



Taccasubosides A–D, four new steroidal glycosides from *Tacca subflabellata*

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ABSTRACT

By analyzing the steroidal content of fresh whole plants of *Tacca subflabellata* (Taccaceae), we isolated one sapogenin and eight glycosides with four kinds of steroidal skeletons including four new glycosides, named taccasubosides A–D (**1–4**), together with five known compounds. Among them, compound **1** is the first pentacyclic sterol glycoside with 6–6–6–5–6 fused rings. The structures of **1–4** were elucidated on the basis of extensive spectroscopic analysis, including that of 2D NMR data, and the results of acidic hydrolysis. The cytotoxicity of the selected steroidal glycosides (**1–4**, **8**, and **9**) was evaluated *in vitro* against five human cancer cell lines. Compound **9** showed significant inhibitory activity against all five cell lines.

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1. Introduction

The genus *Tacca* (Taccaceae) comprising ca. 30 species distributes in tropical regions [1]. In China, there are five species and three of them have been long used as folk medicine for treatment of gastric ulcer, toothache and stomachache, incised wounds [2]. Anthocyanins [3], diarylheptanoids and diarylheptanoid glycosides [4], benzoquinone-type *retro*-dihydrochalcone [5], steroidal sapogenins and steroidal glycosides such as C-27 steroidal saponins, C-28 sterol glycosides and withanolide glycosides [6–15], have been isolated from *Tacca plantaginea*, *Tacca chantrieri*, *Tacca paxiana*, *Tacca subflabellata*, *Tacca leontopetaloides*, and *Tacca aspera*. Perhaps the most unique and well-studied class of compounds isolated from genus *Tacca* are the appropriately named taccalonolides [16–23], which are specially pentacyclic steroidal compounds with microtubule-stabilizing properties and antitumor activity [24,25].

In our continuing searching for new steroids from this genus, we investigated the chemical constituents of *T. subflabellata* collected in Hekou County of Yunnan Province, and isolated one new pentacyclic sterol glycoside, two new spirostanol glycosides, and a new pregnane glycoside, named taccasubosides A–D (**1–4**), together with five known steroidal compounds (**5–9**). To the best

of our knowledge, compound **1** is the first pentacyclic sterol glycoside with 6–6–6–5–6 fused rings. Compounds **1–4**, **8**, and **9** were subjected to cytotoxic activity testing against five human cancer cell lines (HL-60, SMMC-7721, A549, MCF-7, and SW480). This paper deals with the isolation and structural elucidation of these new steroidal glycosides, the cytotoxic activity of the glycosides tested.

2. Experimental

2.1. General methods

Optical rotations were measured in a JASCO P-1020 digital polarimeter. UV spectra were measured using a Shimadzu UV-2401 PC spectrophotometer. IR spectra were obtained on Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. FAB mass spectrum was obtained on a VG Auto spec-3000 spectrometer and ESI-MS, high-resolution ESI mass spectrum was recorded on an API Qstar Pulsar instrument. NMR experiments were performed on Bruker AM-400, DRX-500, and Bruker Avance III 600 instruments with TMS as the internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Column chromatography (CC) was performed over silica gel (200–300 mesh, 10–40 μ m, Qingdao Marine Chemical Co., China), Rp-18 (40–63 μ m, Merck), and Sephadex LH-20 (GE Healthcare, Sweden). TLC was performed on HSGF254 (0.2 mm, Qingdao Marine Chemical Co., China) or RP-18 F₂₅₄ (0.25 mm, Merck). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H₂SO₄ in EtOH. GC analysis was performed on a

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Table 1¹H NMR spectroscopic data of compounds **1–4** (δ in ppm, J in Hz, C₅D₅N).^a

