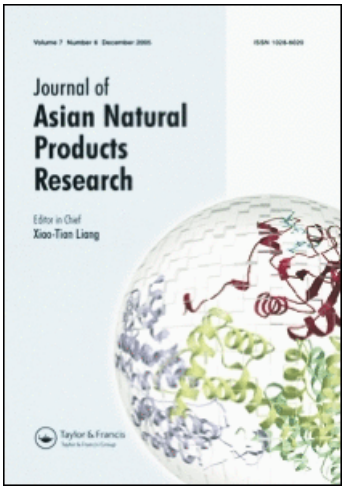

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A new sterol glycoside from *Securidaca inappendiculata*

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A new sterol glycoside from *Securidaca inappendiculata*

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From the roots of *Securidaca inappendiculata*, one new sterol glycoside securisteroside (**1**) has been isolated, along with two known sterols, spinasterol (**2**) and 3-*O*-β-D-glucopyranosyl-spinasterol (**3**). The new sterol was characterized by chemical and spectrometric methods, including EIMS, FABMS and one- and two-dimensional NMR experiments.

Keywords: *Securidaca inappendiculata*; Polygalaceae; Securisteroside; Sterol glycoside

1. Introduction

The genus *Securidaca* (Polygalaceae) consists of two species that are distributed in the tropical zone in East Asia [1]. The chemical constituents include flavonoids [2,3], alkaloids [4,5], saponins [6], organic acids [7], sucrose derivatives [8] and xanthones [9]. In our continuing search for active components for antidepressant and anti-Parkinson's disease activities, one new sterol, securisteroside (**1**) along with two known sterols, spinasterol (**2**) and 3-*O*-β-D-glucopyranosyl- spinasterol (**3**) (figure 1), have been isolated from the roots of Securidaca inappendiculata Hassk. We report here the isolation and structure determination of these sterols.

2. Results and discussion

Compound **1** was obtained as white flake crystals, mp 148–151°C, $[\alpha]_D^{20}$ −25.7 ($c = 0.07$, $CHCl_3$). Lieberman–Burchard and Molish reactions were both positive. A quasi-molecular ion peak at *m/z* 835.6 $[M + Na]^+$ from the FABMS, and a molecular ion peak at *m/z* 812.9 from the EIMS suggested, in association with NMR data, an empirical formula of $C_{51}H_{88}O_7$. Also shown were one main fragment ion peak at *m/z* 574.4 $[M - 239 + H]^+$, which is

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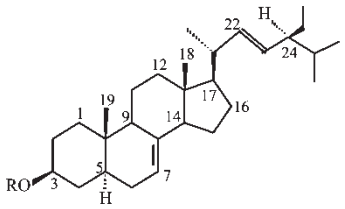

1: R=3-*O*-β-D-(6-hexadecanoyl)-glucopyranosyl
2: R=H
3: R=3-*O*-β-D-glucopyranosyl

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

attributed to loss of hexadecanoyl, and another at *m/z* 412.3 $[M - 239 - 162 + H]^+$ which is attributed to loss of hexadecanoyl and a hexose moiety.

The ^{1}H NMR spectrum displayed signals of seven methyls at δ 1.03 (3H, d, $J = 6.5$ Hz), 0.88 (3H, t, $J = 6.5$ Hz), 0.85 (3H, d, $J = 6.0$ Hz), 0.81 (3H, m, overlap), 0.80 (3H, m, overlap), 0.79 (3H, m, overlap) and 0.55 (3H, s); three double-bond protons at δ 5.15 (2H, m), 5.02 (1H, dd, $J = 8.0$, 15.0 Hz); one anomeric proton of a hexose at δ 4.40 (1H, d, $J = 7.5$ Hz), indicating that sugar moiety should be β-orientated, as well as other protons of the hexose at δ 4.51 (1H, dd, $J = 4.5$, 12.0 Hz), 4.27 (1H, d, $J = 12.0$ Hz), 3.58 (1H, t, $J = 9.0$ Hz), 3.46 (1H, br), 3.40 (1H, m), 3.36 (1H, m), 3.03 (1H, br, s, OH), 2.79 (1H, br, s, OH) and 2.44 (1H, br, s, OH); one proton of a carbon along with oxygen at δ 3.64 (1H, m), and some aliphatic protons at δ 2.0–1.1. The ^{13}C NMR spectrum displayed signals of carbonyl (δ 174.8), four carbons of double bonds (δ 139.6, 138.2, 129.5, 117.3), one anomeric carbon of hexose (δ 101.1), six carbons connected to oxygen (δ 78.9, 75.8, 74.0, 73.6, 70.0, 63.1), seven methyls (δ 21.4, 21.1, 19.0, 14.1, 13.0, 12.2, 12.0) and some aliphatic carbons (δ 55.9–22.7). The ^{1}H and ^{13}C NMR data of compound **1** were assigned by H–H COSY, HMQC and HMBC experiments.

