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Ypsilandrosides C-G, five new spirostanol saponins from *Ypsilandra thibetica*

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ABSTRACT

A further phytochemical investigation on the whole plants of *Ypsilandra thibetica* yielded one sapogenin and 12 spirostanol saponins. Five of these are new compounds, designated as ypsilandrosides C-G (**2**–**6**). Their structures were determined by detailed spectroscopic analysis, including extensive 1D and 2D NMR data, and by the result of a hydrolytic reaction. Compounds **2**–**5** were rare steroidal saponins that an apiofuranosyl unit was directly linked at C-3 of the aglycone. Selected spirostanol saponins (**2**–**6**, **9**) were tested for their cytotoxic activities against K562, SPC-A-1, BGC-823, Eca-109, and AGS cell lines. Compounds **6** and **9** showed moderate inhibitory activity against all five cell lines. The antifungal properties of the new spirostanol saponins (**2**–**6**) against *Candida albicans* were also determined.

1. Introduction

Ypsilandra thibetica Franch. (Liliaceae) is a perennial plant that grows in southwestern China [1], and which has been used in Chinese folk medicine as hemostatic in Sichuan and Yunnan [2]. The anti-tumor activity of furostanol saponins from *Y. thibetica* has been reported [3]. The use of *Ypsilandra* herbs as medicines for treating hemorrhagic diseases have also been reported [4]. Previously, we have reported the structural determination of a new spirostanol sapogenin and of four new spirostanol saponins isolated from the whole plant of *Y. thibetica* [5]. Further phytochemical investigation including the EtOH extraction of *Y. thibetica* yielded five new spirostanol saponins, ypsilandrosides C-G (**2**–**6**), together with a known sapogenin (**1**) and seven known spirostanol saponins (**7**–**13**). The new compounds **2**–**5** were rare steroidal saponins with an apiofuranosyl unit attached to the C-3 of the aglycone. To the best of our knowledge, only one of the steroidal saponin containing an internal apiofuranosyl unit at the C-3 of the aglycone has been isolated from *Heloniopsis orientalis* (Liliaceae) a part for *Y. thibetica* [5,6]. Compounds **2**–**6**, and **9** were subjected to cytotoxic activity testing against five human cancer cell lines (K562, SPC-A-1, BGC-823, Eca-109, and AGS). In addition, all new compounds (**2**–**6**) were tested for their antifungal activities against *Candida albicans*. The present paper describes the isolation and structural elucidation of these new spirostanol saponins, as well as their cytotoxic and antifungal activities.

2. Experimental

2.1. General methods

Melting points were mensurated on a XRC-1 micro-melting point apparatus and were uncorrected. Optical rotations were measured on a SEPA-3000 automatic digital polarimeter. NMR spectra were run on Bruker AM-400 and DRX-500 instruments with TMS as an internal standard. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. FAB-MS spectra were recorded on a VG Auto Spec-300 spectrometer, HRESIMS spectra were recorded on an API Qstar Pulsar instrument. Column chromatography (CC) was performed over silica gel (200–300 mesh, 10–40 μm, Qingdao Marine Chemistry Co. Ltd., PR China), RP-18 (40–63 μm, Merck), and Sephadex LH-20 (20–100 μm, Pharmacia). Thin layer chromatography (TLC) was carried out on plates pre-coated with Merck RP-18 and silica gel GF_{254} (Qingdao Marine Chemistry Co. Ltd., PR China). Semi-preparative HPLC was run on Agilent 1100 liquid chromatograph with diode array detector (DAD) setting at 200 nm and ZORBAX SB-C18 (5 μm) column (25 cm × 9.4 mm i.d.). GC analysis was performed on a Shimadzu GC-2010 gas chromatograph equipped with an H_2 flame ionization detector. The column was SGE AC-10 quartz capillary column (30 m × 0.32 mm × 0.25 μm) with the following conditions: column temperature 180–280 °C; programmed increase, 3 °C/min; carrier gas, N_2 (2 mL/min); injec-

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Table 1
^{1}H NMR data of compounds **2–6** (δ in ppm, J in Hz, C_5D_5N[a]).