Position	1 ^a	2 ^a	3 ^b	4 ^a
1	3.51 m	0.99 m, 1.71 m	0.92 m, 1.69 m	0.89 m, 1.65 m
2	3.85 dd (4.1, 4.6)	1.64 m, 1.97 m	1.83 m, 2.07 m	1.82 m, 2.05 m
3	3.54 br d (3.3)	3.64 m	3.90 m	3.88 m
4	2.91 m, 2.23 d (15.4)	2.31 t (12.3), 2.48 dd (3.0, 12.8)	2.66 t (11.8), 2.73 dd (3.8, 13.4)	2.69 d (9.3) 2.73 dd (4.7, 12.9)
5	1.68 m			
6		5.25 d (4.2)	5.28 d (4.6)	5.31 d (4.5)
7	4.52 br s	1.55 m, 1.88 m	1.54m, 1.86 m	1.55 m, 1.87 m
8	3.17 t (12.3)	1.89 m	1.54 m	1.28 m
9	1.70 m	1.64 m	0.88 m	0.93 m
11	4.95 m	1.55 m, 1.80 m	1.39 m (2H)	1.48 m (2H)
12	5.01 m	3.98 br s	1.12 m, 1.72 m	1.36 m, 2.60 d (11.9)
14	2.44 m	2.08 m	1.09 m	1.30 m
15	1.85 m, 1.76 m	1.57 m, 2.09 m	1.51 m, 2.09 m	1.89 m, 2.13 m
16	2.90 m	4.59 m	4.69 m	6.58 br s
17	1.29 t (12.0)	3.05 dd (7.6, 8.0)	1.93 t (7.7)	
18	1.16 s	0.93 s	1.02 s	0.91 s
19	1.59 s	0.95 s	0.97 s	1.02 s
20	1.47 m	1.98 t (6.9)	3.10 dq (7.0, 13.6)	
21	0.74 d (6.2)	1.17 d (6.9)	1.23 d (7.0)	2.23 s
22	2.47 m, 1.35 m			
23	5.04 d (5.2)	1.70 m (2H)	4.60 (dd, 4.4, 12.0)	
24		1.61 m (2H)	2.28 t (12.0), 2.47 m	
25	1.20 s	1.56 m		
26		3.49 t (10.2), 3.57 d (12.7)	3.71 d (11.0), 3.94 d (11.0)	
27		0.67 d (4.8)	1.27 s	
OAc-12	2.03 s			
OAc-23	1.87 s			
1'	4.97 d (7.8)	5.66 d (3.0)	4.90 d (7.8)	4.89 d (7.8)
2'	4.04m	4.65 d (3.0)	4.06 m	4.05 m
3'	4.27m		4.18 dd (9.0, 9.1)	4.17 dd (9.0, 9.0)
4'	4.25 m	4.26 d (9.4), 4.53 d (9.4)	4.07 m	4.05 m
5'	4.00 m	4.07 d (10.3), 4.43 d (10.3)	3.79 m	3.78 m
6'	4.58 dd (1.9, 11.6), 4.40 dd (5.3, 11.7)		4.34 m, 4.44 d (11.4)	4.34 d (10.9), 4.44 d (10.9)
1''		5.29 br s	5.87 br s	5.88 br s
2''		3.91 dd (2.7, 9.8)	4.85 br s	4.86 m
3''		4.61 t (2.8)	4.52 dd (2.6, 9.1)	4.53 dd (3.3, 9.6)
4''		3.62 m	4.32 dd (8.5, 9.5)	4.31 m
5''		4.24 m	4.87 m	4.87 m
6''		1.54 d (6.2)	1.74 d (6.2)	1.74 d (6.1)
1'''			5.77 br s	5.77 br s
2'''			4.75 br s	4.78 m
3'''			4.49 dd (2.8, 9.3)	4.49 dd (3.3, 9.3)
4'''			4.32 dd (8.5, 9.5)	4.31 m
5'''			4.80 dq (6.1, 9.4)	4.81 dq (6.4, 9.4)
6'''			1.65 d (6.1)	1.64 d (6.1)

^a Recorded at 400 MHz.^b Recorded at 500 MHz.

Shimadzu GC-2010 gas chromatograph equipped with an H₂ flame ionization detector.

2.2. Plant material

The fresh whole plants of *T. subflabellata* were collected in August 2009 from Hekou County, Yunnan Province, People's Republic of China, and identified by Professor Qin Li, Honghe Institute of Tropical Agricultural Science. A voucher specimen (No. HY0005) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China.

2.3. Extraction and isolation

The fresh and chopped plant material (3.2 kg) was percolated four times (4 days per time) with 80% EtOH (4 × 10 L) at room temperature to give the crude extract (116 g). This was partitioned between EtOAc and water to afford EtOAc-soluble

