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Spirostanol tetraglycosides from *Ypsilandra thibetica*

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ABSTRACT

Phytochemical reinvestigation on the whole plants of *Ypsilandra thibetica* obtained five new spirostane glycosides, ypsilandrosides H-L (**1**–**5**), and a known saponin polyphylloside III (**6**). Among them, **1** and **2** are the first spirostane glycosides which possess novel 5(6 → 7) abeo-steroidal aglycones. Compounds **3** and **4** are rare saponins whose aglycones contain a hydroxyl group at C-7. Their structures were elucidated on the basis of MS, 1D and 2D NMR spectroscopic analysis and chemical evidences. The isolated compounds were evaluated for their cytotoxic activity on five tumor cell lines.

1. Introduction

Ypsilandra (Liliaceae) is a small genus including only 5 species which are distributed in southwestern China and Myanmar. *Ypsilandra thibetica* only grows in China and has been used in folk medicine as hemostatic in Sichuan and Yunnan Province, China [1]. Three furostanol saponins obtained from this species reportedly have anticancer activity [2]. Our previous phytochemical investigations on *Y. thibetica* collected from Yunnan Province resulted in the isolation of a new sapogenin and 9 new spirostanol saponins as well as 8 known compounds [3,4].

Many phytochemical investigations have exhibited that the secondary metabolites of one plant often differ when it grows in different ecological environments. With the aim of searching for new saponins, we have reinvestigated the whole plants of *Y. thibetica* collected in Luding County of Sichuan Province and isolated five new spirostane glycosides, ypsilandrosides H-L (**1**–**5**), and a known saponin polyphylloside III (**6**) (Fig. 1) [5]. Here we report the isolation, structure elucidation, and cytotoxic activity testing of the new compounds.

2. Experimental

2.1. General methods

Optical rotations were measured on a SEPA-3000 automatic digital polarimeter. FAB-MS spectra were recorded on a VG Auto Spec-300 spectrometer, HRESIMS spectra were recorded on an API Qstar Pulsar instrument. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were measured using a Shimadzu UV-2401PC spectrophotometer. NMR spectra were run on Bruker AM-400 and DRX-500 instruments with TMS as internal standard. Column chromatography (CC) was performed over silica gel (200–300 mesh, 10–40 μm, Qingdao Marine Chemical Co., China), RP-18 (40–63 μm, Merck), and Sephadex LH-20 (GE Healthcare, Sweden). TLC was performed on HSGF254 (0.2 mm, Qingdao Marine Chemical Co., China) or RP-18 F_{254} (0.25 mm, Merck). Semi-preparative HPLC was run on Agilent 1100 liquid chromatograph with diode array detector (DAD) setting at 200 nm and 254 nm, 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.

2.2. Plant material

The plant material of *Y. thibetica* was collected in November 2006 from Luding County, Sichuan Province, People's Republic of China, and identified by Prof. Xin-Qi Chen, Institute of Botany, Chinese Academy of Sciences, Beijing. A voucher specimen (No. HY0002) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China.

2.3. Extraction and isolation

The whole plants of *Y. thibetica* (10 kg) were extracted three times with 70% EtOH (50 L × 3) under reflux for a total of 6 h and the combined extract was concentrated under reduced pressure. Then the concentrated extract was passed through YWD-3F macro-

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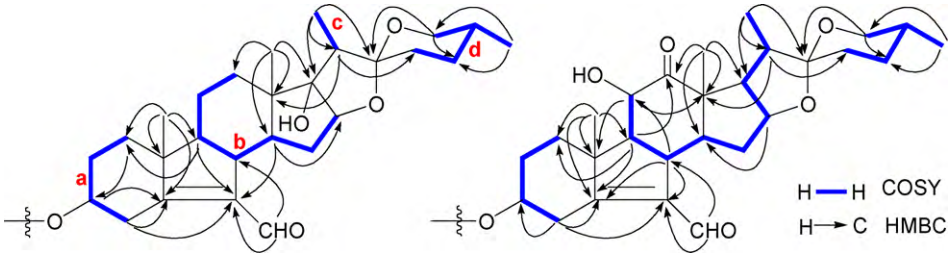

Fig. 1. ^{1}H-^{1}H COSY and key HMBC correlations of the aglycone moieties of **1** and **2**.

porous resin and eluted successively with H_2O, 40% EtOH, 70% EtOH and 95% EtOH, respectively. Vaporated 70% EtOH fractions (70 g) was fractioned by silica gel column and eluted with a gradient of $CHCl_3$–MeOH–H_2O (10:1:0 → 7:3:0.5, v/v). Four fractions were collected. Fraction 4 eluted with $CHCl_3$–MeOH–H_2O (7:3:0.5) was subjected to MPLC (Rp-18, MeOH–H_2O 8:2 → 7:3) to give four subfractions. Subfractions of interest was further chromatographed on Sephadex LH-20 (MeOH) and finally purified by semi-preparative HPLC (MeCN–H_2O 30:70 → 35:65 v/v; flow rate: 3 mL min^{-1}) to yield **1** (17 mg), **2** (14 mg), **3** (13 mg), **4** (14 mg), **5** (8 mg), and **6** (11 mg).

