deshielding effect of carbonyl group and observed NOE correlations in aglycone.

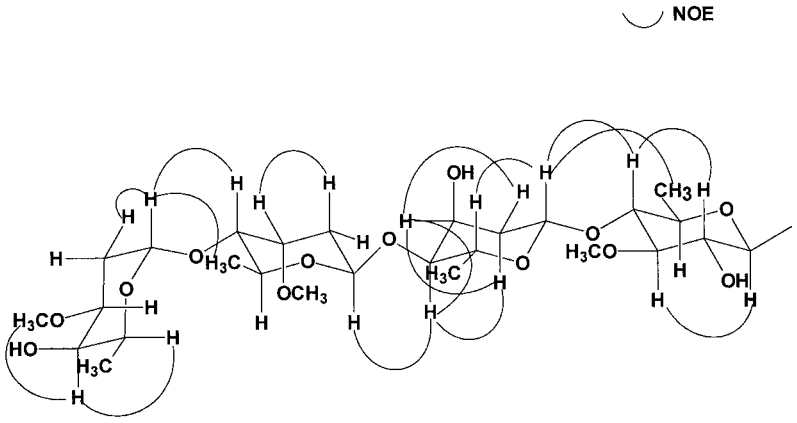

Fig. 3. Observed NOE correlations in the oligosaccharide unit of stauntoside A (**1**).

double doublet protons (H-3′, δ 3.68, *dd*, $J = 8.5$, 8.5 Hz and H-4′, δ 3.72, *dd*, $J = 8.5$, 8.5 Hz), two multiplet protons (H-2′, δ 3.92 and H-5′, δ 3.65), a terminal doublet methyl group (δ 1.45, *d*, $J = 6.3$ Hz) and a methoxyl group (δ 3.94, *s*). Their ^{13}C-NMR data were determined by HMQC as δ 102.0, 74.6, 85.8, 82.9, 71.6, 71.6, 18.7, respectively and 60.5 ($-OCH_3$). Therefore, this sugar was identified as β-D-thevetopyranose, based on D-thevetose being affected by glycosidation shifts of C-4 (+7.0), C-3 (−2.2) and c-5 (−1.0) (Nakagawa et al., 1983). In the same way, other sugars were identified as a β-D-digitoxopyranose (Nakagawa et al., 1983), β-L-cymaropyranose (Tsukamoto et al., 1985) and β-L-diginopyranose (Tsukamoto et al., 1985). Linkage of the sugar units was established from the following HMBC correlations (Table 2): H-4′ with C-1″ and H-1″ with C-4′; H-4″ with C-1‴ and H- 1‴ with C-4″; H-4‴ with C-1⁗ and H-1⁗ with C-4‴. NOESY correlations (Fig. 3) of the sugar sequence yielded the same conclusions as above.

The attachment of the tetrasaccharide moiety to C-3 of the aglycone was deduced from the HMBC spectrum, in which H-1′ (δ 4.79) showed long-range correlation with C-3 (δ 77.4), while H-3 (δ 3.80) showed a correlation with a signal at δ 102.0 assigned to C-1′. This was confirmed by strong NOESY cross peaks from, H-3 (δ 3.80) and H-4eq (δ 2.63) to H-1′ (δ 4.79). The β linkage of the oligosaccharide was deduced from the anomeric proton signal at δ 4.79 exhibiting a coupling constant of 7.6 Hz in the ^{1}H-NMR spectrum. Thus, the structure of stauntoside A was established as stauntogenin 3-*O*-α-L-diginopyranosyl-(1-4)-β-L-cymaropyranosyl-(1-4)-β-D- digitoxopyranosyl-(1-4)-β-D-thevetopyranoside.

Stauntoside B (**2**), an amorphous powder, has a molecular formula of $C_{40}H_{58}O_{15}$, as determined from its FABMS (*m*/*z*: 801 $[M+Na]^+$ and *m*/*z*: 817 $[M+K]^+$), negative APCIMS (*m*/*z*: 778 $[M]^-$) and

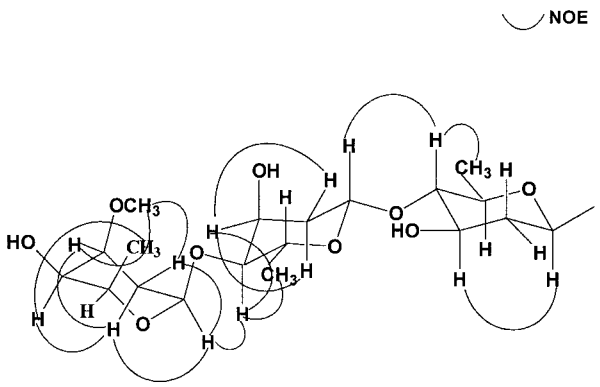

Fig. 4. Observed NOE correlations in the oligosaccharide unit of stauntoside B (**2**).

