New Minor Spirostane Glycosides from *Ypsilandra thibetica*

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A further phytochemical investigation on the whole plants of *Ypsilandra thibetica* yielded three new spirostane glycosides, named ypsilandrosides M–O (**1**–**3**). Their structures were established as (3*β*,11*α*,25*R*)-3,11-dihydroxyspirost-5-en-12-one 3-{*O*-*α*-L-rhanmopyranosyl-(1→4)-*O*-L-rhanmopyranosyl-(1→4)-*O*-[*α*-L-rhanmopyranosyl-(1→2)]-*β*-D-glucopyranoside} (**1**), (3*β*,7*β*,25*R*)-spirost-5-ene-3,7-diol 3-{*O*-*α*-L-rhanmopyranosyl-(1→4)-*O*-*α*-L-rhanmopyranosyl-(1→4)-*O*-[*α*-L-rhanmopyranosyl-(1→2)]-*β*-D-glucopyranoside} (**2**), and (3*β*,7*α*,25*R*)-spirost-5-ene-3,7,17-triol 3-{*O*-*α*-L-rhanmopyranosyl-(1→4)-*O*-*α*-L-rhanmopyranosyl-(1→4)-*O*-[*α*-L-rhanmopyranosyl-(1→2)]-*β*-D-glucopyranoside} (**3**) by means of a combination of MS, 1D- and 2D-NMR spectroscopic methods, and chemical degradation. Among them, compound **3** is the first pennogenin (=(3*β*,25*R*)-spirost-5-ene-3,17-diol) saponin whose aglycone contains an OH group at C(7). Compounds **1**–**3** were evaluated for the inhibition of the growth of five tumor cell lines, but all of them proved to be inactive.

Introduction. – Steroidal saponins, which are almost exclusively present in the monocotyledonous angiosperms, have attracted a growing interest owing to the range of their biological actions including antidiabetic, antitumor, antitussive, and anti-dementia activity and as platelet aggregation inhibitors [1]. *Ypsilandra thibetica* FRANCH. is a perennial herb of the family Liliaceae and grows in southwestern China [2]. The whole plant has been used as hemostatic agent in Chinese folk medicine [3]. In our recent study, we found that this species is a rich source of steroidal saponins. Two sapogenin, 22 spirostanol saponins, and two C(22) steroidal lactone glycosides were isolated from the title plant [4–7]. In continuation of our investigations on the chemistry of this species, we obtained three new minor spirostane glycosides, ypsilandrosides M–O (**1**–**3**; *Fig. 1*). Herein, we report the isolation, structural elucidation, and cytotoxic evaluation of the new spirostane glycosides.

Results and Discussion. – Compound **1**, obtained as a colorless amorphous powder, gave a quasimolecular-ion peak at *m*/*z* 1043.5057 ([*M* − H]$^-$) in its HR-ESI-MS. Combined with ^{13}C-NMR spectroscopic data (*Table*), its molecular formula was determined as $C_{51}H_{80}O_{22}$. The IR spectrum showed absorption for OH groups at 3441 cm^{-1}, a C=O group at 1710 cm^{-1}, and an olefin moiety at 1638 cm^{-1}. The ^{1}H-NMR spectrum of **1** (*Table*) showed signals due to two tertiary Me groups at δ(H) 1.11 and 1.36 (2*s*), two secondary Me groups at δ(H) 0.68 (*d*, *J* = 5.5 Hz) and 1.29 (*d*, *J* = 6.9 Hz), one olefinic H-atom at δ(H) 5.33 (*d*, *J* = 3.9 Hz), as well as two H-atom signals