Position	**2**[b]	**3**[c]	**4**[b]	**5**[b]	**6**[b]
1	1.00 m, 1.78 m	0.95 m, 1.78 m	1.03 m, 1.75 m	1.04 m, 1.76 m	0.88 m, 1.50 m
2	1.77 m, 2.07 m	1.75 m, 2.10 m	1.74 m, 2.13 m	1.78 m, 2.07 m	1.70 m, 2.05 m
3	3.70 m	3.71 m	3.67 m	3.68 m	3.81 m
4	2.46 t (11.4), 2.62 dd (3.0, 11.4)	2.45 m, 2.60 dd (2.9, 10.8)	2.45 t (12.6), 2.61 dd (2.8, 12.6)	2.47 t (11.8), 2.64 dd (3.0, 11.8)	2.40 m, 2.68 m
6	5.27 brd (3.7)	5.27 brs	5.29 brd (4.8)	5.27 brs	5.27 brs
7	1.52 m,1.92 m	1.50 m, 1.90 m	1.58 m, 1.90 m	1.57 m, 1.91 m	1.46 m, 1.86 m
8	1.81 m	1.80 m	1.89 m	1.88 m	1.82 m
9	0.95 m	0.98 m	1.69 m	0.99 m	1.29 m
11	1.49 m, 1.62 m	1.47 m, 1.61 m	1.60 m, 1.86 m	1.18 m, 1.54 m	2.30 dd (5.7, 14.1), 2.54 t (14.1)
12	1.60 m, 1.78 m	1.60 m, 1.77 m	3.98 brs	1.16 m, 1.77 m	
14	2.09 m	2.08 m	2.15 m	1.14 m	1.43 m
15	1.72 m, 2.17 m	1.73 m, 2.18 m	1.57 m, 2.10 m	1.52 m, 1.92 m	1.70 m, 2.11 m
16	4.48 m	4.48 m	4.64 m	4.67 m	4.45 m
17			3.08 dd (7.3, 8.3)	1.10 m	2.76 m
18	0.97 s	0.97 s	0.95 s	0.97 s	1.06 s
19	0.98 s	0.99 s	0.99 s	0.99 s	1.09 s
20	2.29 d (7.1)	2.30 d (7.0)	2.01 m	2.07 m	1.91 m
21	1.24 d (7.1)	1.25 d (7.4)	1.18 d (6.9)	1.15 d (7.4)	1.33 d (6.9)
23	1.80 m (2H)	1.80 m (2H)	1.76 m (2H)	1.81 m (2H)	1.74 m (2H)
24	1.84 m (2H)	1.82 m (2H)	1.65 m (2H)	1.86 m (2H)	1.64 m (2H)
25	2.06 m	2.05 m	1.56 m	2.17 m	1.63 m
26	3.87 t (11.2), 4.05 dd (3.7, 11.2)	3.89 t (11.3), 4.06 dd (3.5, 11.3)	3.59 m, 3.55 m	3.89 t (11.3), 4.06 dd (3.5, 1.3)	3.46 t (10.4), 3.58 m
27	3.65 m, 3.72 m	3.64 m, 3.73 m	0.67 d (4.9)	3.85 m, 3.90 m	0.68 d (5.6)
Api-1′	5.72 d (2.5)	5.70 d (1.9)	5.69 d (2.5)	5.69 d (2.5)	
2′	4.83 d (2.5)	4.75 d (1.9)	4.75 d (2.5)	4.75 d (2.5)	
4′	4.25 d (9.1) 4.48 d (9.1)	4.20 d (9.3), 4.33 d (9.3)	4.18 d (9.3), 4.31 d (9.3)	4.20 d (9.3), 4.33 d (9.3)	
5′	4.13 m (2H)	4.27 d (10.2), 3.94 d (10.2)	4.27 d (10.3), 3.95 d (10.3)	3.94 d (9.8), 4.27 d (9.8)	
Glc-1′					4.93 d (7.8)
2′					4.38 m
3′					4.18 m
4′					4.21 m
5′					3.62 m
6′					4.04 m, 4.21 m
Rha-1″	5.85 brs	5.79 brs	5.79 brs	5.79 brs	6.40 brs
2″	4.70 m	4.68 brs	4.67 m	4.68 m	4.83 m
3″	4.56 m	4.55 m	4.56 m	4.55 m	4.61 m
4″	4.31 m	4.30 m	4.32 m	4.32 m	4.35 m
5″	4.48 m	4.44 m	4.45 m	4.44 m	4.93 m
6″	1.73 d (6.0)	1.70 d (6.2)	1.70 d (6.0)	1.70 d (6.1)	1.75 d (6.2)
Rha-1‴		5.42 brs	5.42 brs	5.42 brs	5.83 brs
2‴		4.65 m	4.65 m	4.64 m	4.51 m
3‴		4.56 m	4.55 m	4.54 m	4.55 m
4‴		4.28 m	4.28 m	4.27 m	4.43 m
5‴		4.31 m	4.30 m	4.31 m	4.92 m
6‴		1.62 d (5.6)	1.61 d (5.3)	1.62 d (5.4)	1.58 d (6.1)
Rha-1⁗					6.27 brs
2⁗					4.90 m
3⁗					4.52 m
4⁗					4.29 m
5⁗					4.96 m
6⁗					1.59 d (5.9)

[a] Assignments based on 2D NMR spectra.
[b] Recorded at 500 MHz.
[c] Recorded at 400 MHz.

tor and detector temperature, 250 °C; injection volume, 2 μL; split ratio, 1/50.