portion (72 g). The fraction of water was passed through D-101 macroporous resin and eluted successively with H₂O and 70% EtOH. Vaporated 70% EtOH eluate gave H₂O-soluble portion (10 g). The EtOAc-soluble portion (72 g) was passed through a MCI gel column chromatography eluted with aqueous EtOH in gradient (50% → 80% → 95%, v/v) to obtain four fractions 1–4 (as monitored by TLC). Fraction 2 was purified by using a reversed-phase C-18 silica gel column (aqueous MeOH, 70%) to give **1** (15 mg), chantriolide A (18 mg), and chantriolide B (14 mg). Fraction 3 was chromatographed on Sephadex LH-20 (CHCl₃–MeOH 1:1) and finally purified by a silica gel column (CHCl₃–MeOH 20:1) to obtain plantagiolide A (16 mg). The H₂O-soluble portion was chromatographed over a silica gel column (CHCl₃–MeOH–H₂O 10:1:0 → 8:2:0.2, v/v) to give two major components subfractions. The two subfractions were repeatedly chromatographed on Rp-18 columns (MeOH–H₂O 5:5 → 8:2 → 7:3, v/v) to yield **2** (12 mg), **3** (21 mg), **4** (20 mg), **8** (25 mg), and **9** (100 mg).

Table 2¹³C NMR spectroscopic data of compounds **1–4** (δ in ppm, C₅D₅N).

Position	1 ^a	2 ^a	3 ^b	4 ^a
1	86.5 (d)	37.4 (t)	37.5 (t)	37.3 (t)
2	55.0 (d)	30.3 (t)	30.1 (t)	30.0 (t)
3	51.2 (d)	77.6 (d)	77.9 (d)	77.7 (d)
4	30.0 (t)	39.3 (t)	38.7 (t)	38.7 (t)
5	44.1 (d)	141.0 (s)	140.8 (s)	141.2 (s)
6	209.7 (s)	122.0 (d)	121.8 (d)	121.6 (d)
7	79.2 (d)	31.9 (t)	32.4 (t)	31.8 (t)
8	43.3 (d)	32.0 (d)	31.6 (d)	30.3 (d)
9	57.6 (d)	44.4 (d)	50.3 (d)	50.7 (d)
10	42.9 (s)	36.9 (s)	37.2 (s)	37.1 (s)
11	71.3 (d)	29.4 (t)	21.1 (t)	20.9 (t)
12	75.5 (d)	71.4 (d)	40.2 (t)	35.1 (t)
13	44.6 (s)	45.1 (s)	41.1 (s)	46.3 (s)
14	47.6 (d)	48.3 (d)	56.7 (d)	56.4 (d)
15	26.1 (t)	32.4 (t)	32.3 (t)	32.3 (t)
16	37.8 (d)	81.1 (d)	81.9 (d)	144.8 (d)
17	54.1 (d)	53.9 (d)	62.6 (d)	155.2 (s)
18	14.8 (q)	17.5 (q)	16.6 (q)	15.9 (q)
19	16.7 (q)	19.4 (q)	19.4 (q)	19.3 (q)
20	31.4 (d)	42.3 (d)	35.8 (d)	196.3 (s)
21	20.0 (q)	15.1 (q)	14.9 (q)	27.1 (q)
22	45.0 (t)	109.4 (s)	112.2 (s)	
23	73.0 (d)	32.4 (t)	64.6 (d)	
24	81.3 (s)	29.4 (t)	43.6 (t)	
25	15.7 (q)	30.7 (d)	70.0 (s)	
26		66.9 (t)	69.3 (t)	
27		17.3 (q)	26.9 (q)	
OAc-12	170.3 (s), 20.4 (q)			
OAc-23	170.0 (s), 21.0 (q)			
1'	106.6 (d)	108.2 (d)	99.9 (d)	99.8 (d)
2'	75.8 (d)	77.6 (d)	78.4 (d)	78.4 (d)
3'	78.7 (d)	79.0 (s)	87.5 (d)	87.3 (d)
4'	71.9 (d)	74.8 (t)	69.9 (d)	69.8 (d)
5'	78.4 (d)	72.8 (t)	78.1 (d)	78.2 (d)
6'	63.0 (t)		62.3 (t)	62.2 (t)
1''		102.8 (d)	102.7 (d)	102.7 (d)
2''		72.5 (d)	72.5 (d)	72.5 (d)
3''		73.0 (d)	72.9 (d)	72.9 (d)
4''		74.4 (d)	73.9 (d)	73.8 (d)
5''		70.6 (d)	69.9 (d)	69.9 (d)
6''		18.7 (q)	18.7 (q)	18.7 (q)
1'''			103.9 (d)	103.9 (d)
2'''			72.5 (d)	72.6 (d)
3'''			72.7 (d)	72.7 (d)
4'''			73.6 (d)	73.6 (d)
5'''			70.7 (d)	70.6 (d)
6'''			18.5 (q)	18.5 (q)

^a Recorded at 100 MHz.^b Recorded at 125 MHz.**2.3.1. Taccasuboside A (1)**

White amorphous powder; $[\alpha]_D^{17} +1.2$ (c 0.11, MeOH–CHCl₃ 1:1); ESI: m/z 711 [M–H][–]; HRESI-MS: m/z 747.2973 [M+Cl][–] (calcd. for C₃₅H₅₂O₁₅Cl 747.2994); IR(KBr) $\nu_{\max}$ (cm^{–1}): 3432, 2972, 2952, 2907, 1730, 1639, 1378, 1253, 1072, 1030, 855, 612; ¹H NMR data see Table 1; ¹³C NMR data see Table 2.