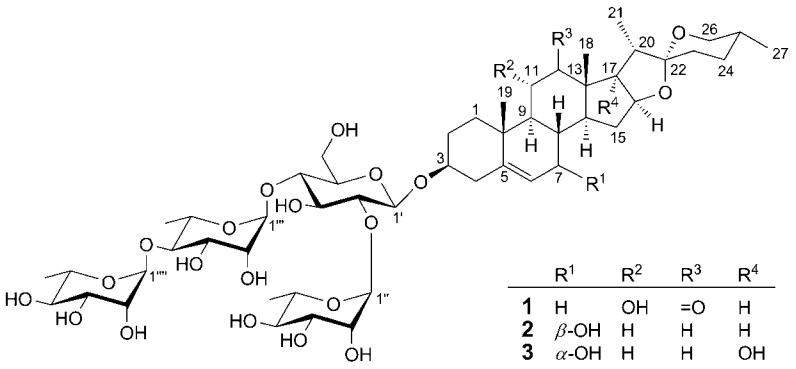


Fig. 1. *Compounds* **1**–**3**, *isolated from* Ypsilandra thibetica

attributable to an O-bearing CH_2(26) at δ(H) 3.45 (*t*, $J = 10.5$ Hz) and 3.54–3.57 (*m*). The ^{13}C-NMR and DEPT spectrum (*Table*) showed a total of 27 C-atoms arising from the aglycone moiety. Furthermore, a dioxygenated quaternary C-atom at δ(C) 109.4 and olefinic C-atoms at δ(C) 141.5 and 121.3 suggested that **1** possessed a hydroxyspirost-5-ene skeleton. Comparison of the ^{1}H- and ^{13}C-NMR spectra indicated that **1** differed from ypsilandroside J (=(3β,7α,25*R*)-3-[(*O*-6-deoxy-α-L-mannopyranosyl-(1 → 4)-*O*-6-deoxy-α-L-mannopyranosyl-(1 → 4)-*O*-[6-deoxy-α-L-mannopyranosyl-(1 → 2)]-β-D-glucopyranosyl)oxy]-7-hydroxyspirost-5-en-12-one) [6] by the presence of a CH_2 (δ(C) 32.0; δ(H) 1.48–1.50 (*m*) and 1.80–1.83 (*m*)) instead of an O-bearing CH moiety (δ(C) 64.3; δ(H) 4.06 (*m*)) comprising C(7) in the latter, which was confirmed by the ^{1}H,^{1}H correlations H–C(6) (δ(H) 5.33)/CH_2(7) in the ^{1}H,^{1}H-COSY plot of **1**. The ROESY correlations H–C(11) (δ(H) 4.72)/Me(18) (δ(H) 1.11) confirmed that OH–C(11) was α-oriented. Other ROESY correlations suggested that compound **1** has the same ring junctions as those of ypsilandroside J. The stronger intensity of the band at 899 compared with that at 919 cm^{-1} in its IR spectrum suggested the (*R*) absolute configuration at C(25) [8]. The anomeric region in the ^{1}H- and ^{13}C-NMR spectra of **1** showed signals for four anomeric H-atoms at δ(H) 4.97 (*d*, $J =$ 7.0 Hz), 5.85 (br. *s*), 6.29 (br. *s*), and 6.41 (br. *s*) with their corresponding anomeric C-atoms at δ(C) 100.4, 102.3, 103.3, and 102.2, respectively (*Table*). Acid hydrolysis of **1** produced D-glucose and L-rhamnose (=6-deoxy-L-mannose) as sugar residues, which were determined by GC analysis of their corresponding trimethylsilated L-cysteine adducts. The ^{1}H-NMR coupling constant ($^3J(1,2) \geq 7$ Hz) for the anomeric H-atom revealed that the glucose unit has a β-configuration, while the three rhamnose units have the α-configuration according to the chemical-shift values of C(3″) (δ(C) 72.9 (*d*)), C(5″) (δ(C) 69.6 (*d*)), C(3‴) (δ(C) 73.3 (*d*)), C(5‴) (δ(C) 68.4 (*d*)), C(3⁗) (δ(C) 72.9 (*d*)), and C(5⁗) (δ(C) 70.4 (*d*)), compared with the corresponding C-atoms of methyl α- and β-rhamnopyranoside [9][10]. In the HMBC spectrum of **1**, the correlations H–C(1′) (δ(H) 4.97)/C(3) (δ(C) 77.9), H–C(1″) (δ(H) 6.41)/C(2′) (δ(C) 78.2), H–C(1‴) (δ(H) 5.85)/C(4′) (δ(C) 78.0), and H–C(1⁗) (δ(H) 6.29)/C(4‴) (δ(C) 80.5) revealed an inner glucopyranosyl unit linked at C(3) of the aglycone, a terminal rhamnopyranosyl unit at C(2′) of the glucopyranosyl unit, an inner rhamnopyranosyl

Table. *[1]H- and [13]C-NMR Data* (C_5D_5N) *of Compounds* **1**–**3**. δ in ppm, J in Hz.