From the data shown we concluded that compound **1** consists of sterol, hexosyl and hexadecanoyl moieties.

The ^{13}C NMR spectrum signals of hexose of **1** and methyl-β-D-glucopyranoside [10] are similar except for C-6′ and C-5′; the hexose moiety of **1** was deduced as glucose, which was confirmed by TLC after acid hydrolysis, but C-6′ of glucose appeared at downfield (2 ppm) and C-5′ of glucose appeared upfield (2 ppm), suggesting that the hexadecanoyl was connected with the C-6′. The spectral data of **1** were similar to those of spinasterol (**2**), except that C-3 of **1** is shifted downfield to 7.8 ppm, and C-4 of **1** is upfield to 3.6 ppm. These results suggest that the aglycone of **1** is spinasterol, and the β-glucose is connected to C-3. The HMBC spectrum shows correlations between the signal of the glucose anomeric proton at δ 4.40 and that of aglycone C-3 at δ 78.9, and the signals of glucose H-6′ at δ 4.40, 4.27 and that of hexadecanoyl C-1″ at δ 174.8. These also indicated that β-glucose is connected to aglycone at C-3, and that hexadecanoyl is connected to glucose at C-6′. The HMBC spectrum also shows correlations between the signal of Me-21 at δ 1.03 and those of C-17 at δ 55.9, C-20 at δ 40.8, the signal of Me-27 at δ 0.88 and those of C-24 at δ 51.2, C-25 at δ 31.9, C-26 at δ 19.0 (figure 2). The HMQC spectrum displays correlations between H-21 (δ 1.03) and C-21 (δ 21.4), H-27 (δ 0.88) and C-27 (δ 21.1). Thus, we revised the assignments of C-21 and C-27 of **2** and **3** of the reference data [11].

On the basis of these chemical and spectral evidences, **1** was identified as 3-*O*-β-D-(6-hexadecanoyl)-glucopyranosyl-spinasterol, named securisteroside.

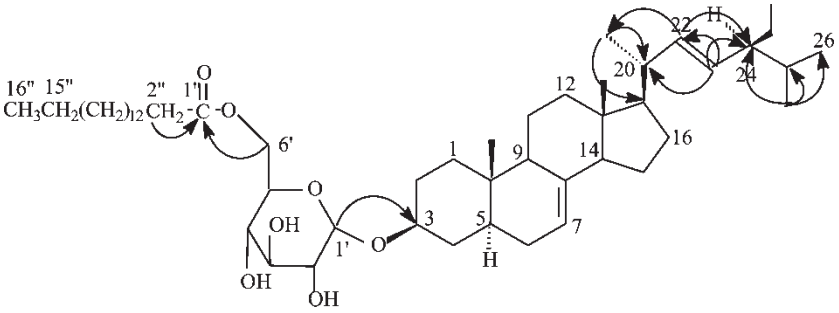

Figure 2. Key HMBC correlations for compound **1**.

3. Experimental

3.1 General experimental procedures

Melting points were determined using a Fisher Johns apparatus and are uncorrected. Polarimetric analysis was performed on a Perkin-Elmer 241 polarimeter. One- and two-dimensional NMR spectra were recorded on a Bruker AM 500 spectrometer. The FABMS (positive mode) spectrum was recorded on a Zabspec E mass spectrometer. EIMS data were obtained using Zabspec E mass spectrometer. For the column chromatography, silica gel (Qingdao Haiyang) and Sephadex LH 20 (Pharmacia) were used. TLC and HPTLC employed precoated silica gel plates (Qingdao Haiyang). MPLC were performed on a system equipped with a Büchi pump B-688, Büchi B-684 collector, UVOLOG-5IIIA UV-Detector, Büchi columns and precolumns, with the stationary phase silica gel 60 (15–40 μm, Qingdao Haiyang).