2.3.2. Taccasuboside B (2)

White amorphous powder; $[\alpha]_D^{17} -18.8$ (c 0.2, MeOH); positive FAB-MS: m/z 709 [M+H]⁺; HRESI-MS: m/z 743.3767 [M+Cl][–] (calcd. for C₃₈H₆₀O₁₂Cl 743.3773); IR(KBr) $\nu_{\max}$ (cm^{–1}): 3442, 2926, 2854, 2235, 1632, 1458, 1379, 1243, 1079, 1044, 982, 921, 899 (intensity: 899 > 921), 609; ¹H NMR data see Table 1; ¹³C NMR data see Table 2.

2.3.3. Taccasuboside C (3)

White amorphous powder; $[\alpha]_D^{17} -89.9$ (c 0.15, MeOH); ESI: m/z 899 [M–H][–]; HRESI-MS: m/z 935.4385 [M+Cl][–] (calcd. for C₄₅H₇₂O₁₈Cl 935.4407); IR(KBr) $\nu_{\max}$ (cm^{–1}): 3418, 2936, 2848, 2364, 2116, 1631, 1453, 1381, 1138, 1045, 954, 920, 838, 811; ¹H NMR data see Table 1; ¹³C NMR data see Table 2.

2.3.4. Taccasuboside D (4)

White amorphous powder; $[\alpha]_D^{17} -71.5$ (c 0.11, MeOH); negative FAB: m/z 767 [M–H][–]; HRESI-MS: m/z 767.3839 [M–H][–] 767.3839 (calcd. for C₃₉H₅₉O₁₅ 767.3853); IR(KBr) $\nu_{\max}$ (cm^{–1}): 3423, 2934, 2364, 2044, 1656, 1587, 1454, 1434, 1371, 1238, 1125, 1046, 947, 912, 840, 811, 692; UV(MeOH) $\lambda_{\max}$ (log ϵ) 239 (2.19) nm; ¹H NMR data see Table 1; ¹³C NMR data see Table 2.

2.3.5. Acid hydrolysis of compounds 1–4 and GC analysis

Compounds **1**, **3**, and **4** (2 mg each) were refluxed with 4 M TFA-dioxane (1:1 (v/v), 2 mL) on water bath for 4 h. The reaction mixture was neutralized with 1 M NaOH and filtered. The filtrate was extracted with CHCl₃ and H₂O. The H₂O-soluble fraction was evaporated to dryness. The dried sugar residues was diluted in 1 mL pyridine without water and treated with 0.5 mL trimethyl-chlorosilan (TMCS) and stirred at 60 °C for 5 min. After drying the solution with a stream of N₂, the residue was extracted with ether (1 mL). The ether layer was subjected to GC analysis under the following conditions: H₂ flame ionization detector. Column: SGE AC-10 quartz capillary column (30 m × 0.32 mm × 0.25 μ m). Column temperature: 180–280 °C with the rate of 3 °C/min, and the carrier gas was N₂ (2 mL/min); injector temperature: 250 °C; injection volume: 2 μ L; split ratio: 1/50. The retention times of D-glucose and L-rhamnose were 14.21 min, and 7.66 min, respectively. By the same procedures carried out for compounds **2** (2 mg). The derivatives of D-apiose and L-rhamnose were detected; t_R (min): D-apiose (9.06), and L-rhamnose (7.66). Under the same hydrolysis conditions, compounds **1–4** (8 mg each) were separately subjected to acid hydrolysis to give sugar fractions. After preparative TLC of sugar fractions eluted with CHCl₃–MeOH–H₂O (7:3:0.5), the optical rotation of each purified sugar was measured. The optical rotations were determined after dissolving the sugars in H₂O: $[\alpha]_D^{13} +38.7$ (c 0.09, H₂O), D-apiose: $[\alpha]_D^{13} +7.6$ (c 0.06, H₂O), and L-rhamnose: $[\alpha]_D^{13} -5.1$ (c 0.08, H₂O).