*2.3.1. Ypsilandroside H (**1**)*

White amorphous powder; $[\alpha]_D^{18}$ −127.8 (*c* 0.18, C_5D_5N); negative FAB-MS: *m/z* 1044 $[M]^-$; HRESI-MS: *m/z* 1043.5029 $[M-H]^-$ (calcd. for $C_{51}H_{79}O_{22}$ 1043.5063); IR (KBr) ν_{max} (cm^{-1}): 3440, 2932, 2875, 1794, 1661, 1457, 1382, 1129, 1054, 980, 917, 895, 867, 838, 804 (intensity: 895 > 917); UV (MeOH) λ_{max} (log ε) 255 (3.7) nm; ^{1}H NMR data see Table 1; ^{13}C NMR data see Table 2.

*2.3.2. Ypsilandroside I (**2**)*

White amorphous powder; $[\alpha]_D^{18}$ −79.7 (*c* 0.21, C_5D_5N); negative FAB-MS: *m/z* 1058 $[M]^-$; HRESI-MS: *m/z* 1093.4634 $[M+Cl]^-$ (calcd. for $C_{51}H_{78}O_{23}Cl$ 1093.4622); IR (KBr) ν_{max} (cm^{-1}): 3429, 2931, 1713, 1634, 1456, 1384, 1130, 1052, 981, 919, 899, 867, 840, 803 (intensity: 899 > 919); UV (MeOH) λ_{max} (log ε) 254 (3.6) nm; ^{1}H NMR data see Table 1; ^{13}C NMR data see Table 2.

*2.3.3. Ypsilandroside J (**3**)*

White amorphous powder; $[\alpha]_D^{18}$ −105.1 (*c* 0.18, MeOH); negative FAB-MS: *m/z* 1043 $[M-H]^-$, 897 $[M-H-146]^-$, 751 $[M-H-146-146]^-$; HRESI-MS: *m/z* 1043.5067 $[M-H]^-$ (calcd. for $C_{51}H_{79}O_{22}$ 1043.5063); IR (KBr) ν_{max} (cm^{-1}): 3432, 2934, 1706, 1630, 1460, 1380, 1133, 1044, 981, 919, 898, 868, 839, 803 (intensity: 898 > 919); ^{1}H NMR data see Table 1; ^{13}C NMR data see Table 2.

*2.3.4. Ypsilandroside K (**4**)*

White amorphous powder; $[\alpha]_D^{18}$ −111.9 (*c* 0.19, MeOH); negative FAB-MS: *m/z* 1059 $[M-H]^-$, 913 $[M-H-146]^-$, 767 $[M-H-146-146]^-$; HRESI-MS: *m/z* 1095.4763 $[M+Cl]^-$ (calcd. for $C_{51}H_{80}O_{23}Cl$ 1095.4778); IR (KBr) ν_{max} (cm^{-1}): 3440, 2934, 1710, 1630, 1460, 1383, 1133, 1046, 981, 920, 897, 866, 838, 801 (intensity: 897 > 920); ^{1}H NMR data see Table 1; ^{13}C NMR data see Table 2.

*2.3.5. Ypsilandroside L (**5**)*

White amorphous powder; $[\alpha]_D^{18}$ −113.6 (*c* 0.17, MeOH); negative FAB-MS: *m/z* 1046 $[M]^-$, 900 $[M-146]^-$, 753 $[M-H-146-146]^-$; HRESI-MS *m/z* 1081.4787 $[M+Cl]^-$ (calcd. for $C_{51}H_{82}O_{22}Cl$ 1081.4986); IR (KBr) ν_{max} (cm^{-1}): 3425, 2932, 1456, 1382, 1129, 1034, 980, 917, 895, 867, 838, 804; ^{1}H NMR data see Table 1; ^{13}C NMR data see Table 2.

*2.3.6. Acid hydrolysis of compounds **1**–**5** and GC analysis*

Compounds **1**–**5** (3 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-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-chlorsilan (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 under the following conditions: column, SGE AC-10 quartz capillary column (30 m × 0.32 mm × 0.25 μm); column temperature 180–280 °C; programmed increase, 3 °C/min; carrier gas, N_2 (2 mL/min); injector and detector temperature, 250 °C; injection volume, 2 μL; split ratio, 1/50. Peaks of the hydrolysate were detected by comparison with retention times of authentic samples of glucose and rhamnose after treatment with trimethyl-chlorsilan (TMCS) in pyridine. The absolute configurations of the sugar residues were determined to be L-rhamnose (t_R 7.67 min) and D-glucose (t_R 14.22 min).