2.2. Plant material

The whole plants of *Y. thibetica* Franch. were collected in Zhaotong, Yunnan Province, People's Republic of China, in October 2003 and identified by Prof. Chen Xin-Qi, the Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 0303771) is deposited at the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The powdered air-dried whole plants of *Y. thibetica* (30.0 kg) were extracted with 70% EtOH (3 × 150 L) under reflux for a total of 6 h. After removal of the solvent *in vacuo*, the residue was suspended in H_2O and successively extracted with petroleum ether (60–90 °C), and *n*-BuOH. Solvent was removed to give the *n*-BuOH extracts (2.0 kg), of which 1.8 kg was subjected to CC (silica gel, 200–300 mesh; gradient $CHCl_3$/MeOH 1:0 → 0:1) to afford five fractions: fraction 1 (20 g), fraction 2 (100 g), fraction 3 (500 g), fraction 4 (300 g), and fraction 5 (860 g). Fraction 2 (40 g) was subjected to repeated CC (silica gel (1) gradient petroleum ether/EtOAc 4:1 → 0:1; (2) $CHCl_3$/MeOH 9:1) to give four fractions (2.1–2.4). Fraction 2.1 was purified on CC (silica gel, petroleum ether/EtOAc 6:1) to give **1** (80 mg). Fraction 2.4 was further separated by RP-18 gel (gradient MeOH/H_2O 8:2 → 9:1), and semi-preparative HPLC (MeOH/H_2O 65:35; flow rate: 3 mL/min) to afford **2** (15 mg). Fraction 3 (150 g) was further chromatographed on CC (silica gel, gradient $CHCl_3$/MeOH/H_2O 9:1:0 → 8:2:0.1), RP-18 gel (gradient MeOH/H_2O 6:4 → 9:1), Sephadex LH-20 (MeOH), and semi-preparative HPLC (MeOH/H_2O 50:50 → 60:40; flow rate: 3 mL/min) to give **3** (14 mg), **4** (200 mg), **5** (15 mg), **7** (35 mg), **8** (40 mg), **10** (20 mg), **11** (56 mg), and **12** (15 mg). Fraction 4 (100 g) was further subjected to repeated CC (silica gel, $CHCl_3$/MeOH/H_2O 9:1:0 → 7:3:0.5), RP-18 gel (gradient MeOH/H_2O 6:4 → 9:1), and Sephadex LH-20 (MeOH) to give **6** (400 mg), **9** (1.0 g), and **13** (200 mg).

2.3.1. Ypsilandroside C (**2**)

Colorless needles (MeOH); mp 243–244 °C; $[\alpha]_D^{25}$ −99.6° (*c* 0.4, pyridine); FAB-MS (negative ion mode): *m/z* 723 $[M-H]^-$ (100), 577 $[M-H-C_6H_{10}O_4]^-$ (24), 445 $[M-H-C_6H_{10}O_4-C_5H_8O_4]^-$ (1.4); HRESI-MS (negative ion mode): *m/z* 723.3941 $[M-H]^-$ (calcd. for $C_{38}H_{59}O_{13}$ 723.3955); IR (KBr) ν_{max} (cm^{-1}): 3442, 2939, 1634, 1059; ^1H NMR data see Table 1; ^{13}C NMR data see Table 2.

2.3.2. Ypsilandroside D (**3**)

White amorphous powder; mp 292–293 °C; $[\alpha]_D^{25}$ −128.6° (*c* 0.91, pyridine); FAB-MS (negative ion mode): *m/z* 870 $[M]^-$ (100), 723 $[M-H-C_6H_{10}O_4]^-$ (18), 577 $[M-H-C_6H_{10}O_4-C_6H_{10}O_4]^-$ (3), 445 $[M-H-C_6H_{10}O_4-C_6H_{10}O_4-C_5H_8O_4]^-$ (0.7); HRESI-MS (negative ion mode): *m/z* 869.4528 $[M-H]^-$ (calcd. for $C_{44}H_{69}O_{17}$ 869.4515). IR (KBr) ν_{max} (cm^{-1}): 3423, 2935, 1656, 1058; ^1H NMR data see Table 1; ^{13}C NMR data see Table 2.

2.3.3. Ypsilandroside E (**4**)

Colorless needles (MeOH); mp 264–266 °C; $[\alpha]_D^{25}$ −118.5° (*c* 0.54, pyridine); FAB-MS (negative ion mode): *m/z* 853 $[M-H]^-$ (100), 707 $[M-H-C_6H_{10}O_4]^-$ (17); HRESI-MS (negative ion mode): *m/z* 853.4624 $[M-H]^-$ (calcd. for $C_{44}H_{69}O_{16}$ 853.4585); IR (KBr) ν_{max} (cm^{-1}): 3442, 2932, 1633, 1056, 981, 913, 895, 869 (895 > 913, for 25*R*-spiroketal); ^1H NMR data see Table 1; ^{13}C NMR data see Table 2.

Table 2
^{13}C NMR data of compounds **1–6** (δ in ppm, C_5D_5N[a]).