Position	**1**[a])		**2**[a])		**3**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.23–1.25, 1.87–1.88 (2*m*)	39.1 (*t*)	0.96–0.99, 1.72–1.74 (2*m*)	37.1 (*t*)	0.93 (*dd*, *J* = 4.0, 14.2), 1.69–1.71 (*m*)	38.0 (*t*)
CH_2(2)	1.92–1.95, 2.04–2.06 (2*m*)	29.2 (*t*)	1.83–1.85, 2.07–2.09 (2*m*)	30.3 (*t*)	1.84–1.87, 2.00–2.03 (2*m*)	30.0 (*t*)
H–C(3)	3.86–3.88 (*m*)	77.9 (*d*)	3.92–3.95 (*m*)	77.9 (*d*)	3.75–3.77 (*m*)	77.9 (*d*)
CH_2(4)	2.77 (*t*, *J* = 11.8), 2.81–2.83 (*m*)	39.4 (*t*)	2.75 (*t*, *J* = 12.3), 2.84–2.87 (*m*)	38.6 (*t*)	2.69–2.71 (*m*), 2.82 (*dd* , *J* = 4.7, 12.2)	39.0 (*t*)
C(5)		141.5 (*s*)		141.5 (*s*)		143.9 (*s*)
H–C(6)	5.33 (*d*, *J* = 3.9)	121.3 (*d*)	5.68 (br. *s*)	128.8 (*d*)	5.81 (*d*, *J* = 4.8)	126.1 (*d*)
CH_2(7) or H–C(7)	1.48–1.50 (*m*), 1.80–1.83 (*m*)	32.0 (*t*)	4.00–4.03 (*m*)	72.5 (*d*)	4.03–4.05 (*m*)	64.7 (*d*)
H–C(8)	1.44–1.46 (*m*)	31.7 (*d*)	1.81–1.83 (*m*)	40.8 (*d*)	1.66–1.68 (*m*)	38.7 (*d*)
H–C(9)	1.38–1.40 (*m*)	60.4 (*d*)	1.08–1.11 (*m*)	48.7 (*d*)	1.65–1.67 (*m*)	42.6 (*d*)
C(10)		39.7 (*s*)		37.3 (*s*)		37.3 (*s*)
H–C(11) or CH_2(11)	4.72 (*d*, *J* = 9.9)	73.9 (*d*)	1.45–1.47 (*m*)	21.3 (*t*)	1.63–1.65 (*m*)	20.9 (*t*)
C(12) or CH_2(12)		213.4 (*s*)	1.12–1.15, 1.70–1.72 (2*m*)	40.0 (*t*)	1.67–1.69 (*m*)	32.1 (*t*)
C(13)		54.0 (*s*)		41.0 (*s*)		45.0 (*s*)
H–C(14)	1.41–1.43 (*m*)	56.0 (*d*)	1.34–1.36 (*m*)	56.4 (*d*)	3.03–3.05 (*m*)	46.3 (*d*)
CH_2(15)	1.77–1.80, 2.06–2.09 (2*m*)	31.8 (*t*)	2.03–2.05, 2.83–2.85 (2*m*)	35.3 (*t*)	2.12–2.15, 2.72–2.74 (2*m*)	32.1 (*t*)
H–C(16)	4.42–4.44 (*m*)	79.9 (*d*)	4.63–4.66 (*m*)	81.7 (*d*)	4.55–4.58 (*m*)	90.4 (*d*)