2.4. MTT cytotoxicity assays

The bioassay was carried out as described elsewhere [6], with five human cancer cell lines (A-549, HL-60, PANC-1, SMMC-7721, and SK-BR-3). The experiments were repeated twice. Cisplatin was used as the positive control antitumor drug.

3. Results and discussion

The EtOH extract of *Y. thibetica* was subjected to YWD-3F macroporous resin eluted successively with an EtOH–H_2O gradient system. 70% EtOH fraction was subjected to subsequent silica gel, Sephadex LH-20, and Rp-18 silica gel column chromatography and finally purified by semi-preparative HPLC to yield five new spirostane glycosides, ypsilandrosides H-L (**1**–**5**), and polyphylloside III (**6**). Each of the isolates was subjected to detailed spectroscopic analysis to establish their chemical structures.

Ypsilandroside H (**1**) was obtained as white amorphous powder. Its negative-ion HRESI-MS displayed a quasi-molecular ion peak at *m/z* 1043.5029 $[M-H]^-$ (calcd. for 1043.5063) in accord with the molecular formula $C_{51}H_{80}O_{22}$, requiring 12 degrees of unsaturation. The UV spectrum of **1** showed absorption maxima at 255 nm, indicating the presence of a conjugated enal system, while its IR spectrum showing absorptions at 3440, 1794, and 1661 cm^{-1} suggested the existence of hydroxyl, α,β-unsaturated aldehyde, and olefin groups, respectively. The ^{1}H NMR spectrum (Table 1) of **1** exhibited the signals due to an aldehyde proton at δ_H 10.2, two tertiary methyls at δ_H 0.83, 1.00, and two secondary methyls at δ_H 0.69 (d, *J* = 5.5 Hz), 1.21 (d, *J* = 7.5 Hz), which were recognized as typical steroid methyls. The ^{13}C NMR signals (Table 2) showed 51 carbon signals, 27 of which were assigned to the aglycone moiety consisted in two tertiary methyls, two secondary methyls, nine methylenes (including an oxygenated one at δ_C 66.7), seven methines (including

Table 1
^{1}H NMR spectroscopic data of compounds **1**–**5** (500 MHz, δ in ppm, J in Hz, C_5D_5N)[a].