Position	**1**[b]	**2**[b]	**3**[c]	**4**[b]	**5**[b]	**6**[b]
1	37.9 (t)	37.1 (t)	37.2 (t)	37.4 (t)	37.5 (t)	37.1 (t)
2	32.6 (t)	30.3 (t)	30.3 (t)	30.2 (t)	30.3 (t)	29.9 (t)
3	71.3 (d)	77.7 (d)	77.7 (d)	77.6 (d)	77.6 (d)	77.8 (d)
4	43.5 (t)	39.3 (t)	39.3 (t)	39.3 (t)	39.2 (t)	38.8 (t)
5	142.0 (s)	140.8 (s)	140.8 (s)	140.9 (s)	140.8 (s)	140.6 (s)
6	121.1 (d)	121.9 (d)	121.9 (d)	122.0 (d)	121.8 (d)	121.6 (d)
7	31.8 (t)	32.4 (t)	32.4 (t)	31.9 (t)	32.3 (t)	31.8 (t)
8	32.4 (d)	32.4 (d)	32.4 (d)	32.0 (d)	31.7 (d)	31.0 (d)
9	50.4 (d)	50.2 (d)	50.3 (d)	44.4 (d)	50.3 (d)	52.4 (d)
10	37.1 (s)	37.6 (s)	37.6 (s)	36.9 (s)	37.1 (s)	37.6 (s)
11	21.1 (t)	21.0 (t)	21.0 (t)	29.3 (t)	21.2 (t)	37.7 (t)
12	32.5 (t)	31.9 (t)	31.9 (t)	71.4 (d)	39.9 (t)	212.8 (s)
13	45.2 (s)	45.2 (s)	45.2 (s)	45.1 (s)	40.5 (s)	55.0 (s)
14	53.2 (d)	53.1 (d)	53.1 (d)	48.3 (d)	56.7 (d)	56.0 (d)
15	31.8 (t)	32.1 (t)	32.1 (t)	32.4 (t)	32.3 (t)	31.8 (t)
16	90.1 (d)	90.1 (d)	90.1 (d)	81.1 (d)	81.2 (d)	79.8 (d)
17	90.2 (s)	90.2 (s)	90.2 (s)	53.9 (d)	62.9 (d)	54.1 (d)
18	17.2 (q)	17.2 (q)	17.2 (q)	17.4 (q)	16.4 (q)	15.9 (q)
19	19.7 (q)	19.5 (q)	19.5 (q)	19.3 (q)	19.4 (q)	18.9 (q)
20	44.9 (d)	44.9 (d)	45.0 (d)	42.3 (d)	42.1 (d)	42.7 (d)
21	9.8 (q)	9.9 (q)	9.8 (q)	15.0 (q)	15.1 (q)	14.0 (q)
22	110.3 (s)	110.3 (s)	110.3 (s)	109.3 (s)	110.0 (s)	109.4 (s)
23	32.2 (t)	31.9 (t)	31.9 (t)	32.4 (t)	31.6 (t)	31.7 (t)
24	23.6 (t)	23.6 (t)	23.7 (t)	29.3 (t)	24.1 (t)	29.3 (t)
25	39.1 (d)	39.1 (d)	39.1 (d)	30.7 (d)	39.2 (d)	30.6 (d)
26	64..0 (t)	64.0 (t)	64.0 (t)	66.9 (t)	64.1 (t)	67.0 (t)
27	64.4 (t)	64.4 (t)	64.4 (t)	17.3 (q)	64.5 (t)	17.4 (q)
Api-1′		107.1 (d)	107.0 (d)	106.9 (d)	106.9 (d)	
2′		82.5 (d)	82.4 (d)	82.2 (d)	82.3 (d)	
3′		80.6 (s)	79.4 (s)	79.4 (s)	79.4 (s)	
4′		75.0 (t)	75.1 (t)	75.1 (t)	75.1 (t)	
5′		65.9 (t)	71.3 (t)	71.2 (t)	71.3 (t)	
Glc-1′						100.4 (d)
2′						78.0 (d)
3′						77.7 (d)
4′						77.8 (d)
5′						77.1 (d)
6′						61.2 (t)
Rha-1″		102.0 (d)	102.0 (d)	102.0 (d)	102.0 (d)	102.2 (d)
2″		72.0 (d)	71.9 (d)	71.9 (d)	71.9 (d)	72.5 (d)
3″		72.7 (d)	72.7 (d)	72.6 (d)	72.7 (d)	72.9 (d)
4″		74.1 (d)	74.0 (d)	74.0 (d)	74.0 (d)	74.2 (d)
5″		70.3 (d)	70.1 (d)	70.1 (d)	70.1 (d)	69.6 (d)
6″		18.8 (q)	18.7 (q)	18.7 (q)	18.7 (q)	18.7 (q)
Rha-1‴			102.4 (d)	102.4 (d)	102.4 (d)	102.3 (d)
2‴			72.1 (d)	72.1 (d)	72.1 (d)	72.9 (d)
3‴			72.8 (d)	72.8 (d)	72.8 (d)	73.3 (d)
4‴			74.1 (d)	74.0 (d)	74.0 (d)	80.5 (d)
5‴			70.4 (d)	70.4 (d)	70.4 (d)	68.4 (d)
6‴			18.7 (q)	18.7 (q)	18.7 (q)	18.9 (q)
Rha-1⁗						103.4 (d)
2⁗						72.7 (d)
3⁗						72.9 (d)
4⁗						74.1 (d)
5⁗						70.5 (d)
6⁗						18.5 (q)

[a] Assignments based on 2D NMR spectra.
[b] Recorded at 125 MHz.
[c] Recorded at 100 MHz.