2.4. MTT cytotoxicity assays

The bioassay was carried out as described elsewhere [26], with five human cancer cell lines (HL-60, SMMC-7721, A549, MCF-7, and SW480). The experiments were repeated twice. Cisplatin was used as the positive control antitumor drug.

3. Results and discussion

The EtOH extract of *T. subflabellata* was partitioned between EtOAc and water to afford EtOAc-soluble portion and then the fraction of water was passed through D-101 macroporous resin to get H₂O-soluble portion. The two portions were subjected to subsequent silica gel, Sephadex LH-20, and Rp-18 silica gel column chromatography to yield four new steroidal glycosides, taccasubosides A–D (**1–4**) and five known compounds, which were identified by comparison of their NMR and MS data with reported values in the literatures as plantagiolide A (**5**) [15], chantriolide A (**6**) [10], chantriolide B (**7**) [10], diosgenin 3-O- α -L-rhamnopyranosyl (1 $\rightarrow$ 2)-[α -L-rhamnopyranosyl (1 $\rightarrow$ 3)]- β -D-glucopyranoside (**8**) [6], and 26-O- β -D-glucopyranosyl-(25S)-3 β ,22 ξ ,26-triol-furost-5-ene 3-O- α -L-rhamnopyranosyl (1 $\rightarrow$ 2)-[α -L-rhamnopyranosyl (1 $\rightarrow$ 3)]- β -D-glucopyranoside (**9**) [14], respectively.

Taccasuboside A (**1**) was isolated as white amorphous powder. Its molecular formula C₃₅H₅₂O₁₅ was determined by HRESI-MS at m/z 747.2973 [M+Cl][–] (calcd. for 747.2994), corresponding to 10 degrees of unsaturation. The absorptions in the IR spectrum at 3432, 1730, and 1639 cm^{–1} suggested the presence of hydroxyl, acyl, and ketone groups, respectively. The ¹H NMR spectrum (Table 1) of **1** exhibited the signals due to an anomeric proton at δ_H 4.97 (d,