Position	**1**	**2**	**3**	**4**	**5**
1a	1.65 (o)	2.39 (br d, 13.7)	1.51 (o)	1.90 (o)	1.72 (o)
1b	1.06 (m)	1.44 (m)	0.83 (t, 13.2)	1.26 (m)	0.96 (o)
2a	2.14 (o)	2.18 (o)	1.92 (o)	2.01 (o)	2.03 (o)
2b	1.87 (o)	1.96 (o)	1.80 (m)	1.90 (o)	1.83 (o)
3	4.00 (m)	4.22 (m)	3.67 (m)	3.73 (m)	3.85 (m)
4a	3.97 (o)	4.05 (dd, 3.3, 12.6)	2.85 (dd, 4.0, 13.8)	2.88 (dd, 4.1, 13.6)	2.78 (dd, 4.2, 13.7)
4b	2.46 (t, 12.1)	2.55 (t, 11.9)	2.74 (t, 11.9)	2.83 (t, 13.4)	2.71 (t, 11.9)
6	10.2 (s)	10.2 (s)	5.77 (d, 5.1)	5.82 (d, 5.0)	5.29 (d, 3.9)
7a			4.06 (o)	4.07 (o)	1.92 (o)
7b					1.51 (dd, 5.9, 14.0)
8	2.72 (m)	3.30 (m)	1.96 (o)	1.98 (m)	1.89 (o)
9	1.11 (m)	1.62 (m)	2.10 (m)	2.22 (t, 11.5)	0.96 (o)
11a	1.41 (2H, m)	4.89 (d, 6.2)	2.62 (t, 14.2)	4.82 (d, 8.8)	1.56 (o)
11b			2.38 (m)		1.48 (o)
12a	2.14 (o)				1.85 (o)
12b	1.50 (m)				1.60 (o)
14	2.39 (m)	1.84 (m)	2.41 (m)	2.43 (m)	2.04 (o)
15a	2.83 (ddd, 6.0, 7.4, 13.4)	2.68 (m)	2.67 (m)	2.63 (m)	2.18 (o)
15b	2.06 (6.2, 7.2, 13.4)	2.21 (o)	1.81 (m)	1.83 (m)	1.61 (o)
16	4.44 (t, 8.3)	4.56 (m)	4.44 (t, 9.3)	4.45 (t, 9.4)	4.47 (dd, 6.8, 13.5)
17		2.72 (t, 7.5)	2.92 (t, 8.3)	3.00 (t, 7.6)	
18	0.83 (s)	1.20 (s)	1.19 (s)	1.20 (s)	0.93 (s)
19	1.00 (s)	1.23 (s)	1.08 (s)	1.37 (s)	1.06 (s)
20	2.26 (d, 7.5)	1.94 (o)	1.95 (o)	1.92 (o)	2.21 (d, 7.1)
21	1.21 (d, 7.5)	1.36 (d, 7.0)	1.33 (d, 6.8)	1.28 (d, 6.9)	1.19 (d, 7.1)
23a	1.70 (2H, m)	1.67 (2H, m)	1.67 (2H, m)	1.64 (2H, m)	1.91 (o)
23b					1.54 (o)
24a	1.57 (2H, o)	1.59 (2H, o)	1.54 (2H, o)	1.54 (2H, o)	2.19 (o)
24b					1.87 (o)
25	1.58 (o)	1.60 (o)	1.56 (o)	1.55 (o)	1.88 (o)
26a	3.48 (br s)	3.58 (dd, 2.0, 9.9)	3.53 (br d, 11.0)	3.53 (dd, 2.8, 11.2)	4.10 (o)
26b	3.47 (br s)	3.47 (t, 10.4)	3.43 (t, 10.0)	3.43 (t, 10.7)	3.92 (o)
27a	0.69 (d, 5.5)	0.71 (d, 5.0)	0.65 (d, 4.6)	0.65 (d, 5.4)	4.13 (o)
27b					3.95 (o)
3-Glc					
1′	5.00 (d, 7.3)	5.04 (d, 7.5)	4.94 (d, 7.3)	4.88 (d, 6.8)	4.93 (d, 6.9)
2′	4.18 (o)	4.19 (o)	4.20 (o)	4.20 (o)	4.21 (o)
3′	4.21 (o)	4.23 (o)	4.22 (o)	4.22 (o)	4.22 (o)
4′	4.38 (t, 9.1)	4.38 (t, 8.9)	4.37 (t, 9.0)	4.39 (t, 9.3)	4.40 (t, 9.9)
5′	3.62 (br d, 9.4)	3.36 (m)	3.60 (m)	3.59 (m)	3.61 (m)
6′a	4.22 (br d, 12.5)	4.18 (br d, 10.5)	4.18 (br d, 12.1)	4.16 (br d, 12.8)	4.21 (o)
6′b	4.04 (t, 11.1)	4.03 (d, 3.0, 10.5)	4.06 (br d, 12.1)	4.05 (br d, 12.8)	4.04 (br d, 12.2)
2′-Rha					
1″	6.43 (br s)	6.45 (br s)	6.43 (br s)	6.43 (br s)	6.40 (br s)
2″	4.83 (br s)	4.86 (br s)	4.87 (br s)	4.84 (br s)	4.85 (br s)
3″	4.61 (dd, 3.1, 9.2)	4.62 (dd, 3.1, 9.3)	4.62 (dd, 3.0, 9.2)	4.66 (dd, 3.1, 9.2)	4.63 (dd, 3.2, 9.3)
4″	4.35 (t, 9.2)	4.37 (t, 9.4)	4.36 (t, 9.1)	4.36 (t, 9.2)	4.36 (t, 9.3)
5″	4.93 (m)	4.96 (m)	4.94 (m)	4.95 (m)	4.96 (m)
6″	1.58 (d, 6.0)	1.59 (d, 6.5)	1.59 (d, 6.0)	1.59 (d, 6.0)	1.59 (d, 6.0)
4′-Rha					
1‴	5.82 (br s)	5.82 (br s)	5.85 (br s)	5.85 (br s)	5.84 (br s)
2‴	4.51 (o)	4.51 (o)	4.53 (o)	4.52 (o)	4.52 (o)
3‴	4.55 (o)	4.55 (o)	4.57 (o)	4.56 (o)	4.57 (o)
4‴	4.43 (t, 9.0)	4.45 (t, 9.2)	4.46 (t, 9.3)	4.46 (t, 9.4)	4.46 (t, 9.6)
5‴	4.92 (m)	4.93 (m)	4.92 (m)	4.91 (m)	4.92 (m)
6‴	1.58 (d, 6.0)	1.59 (d, 6.5)	1.59 (d, 6.0)	1.59 (d, 6.0)	1.58 (d, 6.0)
4″-Rha					
1⁗	6.28 (br s)	6.29 (br s)	6.30 (br s)	6.31 (br s)	6.29 (br s)
2⁗	4.90 (br s)	4.92 (br s)	4.92 (br s)	4.91 (br s)	4.92 (br s)
3⁗	4.52 (dd, 3.0, 9.0)	4.53 (dd, 2.7, 8.9)	4.53 (dd, 3.0, 9.5)	4.52 (dd, 3.0, 9.2)	4.53 (dd, 3.1, 9.1)
4⁗	4.29 (t, 9.8)	4.31 (t, 8.9)	4.29 (t, 9.0)	4.30 (t, 9.2)	4.31 (t, 9.1)
5⁗	4.95 (o)	4.97 (o)	4.95 (o)	4.95 (o)	4.96 (o)
6⁗	1.72 (d, 6.1)	1.74 (d, 6.0)	1.76 (d, 6.2)	1.77 (d, 6.2)	1.76 (d, 6.2)