H–C(17) or C(17)	2.85–2.87 (*m*)	54.2 (*d*)	1.80–1.82 (*m*)	62.6 (*d*)		90.3 (*s*)
Me(18)	1.11 (*s*)	15.7 (*q*)	0.86 (*s*)	16.5 (*q*)	1.03 (*s*)	17.2 (*q*)
Me(19)	1.36 (*s*)	19.1 (*q*)	1.00 (*s*)	19.0 (*q*)	1.08 (*s*)	19.6 (*q*)
H–C(20)	1.85–1.88 (*m*)	42.5 (*d*)	1.97 (*dd*, *J* = 7.0, 13.5)	42.1 (*d*)	2.21–2.24 (*m*)	45.0 (*d*)
Me(21)	1.29 (*d*, *J* = 6.9)	13.9 (*q*)	1.15 (*d*, *J* = 7.0)	15.2 (*q*)	1.25 (*d*, *J* = 7.1)	9.7 (*q*)
C(22)		109.4 (*s*)		109.3 (*s*)		109.9 (*s*)
CH_2(23)	1.59–1.61, 1.62–1.65 (2*m*)	31.8 (*t*)	1.62–1.65, 1.65–1.68 (2*m*)	31.9 (*t*)	1.67–1.69 (*m*, 2 H)	32.1 (*t*)
CH_2(24)	1.52–1.54 (*m*)	30.2 (*t*)	1.53–1.55 (*m*)	29.3 (*t*)	1.57–1.59 (*m*)	28.9 (*t*)
H–C(25)	1.57–1.60 (*m*)	30.5 (*d*)	1.57–1.59 (*m*)	30.7 (*d*)	1.72–1.75 (*m*)	30.5 (*d*)
CH_2(26)	3.45 (*t*, *J* = 10.5), 3.54–3.57 (*m*)	67.0 (*t*)	3.49 (*t*, *J* = 10.5), 3.55–3.57 (*m*)	66.9 (*t*)	3.44–3.46, 3.47–3.49 (2*m*)	66.7 (*t*)
Me(27)	0.68 (*d*, *J* = 5.5)	17.3 (*q*)	0.67 (*d*, *J* = 5.2)	17.4 (*q*)	0.64 (*d*, *J* = 5.8)	17.3 (*q*)
Glc:						
H–C(1′)	4.97 (*d*, *J* = 7.0)	100.4 (*d*)	4.95 (*d*, *J* = 7.5)	100.4 (*d*)	4.87 (*d*, *J* = 7.7)	100.4 (*d*)
H–C(2′)	4.20–4.22 (*m*)	78.2 (*d*)	4.19–4.22 (*m*)	78.1 (*d*)	4.18–4.21 (*m*)	78.1 (*d*)
H–C(3′)	4.22–4.25 (*m*)	77.8 (*d*)	4.22–4.25 (*m*)	77.6 (*d*)	4.21–4.23 (*m*)	77.8 (*d*)
H–C(4′)	4.40–4.43 (*m*)	78.0 (*d*)	4.40–4.42 (*m*)	77.7 (*d*)	4.40–4.43 (*m*)	78.1 (*d*)
H–C(5′)	3.52–3.55 (*m*)	77.0 (*d*)	3.58–3.60 (*m*)	77.1 (*d*)	3.60–3.62 (*m*)	77.0 (*d*)
CH_2(6′)	4.02 (*d*, *J* = 11.1), 4.15 (*d*, *J* = 11.6)	61.2 (*t*)	4.05 (*dd*, *J* = 3.0, 13.0), 4.21–4.23 (*m*)	61.3 (*t*)	4.05–4.07 (*m*), 4.19 (*d*, *J* = 13.0)	61.4 (*t*)