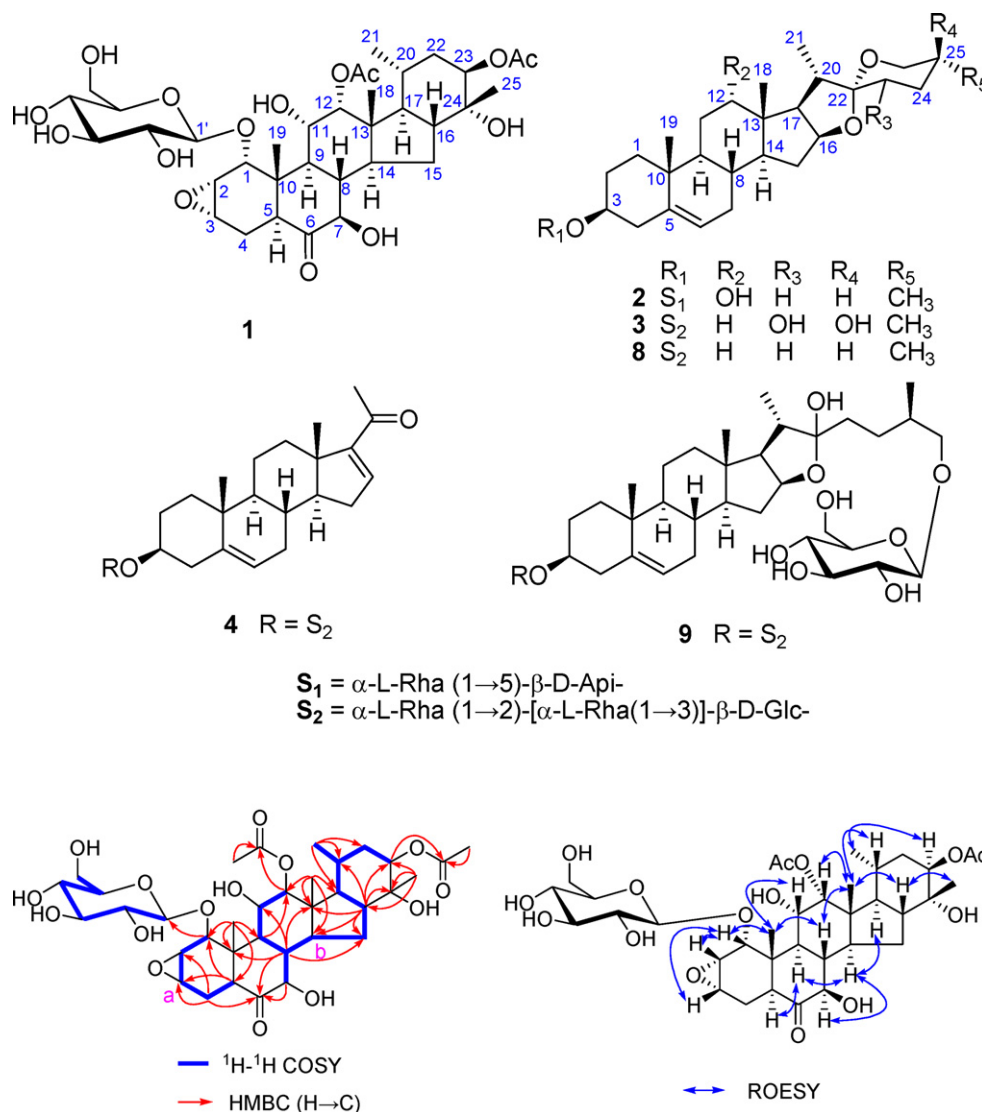


Fig. 1. Selected 2D NMR correlations of **1**.

$J=7.8$ Hz), three tertiary methyls at δ_{H} 1.16, 1.20, and 1.59, one secondary methyls at δ_{H} 0.74 (d, $J=6.2$ Hz), and two methyls of acetyl groups at δ_{H} 1.87 and 2.03. The ^{13}C NMR and DEPT spectra of **1** (Table 2) displayed signals including one ketone group (δ_{C} 209.7), two acetyl groups (δ_{C} 20.4, 21.0, 170.0, and 170.3), four methyls, three methylenes, fourteen methines (seven oxygenated), and three quaternary carbons (one oxygenated), as well as a hexose signals at δ_{C} 106.6 (d), 75.8 (d), 78.7 (d), 71.9 (d), 78.4 (d), and 63.0 (t) assigned by HMQC, ^1H , ^1H -COSY, and HMBC correlations. The monosaccharide of the acidic hydrolysate of **1** was identified as D-glucose on the basis of GC analysis of the silylated sugar residue and the optical rotation value of sugar residue ($[\alpha]_{\text{D}}^{13} +38.7$, H_2O). A comparison of the ^1H and ^{13}C spectroscopic signals of the aglycone moiety of **1** with taccalonolides [16–23] isolated from *Tacca* genus indicated that the aglycone moiety of **1** was a pentacyclic steroid except for the disappearance of F-ring. The presence of a 2,3-epoxide was supported by the COSY correlations between H-2 (δ_{H} 3.85, dd, $J=4.1$, 4.6) and H-3 (δ_{H} 3.54, br d, $J=3.3$), and HMBC correlations of H₂-4 (δ_{H} 2.23 and 2.91) with C-2 (δ_{C} 55.0, d) and C-3 (δ_{C} 51.2, d). The ^1H , ^1H -COSY spectra of the aglycone of **1** revealed connectivity of two partial structures **a** (C-1 to C-5) and **b** (C-7 to 9, C-9 to C-11, C-11 to C-12, C-8 to C-14, C-14 to 17, C-17 to C-20, C-20 to C-21 and C-22), as drawn with bold bonds in Fig. 1.

The connectivities of fragments **a** and **b** through C-6 was suggested by the critical HMBC correlations between the C-6 (δ_{C} 209.7) with H₂-4 (δ_{H} 2.23 and 2.91), H-7 (δ_{H} 4.52), and H-8 (δ_{H} 3.17), and was further confirmed by correlations H-5 (δ_{H} 1.68) with C-7 (δ_{C} 79.2). Similarly, the connectivities of the two tertiary methyls were set up by the aid of the HMBC correlations of Me-18 (δ_{H} 1.16) with C-12 (δ_{C} 75.5), C-13 (δ_{C} 44.6), C-14 (δ_{C} 47.6), and C-17 (δ_{C} 54.1), and Me-19 (δ_{H} 1.59) with C-1 (δ_{C} 86.5), C-5 (δ_{C} 44.1), C-9 (δ_{C} 57.6) and C-10 (δ_{C} 42.9). Furthermore, the HMBC correlations between Me-25 (δ_{H} 1.20) with C-16 (δ_{C} 37.8), C-23 (δ_{C} 73.0), and C-24 (δ_{C} 81.3) revealed the oxygenated quaternary carbon at E-ring located between C-16 and C-23. In addition, HMBC correlations of H-12 (δ_{H} 5.01) with acetyloxy group (δ_{C} 170.3) and H-23 (δ_{H} 5.04) with acetyloxy group (δ_{C} 170.0) suggested the two acetyloxy groups were linked to C-12 and C-23, respectively. The glucopyranosyl attachment to C-1 could be determined by the HMBC correlations from H-1 (δ_{H} 3.51) to C-1 (δ_{C} 106.6). Therefore, the planar structure of **1** was assigned as shown in Fig. 1.