[a] s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, multiplet; o, overlapped.

two oxygenated ones at δ_C 90.0 and 78.0), four quaternary carbons (including two oxygenated ones δ_C 109.8 and 89.1), a tetrasubstituted double bond (δ_C 139.9 and 169.7), and an aldehyde (δ_C 189.4); the remaining signals were due to the carbons of the four sugar units. All the information mentioned above was in support of the aglycone of **1** being a spirotanol skeleton with a hydroxyl group at C-17, which possessed the same A, C, D, E, F and C rings as pennogenin isolated from the genus *Paris* (Trilliaceae) and the differences were in the B ring [7,8]. Analysis of COSY connectivities allowed to identify in the aglycone of **1** four spin systems (**a**–**d**) as shown with bold bonds in Fig. 1. Furthermore, the HMBC correlations of C-5 with H-3, H_2-4, H-8, H-9 and Me-19, C-7 with H_2-4, H-6, H-

Table 2
^{13}C NMR spectroscopic data of compounds **1–5** (125 MHz, δ in ppm, C_5D_5N).

Position	1	2	3	4	5
1	36.3 (t)	38.0 (t)	36.7 (t)	38.5 (t)	37.6 (t)
2	29.9 (t)	29.9 (t)	29.2 (t)	29.2 (t)	30.2 (t)
3	78.0 (d)	77.9 (d)	77.9 (d)	77.8 (d)	77.8 (d)
4	30.8 (t)	30.9 (t)	38.7 (t)	39.3 (t)	39.0 (t)
5	169.7 (s)	170.4 (s)	143.5 (s)	144.3 (s)	140.9 (s)
6	189.4 (d)	188.9 (d)	125.8 (d)	125.4 (d)	121.9 (d)
7	139.9 (s)	137.4 (s)	64.3 (d)	64.4 (d)	32.5 (t)
8	46.4 (d)	43.8 (d)	37.2 (d)	37.3 (d)	32.4 (d)
9	60.2 (d)	66.1 (d)	45.2 (d)	53.6 (d)	50.3 (d)
10	46.6 (s)	47.3 (s)	38.5 (s)	40.3 (s)	37.2 (s)
11	20.4 (t)	73.8 (d)	37.3 (t)	73.9 (d)	21.0 (t)
12	32.6 (t)	213.0 (s)	212.8 (s)	213.6 (s)	31.9 (t)
13	47.9 (s)	56.5 (s)	54.7 (s)	53.7 (s)	45.4 (s)
14	50.5 (d)	54.1 (d)	50.6 (d)	51.2 (d)	53.1 (d)
15	34.5 (t)	34.5 (t)	31.8 (t)	31.8 (t)	32.1 (t)
16	90.0 (d)	80.4 (d)	80.0 (d)	80.4 (d)	90.2 (d)
17	89.1 (s)	54.6 (d)	54.1 (d)	54.2 (d)	90.1 (s)
18	17.6 (q)	16.1 (q)	15.9 (q)	15.7 (q)	17.2 (q)
19	15.5 (q)	14.8 (q)	17.6 (q)	18.0 (q)	19.5 (q)
20	44.7 (d)	42.4 (d)	42.8 (d)	42.6 (d)	45.2 (d)
21	9.9 (q)	14.0 (q)	13.9 (q)	13.9 (q)	9.6 (q)
22	109.8 (s)	109.2 (s)	109.3 (s)	109.3 (s)	110.5 (s)
23	32.2 (t)	31.9 (t)	31.8 (t)	31.8 (t)	27.5 (t)
24	28.9 (t)	29.2 (t)	29.7 (t)	30.0 (t)	21.3 (t)
25	30.5 (d)	30.6 (d)	30.5 (d)	30.5 (d)	36.1 (d)
26	66.7 (t)	67.0 (t)	66.9 (t)	66.9 (t)	60.7 (t)
27	17.4 (q)	17.4 (q)	17.3 (q)	17.3 (q)	61.5 (t)
3-Glc					
1′	100.9 (d)	100.9 (d)	100.4 (d)	100.4 (d)	100.4 (d)
2′	77.9 (d)	77.8 (d)	77.9 (d)	77.8 (d)	78.1 (d)
3′	77.5 (d)	77.4 (d)	77.5 (d)	77.7 (d)	77.8 (d)
4′	77.8 (d)	77.7 (d)	77.8 (d)	77.8 (d)	78.0 (d)
5′	77.2 (d)	77.1 (d)	77.1 (d)	77.0 (d)	77.0 (d)
6′	61.3 (t)	61.4 (t)	61.3 (t)	61.2 (t)	61.3 (t)
2′-Rha					
1″	101.9 (d)	101.9 (d)	102.1 (d)	102.1 (d)	102.2 (d)
2″	72.5 (d)	72.4 (d)	72.5 (d)	72.5 (d)	72.6 (d)
3″	72.8 (d)	72.8 (d)	72.9 (d)	72.9 (d)	72.9 (d)
4″	74.2 (d)	74.2 (d)	74.2 (d)	74.2 (d)	74.2 (d)
5″	69.5 (d)	69.5 (d)	69.5 (d)	69.5 (d)	69.6 (d)
6″	18.7 (q)	18.6 (q)	18.6 (q)	18.6 (q)	18.7 (q)
4′-Rha					
1‴	102.3 (d)	102.3 (d)	102.3 (d)	102.3 (d)	102.3 (d)
2‴	72.9 (d)	72.9 (d)	72.9 (d)	72.9 (d)	72.9 (d)
3‴	73.3 (d)	73.3 (d)	73.3 (d)	72.3 (d)	73.3 (d)
4‴	80.4 (d)	80.4 (d)	80.4 (d)	80.5 (d)	80.4 (d)
5‴	68.4 (d)	68.4 (d)	68.4 (d)	68.4 (d)	68.4 (d)
6‴	18.9 (q)	18.9 (q)	18.9 (q)	18.9 (q)	18.9 (q)
4″-Rha					
1⁗	103.3 (d)	103.3 (d)	103.3 (d)	103.3 (d)	103.3 (d)
2⁗	72.7 (d)	72.7 (d)	72.7 (d)	72.5 (d)	72.6 (d)
3⁗	72.9 (d)	72.9 (d)	72.9 (d)	72.9 (d)	72.9 (d)
4⁗	74.0 (d)	74.0 (d)	74.0 (d)	74.1 (d)	74.1 (d)
5⁗	70.4 (d)	70.5 (d)	70.5 (d)	70.4 (d)	70.4 (d)
6⁗	18.5 (q)	18.5 (q)	18.4 (q)	18.4 (q)	18.5 (q)