3.2 Plant material

The roots of *Securidaca inappendiculata* were collected in Yunnan province of China and identified by Professors Wen-Yan Lian (Institute of Medicinal Plant Development) and Hong Wang (Menglun Botanical Garden). A voucher specimen (YS-9801) has been deposited in the New Drug Research and Development Center, Institute of Medicinal Plant Development.

3.3 Extraction and isolation

The dried ground roots (340 g) of the plant material were extracted with 95% EtOH (4L × 4, 2 h each) under reflux. The EtOH extract (64 g) was chromatographed on flash column (silica gel H) and eluted with a gradient of C_6H_{12}–$CHCl_3$–MeOH–H_2O (50:50:0:0–0:0:50:50) to give 85 fractions. Fractions 1–5 (50:50:0:0–30:70:1:0) were purified by MPLC (silica gel H) eluted with $CHCl_3$–MeOH (1:0–0:1, gradient) to give 69 parts. Parts 10–14 were purified with a Sephadex LH 20 column to give **2** (20 mg). Fractions 21–35 (30:70:3:0–30:70:10:0) were purified by MPLC (silica gel H) eluted with $CHCl_3$–MeOH (9:1–0:1, gradient) to give 74 parts. Parts 30–44 were purified with a Sephadex LH 20 column and recrystallized by MeOH to give **1** (10 mg). Parts 68–74 were purified with a Sephadex LH 20 column and recrystallized by $CHCl_3$ to give **3** (30 mg).

Securisteroside (**1**) was obtained as white flake crystal from MeOH, mp 148–151°C, $[\alpha]_D^{20}$ −25.71 ($c = 0.07$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm): 5.15 (2H, m, H-7,22), 5.02 (1H, dd, $J = 8.0$, 15.0 Hz, H-23), 4.51 (1H, dd, $J = 4.5$, 12.0 Hz, H-6$_a'$), 4.40 (1H, d, $J = 7.5$ Hz, H-1′), 4.27 (1H, d, $J = 12.0$ Hz, H-6$_b'$), 3.64 (1H, m, H-3), 3.58 (1H, t, $J = 9.0$ Hz, H-3′), 3.46 (1H, br, H-5′), 3.40 (1H, m, H-4′), 3.36 (1H, m, H-2′), 3.03 (1H, br s, OH), 2.79 (1H, br s, OH), 2.44 (1H, br s, OH), 2.36 (2H, t, $J = 7.5$ Hz, H-2″), 1.03 (3H, d, $J = 6.5$ Hz, H-21), 0.88 (3H, t, $J = 6.5$ Hz, H-16″), 0.85 (3H, d, $J = 6.0$ Hz, H-27), 0.81 (3H, m, overlapped, H-29), 0.80 (3H, m, overlapped, H-26), 0.79 (3H, m, overlapped, H-19), 0.55 (3H, s, H-18); ^{13}C NMR data see table 1; FABMS *m/z* 835.6 $[M + Na]^+$; EIMS *m/z* 812.9 [M^+,0.64], 794.8 [0.98], 671.5 [1.0], 574.4 [$M^+ - 239 + H$,11], 412.3 [$M^+ - 239 - 162 + H$,10], 395.3 [85.5],255.2 [48],83.1 [100]. On the basis of these chemical and spectral evidences, compound **1** was identified as 3-*O*-β-D-(6-hexadecanoyl)-glucopyranosyl-spinasterol, named securisteroside.