Table (cont.)

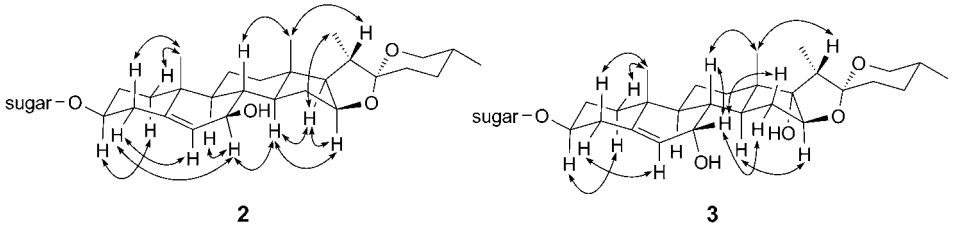
	1[a])		**2**[a])		**3**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
Rha:						
H–C(1″)	6.41 (br. *s*)	102.2 (*d*)	6.39 (br. *s*)	102.2 (*d*)	6.41 (br. *s*)	102.2 (*d*)
H–C(2″)	4.86–4. 88 (*m*)	72.5 (*d*)	4.85–4.87 (*m*)	72.5 (*d*)	4.86–4.88 (*m*)	72.5 (*d*)
H–C(3″)	4.62–4.64 (*m*)	72.9 (*d*)	4.62–4.65 (*m*)	73.0 (*d*)	4.66–4.69 (*m*)	72.9 (*d*)
H–C(4″)	4.35–4.37 (*m*)	74.2 (*d*)	4.37–4.39 (*m*)	74.2 (*d*)	4.38–4.40 (*m*)	74.2 (*d*)
H–C(5″)	4.96–4.98 (*m*)	69.6 (*d*)	4.95–4.97 (*m*)	69.6 (*d*)	4.95–4.98 (*m*)	69.6 (*d*)
Me(6″)	1.59 (*d*, *J* = 5.8)	18.7 (*q*)	1.59 (*d*, *J* = 6.3)	18.7 (*q*)	1.59 (*d*, *J* = 6.0)	18.7 (*q*)
Rha:						
H–C(1‴)	5.85 (br. *s*)	102.3 (*d*)	5.84 (br. *s*)	102.3 (*d*)	5.84 (br. *s*)	102.3 (*d*)
H–C(2‴)	4.53–4.55 (*m*)	72.9 (*d*)	4.52–4.54 (*m*)	72.9 (*d*)	4.52–4.54 (*m*)	72.9 (*d*)
H–C(3‴)	4.55–4.58 (*m*)	73.3 (*d*)	4.56–4.58 (*m*)	73.4 (*d*)	4.57–4.59 (*m*)	73.3 (*d*)
H–C(4‴)	4.42–4.44 (*m*)	80.5 (*d*)	4.44–4.47 (*m*)	80.5 (*d*)	4.45–4.48 (*m*)	80.5 (*d*)
H–C(5‴)	4.94–4.96 (*m*)	68.4 (*d*)	4.93–4.95 (*m*)	68.4 (*d*)	4.93–4.95 (*m*)	68.4 (*d*)
Me(6‴)	1.59 (*d*, *J* = 5.8)	18.9 (*q*)	1.59 (*d*, *J* = 6.3)	18.9 (*q*)	1.59 (*d*, *J* = 6.0)	18.9 (*q*)
Rha:						
H–C(1⁗)	6.29 (br. *s*)	103.3 (*d*)	6.29 (br. *s*)	103.4 (*d*)	6.29 (br. *s*)	103.3(*d*)
H–C(2⁗)	4.91–4.93 (*m*)	72.7 (*d*)	4.90–4.93 (*m*)	72.7 (*d*)	4.92–4.94 (*m*)	72.7 (*d*)
H–C(3⁗)	4.54–4.56 (*m*)	72.9 (*d*)	4.55–4.57 (*m*)	73.0 (*d*)	4.55–4.57 (*m*)	72.9 (*d*)
H–C(4⁗)	4.28–4.30 (*m*)	74.1 (*d*)	4.31–4.33 (*m*)	74.1 (*d*)	4.32–4.35 (*m*)	74.1 (*d*)
H–C(5⁗)	4.95–4.97 (*m*)	70.4 (*d*)	4.96–4.98 (*m*)	70.5 (*d*)	4.97–4.99 (*m*)	70.4 (*d*)
Me(6⁗)	1.77 (*d*, *J* = 6.2)	18.5 (*q*)	1.75 (*d*, *J* = 6.2)	18.5 (*q*)	1.76 (*d*, *J* = 6.1)	18.5 (*q*)

[a]) Recorded at 400 MHz. [b]) Recorded at 500 MHz.

unit at C(4′) of the inner glucopyranosyl unit, and another terminal rhamnopyranosyl unit at C(4‴) of the inner rhamnopyranosyl unit. Therefore, the structure of **1** was established as (3β,11α,25*R*)-3,11-dihydroxyspirost-5-en-12-one 3-{*O*-α-L-rhanmopyranosyl-(1 → 4)-*O*-α-L-rhanmopyranosyl-(1 → 4)-*O*-[α-L-rhanmopyranosyl-(1 → 2)]-β-D-glucopyranoside}, and named ypsilandroside M.