8, H-9, and H-14 suggested the presence of the α,β-unsaturated aldehyde at B-ring located between C-5 and C-7, which was the same substructure as that of parguesterol A [9]. In addition, the connectivities of the substructures **a**–**d** and four quaternary carbons, and two tertiary methyls were set up by the aid of the HMBC experiment as shown in Fig. 1. The different ring junctions and configurations at C-3, C-17 and C-20 were deduced from ROESY experiment. Intense correlations between Me-19/H-8/Me-18, Me-18/H-20 and between H-9/H-14/H-16 showed the usual *trans* ring fusion for the rings B/C and C/D and *cis* junction for the rings D/E as well as α-configuration for OH-17 and β-configuration for H-20. Further correlations of H-3 with H_{eq}-1 and H_{eq}-4 indicated α-configuration for H-3. The weaker intensity of the band at 917

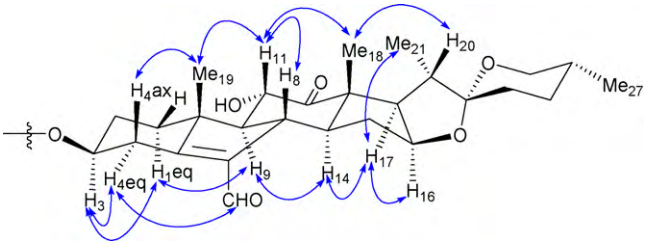

Fig. 2. Key ROESY correlations of the aglycone moiety of **2**.

compared with that at 895 cm^{-1} in its IR spectrum suggested the *R* absolute stereochemistry at C-25 [10].

The ^{1}H NMR and ^{13}C NMR spectra (Tables 1 and 2) of **1** exhibited four sugar anomeric protons at δ_H 5.00 (d, J = 7.3 Hz), 5.82 (br s), 6.28 (br s), and 6.43 (br s) (Table 1), and carbon atoms at δ_C 100.9, 102.3, 103.3, 101.9 (Table 2), respectively. The sugar part of **1** was determined to be a combination of one D-glucose and three L-rhamnose on the basis of the NMR data (including ^{13}C, HSQC-TOCSY, ^{1}H,^{1}H-COSY, and HMBC) and GC analysis of a chiral derivative of the sugars in an acidic hydrolysate [11]. The β-configuration of the anomeric proton of the glucopyranosyl residue was assigned based on its J_{1H-2H} value (J = 7.3 Hz), while the anomeric configuration of the three rhamnopyranosyls were determined as α-oriented on the ground the chemical shift values of the C-3″, C-5″, C-3‴, C-5‴, C-3⁗, and C-5⁗ with those of the corresponding carbons of methyl α- and β-rhamnopyranoside [12,13]. The sequence of the tetrasaccharide chain at C-3 was deduced from the following HMBC correlations: H-1′ (δ_H 5.00) of Glc with C-3 (δ_C 78.0) of the aglycone, H-1″ (δ_H 6.43) of 2′-Rha with C-2′ (δ_C 77.9) of Glc, H-1‴ (δ_H 5.82) of 4′-Rha with C-4′ (δ_C 77.8) of Glc, and H-1⁗ (δ_H 6.28) of 4″-Rha with C-4‴ (δ_C 80.4) of 4′-Rha. Thus, ypsilandroside H (**1**) was determined to be (25*R*)-B-nor(7)-6-carboxaldehyde-spirost-5(7)-en-3β,17α-diol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside.