2.3.4. Ypsilandroside F (**5**)

White amorphous powder; mp 218–220 °C; $[\alpha]_D^{25}$ −102.1° (*c* 1.1, pyridine); FAB-MS (negative ion mode): *m/z* 853 $[M-H]^-$ (100), 707 $[M-H-C_6H_{10}O_4]^-$ (19), 561 $[M-H-C_6H_{10}O_4-C_6H_{10}O_4]^-$ (2); HRESI-MS (negative ion mode): *m/z* 853.4610 $[M-H]^-$ (calcd. for $C_{44}H_{69}O_{16}$ 853.4585); IR (KBr) ν_{max} (cm^{-1}): 3424, 2937, 1654, 1055; ^1H NMR data see Table 1; ^{13}C NMR data see Table 2.

2.3.5. Ypsilandroside G (**6**)

Colorless needles; mp 223–224 °C; $[\alpha]_D^{25}$ −108.2° (*c* 0.73, pyridine); FAB-MS (negative ion mode): *m/z* 1028 $[M]^-$ (100), 882 $[M-C_6H_{10}O_4]^-$ (22), 735 $[M-H-C_6H_{10}O_4-C_6H_{10}O_4]^-$ (5); HRESI-

MS (negative ion mode): *m/z* 1027.5107 $[M-H]^-$ (calcd. for $C_{44}H_{69}O_{16}$ 1027.5113); IR (KBr) ν_{max} (cm^{-1}): 3440, 2934, 2876, 1707, 1633, 1454, 1377, 1046, 981, 918, 899, 868 (899 >918, for 25*R*-spirosketal); ^1H NMR data see Table 1; ^{13}C NMR data see Table 2.

2.3.6. Acid hydrolysis of compounds 2–6

Compounds **2**–**5** (4 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 $CHCl_3$ and H_2O. The H_2O-souble fraction was evaporated to dryness. The dried sugar residues was diluted in 1 mL pyridine without water and treated with 0.5 mL trimethylchlorsilan (TMCS) and stirred at 60°C for 5 min. After drying the solution with a stream of N_2, the residue was extracted with ether (1 mL). The ether layer was analyzed by GC, and the retention times of L-rhamnose and D-apiose standards were 7.67 and 9.06 min, respectively. The same procedures were carried out for compound **6** (5 mg). The derivatives of D-glucose, and L-rhamnose were detected; t_R (min): L-rhamnose (7.67), and D-glucose (14.22).

2.4. Cytotoxicity bioassay

Cytotoxicity evaluations were performed for five human cell lines (K562, SPC-A-1, BGC-823, Eca-109, and AGS) using the MTT method described in the literature [7], with cisplatin as a positive control. Briefly, 4×10^4/mL cells were added to each well (90 μL/well), and incubated with either various concentrations of drugs (100, 30, 10, 3, 1, 0.3 μL/mL) or without drugs, in three replicates, for 48 h at 37 °C and in a humidified atmosphere of 5% CO_2. After 48 h, 10 μL of methyl thiazol tetrazalium (MTT) solution (5 mg/mL) was added to each well, which was then incubated for another 4 h. Then 10% SDS–5% isobutanol–0.012 M HCl was added to each well (100 μL/well). After 12 h at room temperature, the OD value of each well was recorded on a Model 680 reader at 570 nm.

2.5. Antifungal bioassay

An antifungal assay was performed on compounds **2**–**6** according to the methods of the CLSI (formerly NCCLS) (M-27) [8]. The twofold serial broth dilution assay was applied to measure the MIC values and all the compounds were tested at the concentration of 1280, 640, 320, 160, 80, 40, 20, 10, 5, and 2.5 μg/mL. The fungus *C. albicans* ATYY0109 and the clinical isolates of *C. albicans* resistant to FLC (10^3 CFUs/mL) were incubated in Sabouraud's dextrose broth at 35 °C for 24–48 h, with the respective compounds and the positive control dissolved in DMSO. The negative controls of the fungal culture were incubated with limited DMSO under the same conditions. DMSO was defined not to be toxic at a limited amount (<5%) under these experimental conditions. MIC was defined as the lowest concentration that inhibited visible growth and the MIC > 100 mg/mL was considered to be inactive. FLC was used as a positive control.

3. Results and discussion

The *n*-BuOH extract of the whole plant of *Y. thibetica* was repeatedly chromatographed on silica gel and reversed phase silica gel (RP-18) columns and finally purified by semi-preparative HPLC to afford five new compounds (**2**–**6**) and eight known ones. These known compounds, as determined by comparison of their NMR and MS data to reported values in the literatures, were identified as (25*S*)-spirost-5-en-3β,17α,27-triol (**1**) [9], pennogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**7**) [10], pennogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside (**8**) [11], pennogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside (**9**) [12,13], diosgenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**10**) [14], diosgenin 3-*O*-α-L-rhamnopyranosyl (1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside (**11**) [13,15], diosgenin 3-*O*-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**12**) [16], and diosgenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside (**13**) [15], respectively.