The relative configuration of **1** was established by analysis of key correlations observed in the ROESY spectrum. NOE correlations of Me-18/H-8/Me-19, Me-18/H-16/H-20, H-5/H-9, and of H-17/H-14/H-9 in the ROESY spectrum indicated the *trans* ring fusion for the five rings as well as α -configuration for Me-21. Further NOEs

from Me-19 to H-1 and H-11, from H-1 to H-2 and H-3, and from Me-18 to H-12, were consistent with the 1α , 2α , 3α , 11α , and 12α configurations, while correlations of H-7/H-14, H-16/Me-25, and Me-21/H-23 indicated that the α -orientation for OH-24, and the β -orientation for OH-7 and AcO-23. Consequently, the structure of compound **1** was assigned as shown.

Taccasuboside B (**2**) was isolated as a white amorphous powder. The molecular formula of **2** was deduced from negative-ion HRESI-MS at m/z 743.3767 $[M+Cl]^-$ (calcd. for 743.3773), compatible with the molecular formula $C_{38}H_{60}O_{12}$, which was confirmed by data from the ^{13}C NMR spectrum. The IR spectrum, showing absorptions at 3442, 2962, and 1632 cm^{-1} , implied the existence of OH groups, C=C bonds, and CH groups, respectively. The 1H NMR spectrum showed signals for four steroid methyl groups at δ_H 0.67 (d, $J=4.8\text{ Hz}$), 0.93 (s), 0.95 (s), 1.17 (d, $J=6.9\text{ Hz}$), an olefinic proton at δ_H 5.25 (d, $J=4.2\text{ Hz}$). The above 1H NMR data, together with olefinic carbons signals at δ_C 141.0 (s, C-5) and 122.0 (d, C-6) and an acetalic carbon signal at δ_C 109.4 (s, C-22) in the ^{13}C NMR spectrum, indicated **2** to be a $\Delta^{5,6}$ – spirostanol skeleton in the aglycone. The *R*-configuration of C-25 was deduced from the intensity of the absorptions ($899 > 921\text{ cm}^{-1}$) in its IR spectrum [27]. Furthermore, the NMR spectroscopic data attributed to the aglycone of **2** were in good agreement with those of heloniogenin [28]. Anomeric region in the 1H - and ^{13}C NMR spectra of showed signals for two anomeric protons at δ_H 5.29 (br s) and 5.66 (d, $J=3.0\text{ Hz}$) with their corresponding anomeric carbons at δ_C 102.8 and 108.2, respectively (see Tables 1 and 2). Sugars obtained on acid hydrolysis of **2** were identified as D-apiose and L-rhamnose on the basis of GC analysis and the optical rotation values of sugar residues. The β -furanoid anomeric form of the apiofuranosyl was indicated by the chemical shifts of C-1' (δ_C 108.2), C-2' (δ_C 77.6), C-3' (δ_C 79.0), and C-4' (δ_C 74.8) with those of the corresponding carbons of methyl α - and β -apiofuranoside [29], while the anomeric configuration of the rhamnopyranosyl was defined as α -oriented on the basis of the chemical shift values of C-3'' (δ_C 73.0), and C-5'' (δ_C 70.6) with those of the corresponding carbons of methyl α - and β -rhamnopyranoside [30]. Long-range correlations from δ_H 5.66 (H-1' of apiose) to δ_C 77.6 (C-3 of aglycone) and from δ_H 5.29 (H-1'' of rhamnose) to δ_C 72.8 (C-5' of apiose) in the HMBC spectrum showed that the sugar chain was attached to C-3 of the aglycone and that the rhamnose was linked at the C-5' of the inner apiose. Therefore, the structure of **2** was established as heloniogenin O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 5)- β -D-apiofuranoside.