Ypsilandroside I (**2**) had the molecular formula $C_{51}H_{78}O_{23}$ based on negative-ion HRESI-MS (m/z 1093.4634 [M+Cl]$^-$, calcd. for 1093.4622) and ^{13}C NMR and DEPT spectral data (Table 2). The absorption bands in the IR spectrum at 1713 and 1634 cm^{-1}, and the UV maxima at 254 nm indicated the presence of α,β-unsaturated aldehyde moiety in **2** similar to those in **1**. The ^{1}H NMR spectrum of **2** (Table 1) showed signals for four anomeric protons (δ_H 5.04, 5.82, 6.29, and 6.45) together with signals for four steroid methyls (δ_H 0.71, 1.20, 1.23, and 1.36). The ^{13}C NMR shifts of **2** (Table 2) were essentially the same as those of **1** except for the disappearance of an oxygenated quaternary carbon and the presence of an oxymethine and a carbonyl group, which was supported by mass difference of 14 units. The oxymethine was determined to be C-11 by the appearance of the ^{1}H-^{1}H COSY correlation of H-9 with H-11 and the HMBC correlations from H-11 to C-9, C-10, C-12, and C-13. Further HMBC cross-peaks between C-12 and H-9, H-11, H-17, and Me-18 established the carbonyl carbon at C-12. The α-orientation of the OH-11 was inferred by the observed NOE correlations of H-11 (δ_H 4.89) with H-8 (δ_H 3.30) and Me-18 (δ_H 1.20) in the ROESY spectrum (Fig. 2). The ^{13}C NMR signals arising from the tetraglycoside moiety composed of one β-D-glucopyranosyl and three α-L-rhamnopyranosyl units were in good agreement with those of **1**. Therefore, the structure of ypsilandroside I (**2**) was established to be (25*R*)-B-nor(7)-6-carboxaldehyde-spirost-5(7)-en-3β,11α-diol-12-one 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside.

Ypsilandroside J (**3**) gave a pseudo-molecular ion peak [M−H]$^-$ at m/z 1043.5067 (calcd. for 1043.5063) in its high-resolution

ESI-MS. Combined with ^{13}C NMR spectroscopic data, its molecular formula was determined as $C_{51}H_{80}O_{22}$. Two tertiary methyl protons at δ_H 1.08 and 1.19 (each s), two secondary methyl protons at δ_H 0.65 (d, J = 4.6 Hz) and 1.33 (d, J = 6.8 Hz) and an olefinic proton at δ_H 5.77 (d, J = 5.1 Hz), as well as protons attributable to an oxymethylene H-26 at δ_H 3.43 (t, J = 10.0 Hz) and δ_H 3.53 (m), observed in its ^1H NMR spectrum (Table 1). These data, when considered with the analysis of its ^{13}C NMR spectrum (two angular methyls at δ_C 15.9 and 17.6, two secondary methyls at δ_C 13.9 and 17.3, a trisubstituted double bond at δ_C 125.8 and 143.5, a methylene group linked to an oxygen atom at δ_C 66.9, and a quaternary carbon bearing oxygen atoms at δ_C 109.3) (Table 2), suggested that the aglycone possessed a $\Delta^{5,6}$-spirostanol skeleton. A comparison of the ^1H and ^{13}C spectroscopic signals of the aglycone moiety of **3** with dioseptemloside D [14] indicated that the signals were similar except for the presence of a carbonyl group (δ_C 212.8). In the HMBC spectrum, the long-range correlations between δ_H 1.19 (Me-18, s) and δ_C 212.8 (C-12, s), 54.7 (C-13, s), 50.6 (C-14, d), 54.1 (C-17, d) indicated that the carbonyl group was attached at C-12 of the aglycone of **3**. A hydroxyl was located at C-7 of the aglycon of **3** due to the ^1H-^1H correlations of H-7 (δ_H 4.06)/H-6 (δ_H 5.77) and H-7 (δ_H 4.06)/H-8 (δ_H 1.96) in the ^1H-^1H COSY spectrum and the HMBC correlations of H-7 with C-5, C-6, C-8, C-9 and C-14. The configuration of 7-OH was assigned to be α-orientation on the ground of the resonance of C-7 (δ_C 64.3, *d*) [14–16], which could be further confirmed by ROESY correlations of H-7 with H-8, H_{ax}-15, and H_{eq}-15. Hence, the aglycone of **3** was identified as spirost-5-en-3β,7α-diol-12-one. Its 25*R* configuration was deduced from the intensity of the absorptions (898 > 910 cm^{-1}) in its IR spectrum [10], which was also confirmed by the F-ring resonances of C-23 (δ_C 31.8), C-24 (δ_C 29.7), C-25 (δ_C 30.5), C-26 (δ_C 66.9), and C-27 (δ_C 17.3) [17]. Analysis of the NMR data (Tables 1 and 2) for the sugar portion of **3** and comparison with those of **1** revealed that these two compounds had the same saccharide chain linked at C-3. On the basis of the above analysis, ypsilandroside J (**3**) was assigned to be (25*R*)-3β-[(*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[*O*-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranosyl)oxy]-7α-hydroxy-spirost-5-en-12-one.