Ypsilandroside C (**2**) was obtained as colorless needles (MeOH). The IR spectrum showed absorption for OH groups at 3442 cm^{-1} and an olefin moiety at 1634 cm^{-1}. The high-resolution negative-ion ESI-MS exhibited an accurate quasi-molecular ion peak at *m/z* 723.3941 $[M-H]^-$, in accordance to the molecular formula $C_{38}H_{60}O_{13}$. The ^1H NMR spectrum (Table 1) of **2** showed two angular methyl groups at δ_H 0.97 and 0.98, a secondary methyl groups at δ_H 1.24 (d, *J* = 7.4 Hz), a trisubstituted olefinic proton signal at δ_H 5.27 (brs), and two anomeric protons at δ_H 5.72 (d, *J* = 2.5 Hz, H-1′), 5.85 (brs, H-1″), which indicated the presence of two sugar units. The ^{13}C NMR spectrum (Table 2) exhibited 38 carbon atoms, 27 of which were assigned to the aglycone moiety while the remaining were due to two sugar units. Furthermore, its ^{13}C NMR spectrum showed the characteristic signals of 27-hydroxylpennogenin [9] at δ_C 140.8 (s, C-5), 121.9 (d, C-6), 90.1 (d, C-16), 90.2 (s, C-17), 110.3 (s, C-22), 64.0 (t, C-26), 64.4 (t, C-27), respectively. Acid hydrolysis of **2** with 4 M TFA–dioxane (1:1) produced D-apiose and L-rhamnose sugar residues determined by GC analysis. All NMR assignments (Tables 1 and 2) were based on the HMQC, ^1H–^1H COSY, HMBC, HMQC-TOCSY and ROESY experiments. The β-furanoid anomeric form of the apiofuranosyl residue was indicated by the chemical shifts of the C-1′ (δ_C 107.1), C-2′ (δ_C 82.5), C-3′ (δ_C 80.6), and C-4′ (δ_C 75.0) to those of the corresponding carbons of methyl α- and β-D-apiofuranoside [17], while the anomeric configuration of the rhamnopyranosyl was determined as α-oriented based on the chemical shift values of the C-3″ (δ_C 72.7), and C-5″ (δ_C 70.3) to those of the corresponding carbons of methyl α- and β-rhamnopyranoside [18]. Furthermore, the ^1H and ^{13}C NMR signal patterns of the aglycone moiety of **2** were superimposable with those of compound **1**. However, the carbon resonance attributed to C-3 (δ 77.7) was found to be shifted downfield by +6.4 ppm, in comparison to that of compound **1**. This suggested that C-3 was the site of glycosidation on the sapogenol moiety. The HMBC correlations observed between the H-1′ (δ_H 5.72) of apiose and the C-3 (δ_C 77.7) further proved this assumption. In addition, a long-range correlation between the H-$1''_{rha}$ (δ_H 5.85) and the C-$2'_{api}$ (δ_C 82.5) was also observed in the HMBC spectrum of **2**, which revealed that the terminal rhamnopyranosyl unit was linked at the C-2′ of the inner apiofuranosyl attached to the C-3 of the sapogenin. Thus, we identified ypsilandroside C (**2**) as (25*S*)-spirost-5-en-3β,17α,27-triol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-apiofuranoside.

Ypsilandroside D (**3**) was obtained as a white amorphous powder. Its HRESI-MS displayed an accurate ion peak at *m/z* 869.4528 $[M-H]^-$, corresponding to the molecular formula $C_{41}H_{58}O_{13}$. The negative-ion FAB-MS of **3** gave the quasi-molecular-ion and fragment-ion peaks at *m/z* 870 $[M]^-$, 723 $[M-H-C_6H_{10}O_4]^-$, 577 $[M-H-2\times C_6H_{10}O_4]^-$, and 445 $[M-H-2\times C_6H_{10}O_4-C_5H_8O_4]^-$, suggesting the presence of three sugar moieties in the molecule. The quasi-molecular-ion peak of FAB-MS established that compound **3** had 146 mass units more than compound **2**. Comparison of the ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **3** with those of **2** indicated that the aglycone of **3** is identical to that of **2** and a set of six additional signals corresponding to a terminal α-L-rhamnopyranosyl unit (Rha‴) in compound **3** could be observed at δ_C 102.4, 72.1, 72.8, 74.1, 70.4, and 18.7. Correspondingly, three anomeric-proton signals at δ_H 5.70 (d, *J* = 1.9 Hz, H-1′), 5.79 (brs, H-1″), and 5.42 (brs, H-1‴) were observed in the ^1H NMR spectrum of **3**. Acid hydrolysis of **3** with 4 M TFA produced D-apiose and L-rhamnose