Taccasuboside D (**3**) was shown to have the molecular formula $C_{45}H_{72}O_{18}$ on the basis of the negative-ion HRESI-MS (m/z 935.4385 $[M+Cl]^-$; calcd. for 935.4407). The 1H and ^{13}C NMR spectra of **3** displayed signals of a glucopyranosyl and two rhamnopyranosyls, whose anomeric protons were observed at δ_H 4.90 (d, $J=7.8\text{ Hz}$), 5.77 (br s), and 5.87 (br s), respectively, together with an aglycone moiety which was closely related to (25S)-isonuatigenin [31]. The ^{13}C NMR spectra of **3** showed the downfield shifts at C-22 (δ_C 112.2, s), C-23 (δ_C 64.6, d), C-24 (δ_C 43.6, t), C-25 (δ_C 70.0, s) related to (25S)-isonuatigenin and indicated that there was a hydroxyl group substituted on the C-23 of **3**. This could be further confirmed by the long-range correlations of C-23 with H-20 and H₂-24, and of Me-27 with C-24, C-25, and C-26 in the HMBC spectrum of **3**. The spin-coupling constants of H-23, $^3J_{H-23, H-24ax} = 12.0\text{ Hz}$, and $^3J_{H-23, H-24eq} = 4.4\text{ Hz}$, gave evidence for the 23S configuration. The 25S configuration was deduced from the ROESY correlation from H-24ax (δ_H 2.28) to Me-27 (δ_H 1.27). The β -configuration of the anomeric center of the glucopyranosyl was supported by the relatively large *J* value of the anomeric proton ($J=7.8\text{ Hz}$). Furthermore, the HMBC spectrum showed the correlations of H-1'' (δ_H 5.87) of rhamnopyranosyl with C-2' (δ_C 78.4) of glucopyranosyl, H-1''' (δ_H 5.77) of rhamnopyranosyl with C-3' (δ_C 87.5) of glucopyranosyl, and H-1' (δ_H 4.90, d, $J=7.8\text{ Hz}$)

of glucopyranosyl with C-3 (d 77.9) of the aglycone, which supported compound **3** had the same saccharide chain linked at C-3 as in the compound **8**. Thus, the structure of **3** was formulated as (23S, 25S)-spirost-5-en-3 β ,23,25-triol 3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 3)]- β -D-glucopyranoside.

Taccasuboside D (**4**) had a molecular formula of $C_{39}H_{60}O_{15}$ on the ground of the negative-ion HRESI-MS at m/z 767.3839 $[M-H]^-$ (calcd. for $C_{39}H_{59}O_{15}$ 767.3853). The 1H NMR spectrum of **4** exhibited two tertiary methyls at δ_H 0.91 and 1.02, and a methyl singlet at δ_H 2.23 attached to a deshielding moiety, as well as three anomeric proton signals at δ_H 4.89 (d, $J=7.8\text{ Hz}$), 5.77 (br s), and 5.88 (br s). The existence of an α , β -unsaturated carbonyl group was verified by the IR (1656 cm^{-1}), UV [239 nm ($\log \epsilon$ 2.19)], and ^{13}C NMR signals at δ_C 144.8 (C-16, d), 155.2 (C-17, s), and 196.3 (C-20, s). All the spectral data mentioned above and comparison with those of previously reported compounds allowed identification of the aglycone of **5** as 3 β -hydroxypregna-5,16-dien-20-one [8]. Analysis of the NMR data (Tables 1 and 2) for the sugar portion of **4** and comparison with those of **3** revealed that these two compounds had the same saccharide chain linked at C-3. Consequently, the structure of **4** was elucidated as pregna-5,16-dien-3 β -ol-20-one 3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 3)]- β -D-glucopyranoside.

The selected compounds **1–4**, **8**, and **9** were evaluated for their cytotoxic activities against five human cancer cell lines (HL-60, SMMC-7721, A549, MCF-7, and SW480), in which cisplatin (DDP) was used as the reference substance and exhibited IC₅₀ values for the cell lines of 1.50, 14.75, 14.75, 15.68, and 25.57 μM , respectively. The new compounds **1–4** were inactive (IC₅₀ > 40 μM). The known compound **8** has been reported having cytotoxicity against HeLa cells and showing a significant microtubule-stabilizing activity *in vitro* [32]. In our assay, compound **8** exhibited moderate activity against the above cell lines with IC₅₀ values of 18.18, 25.08, 17.32, 18.14, and 15.73 μM , while compound **9** showed significant cytotoxicity on the five tumor cell lines with IC₅₀ values of 4.63, 4.34, 3.00, 11.13, and 2.68 μM , respectively. The results suggested that compound **9** had stronger cytotoxicity on the five tumor cell lines than that of the positive control DDP.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.steroids.2011.04.003.

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