Compound **2** was obtained as a colorless amorphous powder. Its molecular formula $C_{51}H_{82}O_{21}$ was determined by the HR-ESI-MS (*m*/*z* 1029.5274 ($[M-H]^-$) and required eleven degrees of unsaturation. The ^{1}H-NMR spectrum of **2** (*Table*) showed signals for four steroid Me groups (δ(H) 0.67 (*d*, *J* = 5.2 Hz), 0.86 (*s*), 1.00 (*s*), and 1.15 (*d*, *J* = 7.0 Hz)), as well as signals for four anomeric H-atoms (δ(H) 4.95 (*d*, *J* = 7.5 Hz), 5.84 (br. *s*), 6.29 (br. *s*), and 6.39 (br. *s*)). Comparison of the NMR spectra of the aglycone moiety of **2** with those of diosgenin (= (3β,25*R*)-spirost-5-en-3-ol) revealed that the δ(H) and δ(C) for the rings of *A* and *C*–*F* of **2** were in good agreement with those of diosgenin, but indicated the lack of a CH_2 group and the presence of an O-bearing CH group (δ(C) 72.5 (*d*)) in ring *B* [11]. This suggested that that the CH_2 group at C(7) was oxygenated to an O-bearing CH group in **2**, which was corroborated by the ^{1}H,^{1}H correlations of the olefinic H-atom at δ(H) 5.68 (br. *s*, H–C(6)) with the proton linked to an O-bearing C-atom (δ(C) 72.5 (*d*)) at δ(H) 4.00–4.03 (*m*, H–C(7)). The location of OH–C(7) also could be confirmed by the HMBCs H–C(7)/C(5), C(6), C(8), C(9), and C(14). The β-orientation of OH–C(7) was determined by the ROESY

correlations of H–C(7)/H_{eq}–C(4), H–C(9), and H–C(14) (*Fig. 2*), which was confirmed by the chemical shift of C(7) (δ(C) 72.5), while the signal for C(7) would appear at δ(C) *ca.* 64.6 for the 7α-isomer [12–15]. Hence, the aglycone of **2** was identified as (3β,7β,25*R*)-spirost-5-ene-3,7-diol. The ^{13}C-NMR signals arising from the tetraasaccharide moiety composed of one β-D-glucopyranosyl and three α-L-rhamnopyranosyl units were in good agreement with those of **1**. On the basis of the above evidence, the structure of **2** was established as (3β,7β,25*R*)-spirost-5-ene-3,7-diol 3-{*O*-α-L-rhanmopyranosyl-(1 → 4)-*O*-α-L-rhanmopyranosyl-(1 → 4)-*O*-[α-L-rhanmopyranosyl-(1 → 2)]-β-D-glucopyranoside}, and named ypsilandroside N.



2 **3**

Fig. 2. *Key ROESY correlations for the aglycones of compounds* **2** *and* **3**

Compound **3** was isolated as colorless amorphous powder. The negative-ion HR-ESI-MS of **3** displayed a peak at *m*/*z* 1045.5199 ([*M* – H]$^-$), corresponding to the molecular formula $C_{51}H_{82}O_{22}$, which differs from that of **2** by one additional O-atom. Its ^{13}C-NMR spectrum showed 51 resonance lines which included 5 quaternary C-atoms, 29 CH groups, 10 CH_2 groups, and 7 Me groups. Among them, 27 signals were assigned to the aglycone part and 24 to four monosaccharide units. The above data revealed that **3** should be a spirostane glycoside similar to **2**. A major difference between the two molecules was the appearance of an O-bearing quaternary C-atom (δ(C) 90.3) in compound **3**. The downfield shifts of C(13) ($\Delta\delta$ + 4.0), C(16) ($\Delta\delta$ + 8.7), and C(20) ($\Delta\delta$ + 2.9) with respect to **2** and the HMBCs C(17) (δ(C) 90.3)/CH_2(12) (δ(H) 1.67–1.69 (*m*)), H–C(14) (δ(H) 3.03–3.05 (*m*)), CH_2(15) (δ(H) 2.12–2.15 and 2.72–2.74 (2*m*)), H–C(16) (δ(H) 4.55–4.58 (*m*)), Me(18) (δ(H) 1.03 (*s*)), H–C(20) (δ(H) 2.21–2.24 (*m*)), and Me(21) (δ(H) 1.25 (*d*, *J* = 7.1 Hz)) confirmed that **3** had an OH group at C(17). A second major difference was the upfield shift of C(7) (δ(C) 64.7) compared to **2** ($\Delta\delta$ – 7.8). This indicated the α-orientation of OH–C(7) which could be further confirmed by the ROESY correlations H–C(7)/H–C(8), H_{ax}–C(15), and H_{eq}–C(15) (*Fig. 2*). Thus, the structure of **3** was identified as (3β,7α,25*R*)-spirost-5-ene-3,7,17-triol 3-{*O*-α-L-rhanmopyranosyl-(1 → 4)-*O*-α-L-rhanmopyranosyl-(1 → 4)-*O*-[α-L-rhanmopyranosyl-(1 → 2)]-β-D-glucopyranoside}, and named ypsilandroside O.