Ypsilandroside K (**4**) was obtained as white amorphous powder with the molecular formula $C_{51}H_{80}O_{23}$, as determined by HRESI-MS exhibiting an $[M+Cl]^-$ peak at *m*/*z* 1095.4763 (calcd. for 1095.4778), requiring 12 degrees of unsaturation. The ^1H NMR spectrum of **4** showed signals for four anomeric protons (δ_H 4.88, 5.85, 6.31, and 6.43) together with signals for four steroid methyls (δ_H 0.65, 1.20, 1.28, and 1.37). Comparison of the ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **4** with those of **3** showed their considerable structural similarity. The differences consisted only in the appearance of a further oxymethine signal (δ_H 4.82,d, J = 8.8; δ_C 73.9, d) in the ^1H and ^{13}C NMR spectra of **4**. The ^1H-^1H correlations of the proton signal at δ 4.82 with the proton at δ 2.22 in the ^1H-^1H COSY spectrum of **4** suggested that the oxygenated function was located at C-11, which was confirmed by the HMBC correlations from H-11 to C-8, C-10, C-12, and C-13. The α-configuration of the hydroxyl group at C-11 was also defined by the cross-peak between H-11 (δ_H 4.82) and Me-18 (δ_H 1.20) in its NOESY spectrum. From the above evidence, the structure of ypsilandroside K (**4**) was defined as (25*R*)-3β-[(*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[*O*-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranosyl)oxy]-7α,11α-dihydroxy-spirost-5-en-12-one.

Ypsilandroside L (**5**) was obtained as white amorphous powder. Its molecular formula was determined as $C_{51}H_{82}O_{22}$ by HRESI-MS (*m*/*z* 1081.4787 $[M+Cl]^-$, calcd. for 1081.4986). Comparison of the ^1H and ^{13}C NMR data of the aglycone of both **5** and polyphylloside III (**6**) [5] revealed that all signals were the same except for the resonances of the F-ring of **5** which were shifted downfield (Tables 1 and 2). Therefore, it was supposed that the differences were induced by the configuration at C-25. The absolution configuration of C-25 was deduced to be *R* on the basis of the resonances of C-23 (δ_C 27.5, t), C-24 (δ_C 21.3, t), C-25 (δ_C 36.1, d), C-26 (δ_C 60.7, t), and C-27 (δ_C 61.5, t), which could be further confirmed by ROESY correlations of H_{ax}-23/H-20, H_{ax}-23/Me-21, H_{ax}-23/H_2-27, and H_{eq}-23/H-25. Therefore, ypsilandroside L (**5**) was identified as (25*R*)-17α,27-dihydroxy-spirost-5-en-3β-yl-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[*O*-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside.

Compounds **1** and **2** are the first examples of 6-5-6-5-5-6 fused rings spirostanol saponins with a α,β-unsaturated aldehyde group in B ring. Additionally, ypsilandrosides J (**3**) and K (**4**) are rare steroidal saponins which aglycones contain hydroxyl group at C-7. This type of compounds was reported previously from *Dioscorea septemloba* (Dioscoreaceae) [14], *Paris pollyphylla* Smith var. *yunnanensis* (Trilliaceae) [16], *Urginea sanguinea* (Hyacynthaceae) [18], and *Tupistra wattii* (Liliaceae) [19]. The discovery of compounds **1**–**5** is a further addition to the diverse and complex class of spirostane glycosides. Since steroidal saponins are reported to possess, to varying degrees, cytotoxic activity against various cancer cell lines [20,21], we have tested compounds **1**–**6** for cytotoxicity against five tumor cell lines (HL-60, SMMC-7721, A-549, SK-BR-3, and PANC-1) with cisplatin as the positive control. However, the results showed that none of the six compounds had considerable cytotoxic activity against these cell lines (IC_{50} > 40 μM).

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