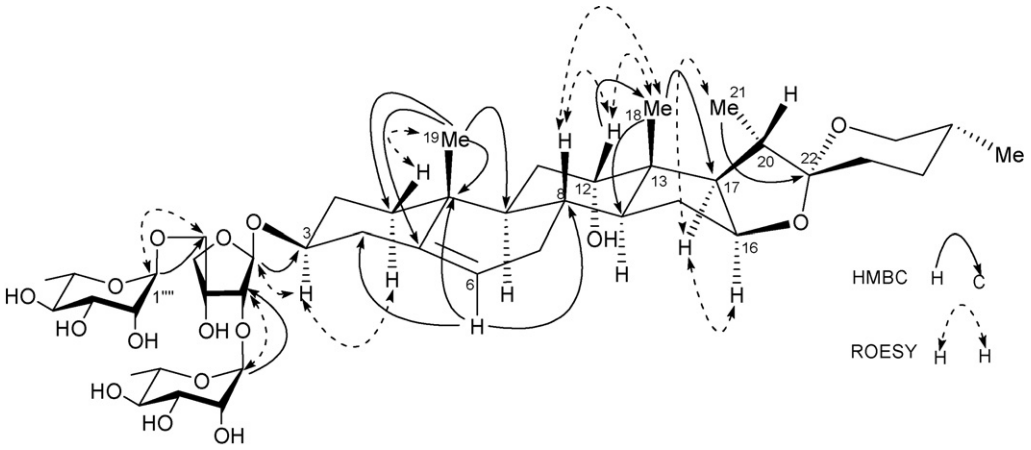

Fig. 1. Key HMBC and ROESY correlations of **4**.

sugar residue as determined by GC analysis. The ^{13}C-NMR data (Table 2) of **3** showed the downfield shift of C-5′$_{api}$ ($\Delta\delta$ + 5.4), suggesting another rhamnosyl residue linked to the C-5′ of the apiofuranosyl attached to the C-3 of sapogenin. This was confirmed by the HMBC correlation of the H-1‴ (δ_H 5.42) with the C-5′ (δ_C 71.3). Consequently, the structure of ypsilandroside D (**3**) was elucidated as (25*S*)-spirost-5-en-3β,17α,27-triol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 5)]-β-D-apiofuranoside.

Ypsilandroside E (**4**) was obtained as colorless needles (MeOH). Its molecular formula was determined to be $C_{44}H_{70}O_{16}$ on the high-resolution negative-ion ESI-MS, which gave an accurate ion peak at *m*/*z* 853.4624 [M−H]$^-$ (calcd. 853.4585). The typical methyl signals at δ_H 0.67 (*d*, *J* = 4.9 Hz), 0.95 (s), 0.99 (s), and 1.18 (*d*, *J* = 6.9 Hz) in the ^{1}H NMR spectrum, and olefinic carbons signals at δ_C 140.9 (s, C-5) and 122.0 (d, C-6) and a quaternary carbon signals at δ_C 109.3 (s, C-22) in the ^{13}C NMR suggested that compound **4** was also a $\Delta^{5,6}$ -spirostanol glycoside. The intensity of the absorptions (895 > 913 cm^{-1}) in its IR spectrum indicated that the absolute configuration of C-25 was *R* [19], which was also confirmed by the ^{13}C NMR chemical shifts of C-23 (δ_C 32.4), C-24 (δ_C 29.3), C-25 (δ_C 30.7), C-26 (δ_C 66.9), and C-27 (δ_C 17.3) [20]. Additionally, the NMR spectroscopic data attributed to the aglycone of **4** were in good agreement with those of 3-O-glycoside derived from heloniogenin [(25*R*)-spirost-5-en-3β,12α-diol] [6], as confirmed by the HMBC and ROESY correlations (Fig. 1). An HMBC experiment of **4** showed the long correlation between the H-12 (δ 3.98) and the Me-18 (δ_C 17.4), suggesting that the hydroxyl group was attached at the C-12. The relative configuration of the hydroxyl group at C-12 was assigned with an α-orientation on the basis of ROESY experiment of H-12 (δ_H 3.98) with H-8 (δ_H 1.89), and Me-18 (δ_H 0.95). According to the consistence of the ^{1}H- and ^{13}C NMR data (Tables 1 and 2) of the sugar moieties in **4** and **3**, these two compounds were considered to have the same sugar sequence and linkage position. Consequently, the structure of ypsilandroside E (**4**) was elucidated as heloniogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 5)]-β-D-apiofuranoside.

Ypsilandroside F (**5**) was isolated as a white amorphous powder. Negative-ion HRESI-MS gave an accurate ion peak at *m*/*z* 853.4610 [M−H]$^-$, corresponding to the molecular formula $C_{44}H_{70}O_{16}$. The ^{13}C NMR data (Table 2) due to the aglycone moieties were superimposable with those of the 3-O-glycoside derived from isonarthogenin [(25*S*)-spirost-5-ene-3β,27-diol] [21]. This suggested that compound **5** had an isonarthogenin unit as aglycone. The remaining ^{1}H- and ^{13}C NMR data (Tables 1 and 2) showed the similar signals ascribable to the sugar moieties to those of **3**, suggesting that the sugar chain of compound **5** and those of **3** had the same linkage sequence and positions. Therefore, the structure of ypsilandroside F (**5**) was established as isonarthogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 5)]-β-D-apiofuranoside.