Spinasterol (**2**) was obtained as colorless needles from $CHCl_3$, mp 171–173°C, $[\alpha]_D^{20}$ +11.43 ($c = 0.07$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm): 5.15 (2H, m, H-7,22), 5.02 (1H dd, $J = 8.0$, 15.0 Hz, H-23), 3.60 (1H m, H-3), 1.03 (3H, d, $J = 6.5$ Hz, H-21), 0.85 (3H, d, $J = 6.0$ Hz, H-27), 0.81 (3H, m, overlapped, H-29), 0.80 (3H, m, overlapped, H-26), 0.79 (3H, m, overlapped, H-19), 0.55 (3H, s, H-18); ^{13}C NMR data see table 1; EIMS *m/z* 412.4 [M^+, 37], 369.3 [21], 300.3 [16], 271 [100]. All of above spectral data were consistent with spinasterol [11].

3-*O*-β-D-Glucopyranosyl-spinasterol (**3**) was obtained as colorless needles from C_5H_5N, mp 279–281°C, $[\alpha]_D^{20}$ −12.59 ($c = 0.07$, C_5H_5N). Lieberman–Burchard and Molish reactions were both positive. ^{1}H NMR (C_5D_5N, 500 MHz): δ (ppm): 5.16 (1H, dd, $J = 9.0$, 15.0 Hz, H-22), 5.11 (1H, m, H-7), 5.02 (1H, dd, $J = 9.0$, 15.0 Hz, H-23), 4.21 (1H, d, $J = 8.0$ Hz, H-1′), 3.63 (1H, d, $J = 11.0$, H-6$_b'$), 3.54 (1H, m, H-3), 3.40 (1H, dd, $J = 5.0$, 11.0 Hz, H-6$_a'$), 3.05 (3H, overlapped), 2.88 (1H, t, $J = 9.0$ Hz) were H-2′, H-3′, H-4′ and H-5′, 0.99 (3H, d, $J = 6.5$ Hz, H-21), 0.82 (3H, d, $J = 6.5$ Hz, H-27), 0.77 (6H, m, overlapped, H-26,29), 0.73 (3H, s, H-19), 0.51 (3H, s, H-18); ^{13}C NMR data see

Table 1. ^{13}C NMR spectral data of compounds **1**–**3** (125 MHz).

C	***1*** *($CDCl_3$)*	***2*** *($CDCl_3$)*	***3*** *(C_5D_5N)*	*C*	***1*** *($CDCl_3$)*	***2*** *($CDCl_3$)*	***3*** *(C_5D_5N)*
1	37.1	37.1	37.3	21	21.4	21.4	21.7
2	31.9	31.5	30.0	22	138.2	138.2	138.7
3	78.9	71.1	77.1	23	129.5	129.4	129.7
4	34.4	38.0	34.6	24	51.2	51.2	51.5
5	40.2	40.2	40.2	25	31.9	31.9	32.2
6	29.7	29.7	30.0	26	19.0	19.0	19.2
7	117.3	117.5	117.9	27	21.1	21.1	21.3
8	139.6	139.6	139.6	28	25.4	25.4	25.7
9	49.3	49.4	49.6	29	12.2	12.2	12.6
10	34.3	34.2	34.8	1′	101.1		102.3
11	21.5	21.5	21.8	2′	73.6		75.4
12	39.4	39.4	39.6	3′	75.8		78.7
13	43.3	43.3	43.5	4′	70.0		71.8
14	55.1	55.1	55.3	5′	74.0		78.6
15	23.0	23.0	23.4	6′	63.1		62.9
16	28.5	28.5	29.0	1″	174.8		
17	55.9	55.9	56.1	2″	34.2		
18	12.0	12.0	12.3	15″	24.9		
19	13.0	13.0	13.1	16″	14.1		
20	40.8	40.8	41.2				

table 1; EIMS *m/z* 574.4 [M^+, 21], 412.3 [$M^+ - 162 + H$, 11], 395.4 [100], 255.2 [53], 83.1 [100]. All of the spectral data were completely consistent with 3-*O*-β-D-glucopyranosyl-spinasterol [11].

3.3.1 TLC Acid Hydrolysis of 1. Compound **1** was spotted on a TLC plate together with sugar references. The plate was exposed to HCL vapor, and then developed with the lower layer of chloroform–methanol–water–glacial acetic acid (30:12:4:0.5), and sprayed with 10% phosphatomolybdic acid in ethanol, and heated at 120°C. From **1**, only glucose was detected [12].

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