Table 3
Oligosaccharide unit NMR spectral data of stauntoside B (**2**)

No.	^{1}H	^{13}C	HMBC(H → C)
1′	4.84 (*dd*, 1.7, 9.5)	98.0	3, 2′
2′a	1.96 (*m*)	40.1	1′, 4′
2′e	2.51 (*m*)	1′, 3′, 4′	
3′	3.96 (*ddd*, 5.3, 8.5, 9.3)	70.0	4′
4′	3.30 (*dd*, 7.6, 9.3)	88.5	6′, 1″
5′	3.51 (*m*)	70.9	1′, 3′, 4′, 6′
6′	1.34 (3H, *d*, 6.1)	18.1	4′, 5′
1″	5.25 (*dd*, 1.7, 9.5)	99.8	4′
2″a	1.95 (*m*)	38.2	1″,
2″e	2.42 (*m*)	3″, 4″	
3″	4.48 (*m*)	67.4	1″
4″	3.42 (*dd*, 2.9, 9.5)	80.7	5″, 6″, 1‴
5″	4.18 (*dd*, 9.5, 6.1)	69.3	1″, 4″, 6″
6″	1.30 (3H, *d*, 6.1)	18.1	4″, 5″
1‴	5.02 (*m*)	98.5	4″, 3‴, 5‴
2‴a	1.82 (*ddd*, 4.1, 4.1, 14.0)	32.2	1‴,
2‴e	2.31 (*ddd*, 2.7, 4.9, 14.0)	1‴, 3‴, 4‴	
3‴	3.68 (*m*)	76.4	3‴-OCH_3
4‴	3.62 (*m*)	72.5	5‴, 6‴
5‴	4.45 (*m*)	67.4	1‴, 3‴, 4‴, 6‴
6‴	1.40 (3H, *d*, 6.4)	18.3	4‴, 5‴
3‴-OCH_3	3.35 (3H, *s*)	56.7	3‴

^{13}C-NMR spectrum. Stauntoside B also showed positive Liebermann–Burchard and Keller–Liliani reactions and its ^{1}H- and ^{13}C-NMR spectra indicated that **2** possesses the same aglycone as **1**, but differs in the sugar component (Tables 1 and 3). The presence of three sugars in **2** was indicated by the three anomeric protons: δ 4.84 (*dd*, $J = 1.7$, 9.5 Hz), δ 5.25 (*dd*, $J = 1.7$, 9.5 Hz), δ 5.02 (*m*) and carbons at δ 98.0, 99.8, and 98.5 respectively. Knowing this, it was apparent that the three sugars are present in one saccharide unit attached to C-3. In the same manner as in **1**, the sugar units of **2** were identified as β-D-3-demethyl-2-deoxy-thevetopyranose, β-D-digitoxopyranose and β-L-cymaropyranose. The sequence of this trisaccharide was also established using HMBC and NOESY spectra (observed long-range and NOE correlations are shown in Table 3 and Fig. 4, respectively). Thus, stauntoside B was identified as stauntogenin 3-*O*-α-L-cymaropyranosyl-(1-4)-β-D-digitoxopyranosyl-(1-4)- β-D-3-demethyl-2-deoxy-thevetopyranoside.

3. Experimental

3.1. General

FABMS: MAT-711 mass spectrometer; Negative APCIMS were obtained on a Micromass Platform II system (Micromass, MA) equipped with a Digital DECPC XL560 computer for data analysis; ^{1}H-NMR, ^{13}C-NMR, ^{1}H – ^{1}H COSY, HMQC, HMBC and NOESY spectra: Varian U-500 Spectrometer with TMS as internal standard and C_5D_5N as solvent; TLC: silica gel $HSGF_{254}$ (Yantai Institute of Chemical Technology, Yantai, China), spots were observed under a UV detector (254 nm) and visualized by 10% H_2SO_4 ethanol solution followed by heating to 110°C; Silica gel H (60μ, Qing Dao) was used for column chromatography; preparative HPLC was performed on an ODS column (10 × 250 mm).

3.2. Extraction and isolation

Fresh roots of *Cynanchum stauntoi* were collected in Anhui Province, P.R. China, in October 1996. A voucher specimen has been deposited at the Department of Drug Discovery, Shanghai Institute of Pharmaceutical Industry. Air-dried roots were extracted with 95% EtOH at room temperature. The extracts were concentrated (220 g) and suspended in water, then partitioned successively by petroleum (10 g), $CHCl_3$ (100 g) and EtOAc (15 g). The CHC_3 fraction (100 g) was subjected to CC on silica gel, using a CH_2Cl_2–MeOH gradient as eluent. The CH_2Cl_2–MeOH (10:1) fraction was repeatedly separated by CC (silica gel), eluted with cyclohexane–acetone (1:1) to give several fractions. Fraction 5 was purified over silica gel chromatography using CH_2Cl_2–MeOH–H_2O (9:1:0.1) affording stauntoside A (15 mg). Fraction 3 was purified by preparative HPLC (70% MeOH in H_2O), giving stauntoside B (13 mg).

3.3. Acidic hydrolysis of stauntosides A and B

To a solution of 3 mg of each glycoside in 1 ml MeOH, 2 ml of 0.1 N H_2SO_4 was added and kept at 65°C for 25 min, then the solution was diluted with water and concentrated. The solution was kept at 60°C for another 30 min, then neutralized with $Ba(OH)_2$. The filtrate was evaporated to dryness for TLC with three solvent systems: $CHCl_3$–MeOH (9:1), CH_2Cl_2–EtOH (9:1) and benzene–acetone (5:3). For comparison with authentic samples (provided by Shanghai Institute of Pharmaceutical Industry, China), stauntoside A afforded cymarose, digitoxose, thevetose and diginose. Stauntoside B afforded cymarose, digitoxose and demethyl-2-deoxythevetose.

3.4. Stauntoside A (*1*)

Amorphous powder, $[\alpha]_D$ – 63.4° ($c = 0.88$, MeOH); APCIMS: m/z 952 $[M]^-$; ^{1}H- and ^{13}C-NMR spectral data (see Tables 1 and 2).

3.5. Stauntoside B (*2*)

Amorphous powder, $[\alpha]_D$ −39.10 ($c = 0.585$, MeOH); APCIMS: m/z 778 $[M]^-$; FABMS: m/z 801 $[M+Na]^+$, 817 $[M+K]^+$; ^{1}H- and ^{13}C-NMR spectral data (see Tables 1 and 2).

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