Ypsilandroside G (**6**) was obtained as colorless needles (MeOH). The IR spectrum showed absorption for OH groups at 3440 cm^{-1}, a carbonyl group at 1707 cm^{-1} and an olefin moiety at 1633 cm^{-1}. Moreover, the characteristic absorptions at 981, 918, 899, 868 cm^{-1} (intensity: 899 > 918) showed a (25*R*)-spiroketal moiety [19]. Its molecular formula was assigned as $C_{51}H_{80}O_{21}$ based on the negative ion HRESI-MS ([M−H]$^-$, *m*/*z* 1027.5107). In the ^{13}C NMR spectrum (Table 2), a carbonyl signal at δ 212.8 was observed. The carbonyl group was placed at C-12 based on the HMBC correlation between δ_H 1.06 (Me-18, s) and δ_C 212.8 (C-12, s), 55.0 (C-13, s), 56.0 (C-14, d), 54.1 (C-17, d) (Fig. 2). Furthermore, the ^{13}C NMR signal patterns of the aglycone moiety (Table 2) of **6** were superimposable with those of gentrogenin [22]. Besides, the downfield shift of C-3 from δ 71.4 to 77.8 suggested that the sapogenin of **6** was

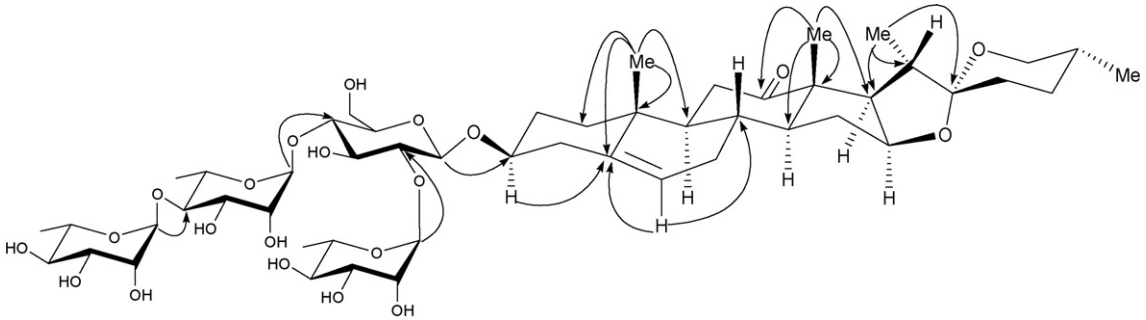

Fig. 2. Key HMBC correlations of **6**.

C-3 glycosided gentrogenin. When treated with 4 M TFA **6** gave D-glucose and L-rhamnose sugar residues, as determined by GC analysis. In the ^{1}H NMR spectrum (Table 1) of **6**, four anomeric-proton signals at δ_H 4.93 (*d*, *J* = 7.8 Hz), 5.83 (brs), 6.27 (brs), and 6.40 (brs) and three methyl proton signals due to the rhamnosyl residues at δ_H 1.75 (d, *J* = 6.2 Hz), 1.59 (d, *J* = 5.9 Hz), and 1.58 (d, *J* = 6.1 Hz) were observed. Correspondingly, four anomeric-carbon signals at δ_C 100.4, 102.3, 103.4, and 102.2 were observed in the ^{13}C NMR spectrum. The relative large coupling constant (*J* > 7) of anomeric-proton δ_H 4.93 (d, *J* = 7.8 Hz) ascribed to a glucose, while the anomeric configuration of the three rhamnopyranosyls were determined as α-oriented based on the chemical shift values of the C-3″, C-5″, C-3‴, C-5‴, C-3⁗, and C-5⁗ to those of the corresponding carbons of methyl α- and *β*-rhamnopyranoside [18,23]. The linkage sites and sequences were determined by analyzing the 2D NMR spectrum of **6**. In the HMBC spectrum, long-range correlations between H-1′ (δ_H 4.93) and C-3 (δ_C 77.8), H-1″ (δ_H 6.40) and C-2′ (δ_C 78.0), H-1‴ (δ_H 5.83) and C-4′ (δ_C 77.8), H-1⁗ (δ_H 6.27) and C-4‴ (δ_C 80.5) revealed that the inner glucopyranosyl unit was linked at the C-3 of aglycone, a terminal rhamnopyranosyl unit at the C-2′ of glucopyranosyl, an inner rhamnopyranosyl unit at the C-4′ of the inner glucopyranosyl, another terminal rhamnopyranosyl unit at the C-4‴ of inner rhamnopyranosyl. Accordingly, the structure of ypsilandroside G (**6**) was assigned as gentrogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside.

Spirostanol saponins **2**–**6**, and **9** were evaluated for their *in vitro* cytotoxicities in five human cell lines (K562, SPC-A-1, BGC-823, Eca-109, and AGS), with cisplatin as a positive control. The new compound **6** showed moderate inhibitory activity against the above cell lines with IC_{50} values of 4.7, 6.3, 6.9, 8.0, and 8.9 μg/mL, respectively. The known compound **9** also exhibited moderate inhibitory activity against the above cell lines with IC_{50} values of 9.4, 2.6, 4.0, 7.2, and 7.3 μg/mL, respectively. Other new compounds (**2**–**5**) were non-cytotoxic to all five cell lines with IC_{50} > 100 μg/mL.

The new compounds (**2**–**6**) were also tested for antifungal activity against *C. albicans*. The results revealed that compound **6** exhibited moderate inhibitory activity against *C. albicans* with a MIC value of 10 μg/mL, while the remaining compounds **2**–**5** were inactive. It appears that the antifungal activity of ypsilandroside G (**6**) has a correlation to its cytotoxicity against mammalian cells.

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