Ypsilandroside N (**2**) is a rare spirostane saponin, whose aglycone contains a 7β-OH group. Only one spirostane sapogenin and three spirostane saponins, which contained 7β-OH group, were previously reported. They were isolated from *Paris pollyphylla* SMITH var. *yunnanensis* (Liliaceae) [13], *Dioscorea septemloba* (Dioscoreaceae) [15], and *Urginea sanguinea* (Hyacynthaceae) [16]. Ypsilandroside O (**3**) is the first report of a 7-hydroxylated pennogenin (=(3β,25*R*)-spirost-5-ene-3,17-diol) saponin. Since steroidal saponins are reported to possess cytotoxic activity against various cancer cell lines [1], the cytotoxic activities of compounds **1**–**3** were evaluated against five tumor

cell lines (MCF-7, SW480, A-549, HL-60, and SMMC-7721). Cisplatin and taxol were used as the positive control. The results showed that none of the tested compounds had any discernible cytotoxic activity against these cell lines ($IC_{50} > 40$ μM).

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Inc.*, P. R. China), SiO_2 *H* (10–40 μm; *Qingdao Marine Chemical Inc.*), or *Lichroprep Rp-18* (43–63 μm; *Merck*). TLC: visualization by heating SiO_2 plates sprayed with 10% H_2SO_4 in EtOH. GC: *Shimadzu-GC-2010* instrument; H_2 flame ionization detector. Semi-prep. HPLC: *Agilent-1100* apparatus; *Zorbax-SB-C-18* column (9.4 mm × 25 cm, 5 μm; *Agilent*); t_R in min. Optical rotations: *Horiba-SEAP-300* polarimeter. IR Spectra: *Bio-Rad-FTS-135* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-AM-400* (400 and 100 MHz) and *-DRX-500* (500 and 125 MHz) instruments; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. FAB-MS: *VG-Auto-Spec-3000* mass spectrometer; in *m*/*z*. ESI-MS and HR-ESI-MS: *API-QSTAR-TOF* spectrometer; in *m*/*z*.

Plant Material. The whole plants of *Y. thibetica* were collected in November 2006 from Luding County, Sichuan Province, P. R. China, and identified by Prof. *Xin-Qi Chen*, Institute of Botany, Chinese Academy of Sciences, Beijing. A voucher specimen (No. HY0002) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany.

Extraction and Isolation. The powdered air-died plants of *Y. thibetica* (10 kg) were exhaustively extracted three times with 70% EtOH (3 × 50 l) under reflux. After evaporation, the resulting residue was passed through a *YWD-3F* macroporous resin column eluted with EtOH/H_2O 0:1, 4:6, 7:3, and 1:0. The 70% EtOH fraction (70 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH/H_2O 10:1:0 → 7:3:0.5 (*v*/*v*)): *Fractions 1–4*. *Fr. 3* (10.5 g) was subjected to MPLC (*Rp-18*, MeOH/H_2O 6:4 → 8:2): *Fr. 3.1–3.3*. Subfractions of interest were purified by semi-prep. HPLC (MeOH/H_2O 65:35; flow rate 3 ml/min): **1** (9 mg; t_R 12.4), **2** (6 mg; t_R 37.0), and **3** (7 mg; t_R 9.6).

Ypsilandroside M (=*(3β,11α,25*R*)-3-[(*O*-6-Deoxy-α-*L*-mannopyranosyl-(1 → 4)*-O*-6-deoxy-α-*L*-mannopyranosyl-(1 → 4)*-O*-[6-deoxy-α-*L*-mannopyranosyl-(1 → 2)]-β-*D*-glucopyranosyl)oxy]-11-hydroxyspirost-5-en-12-one*; **1**): Colorless amorphous powder. $[\alpha]_D^{18} = -113.5$ (*c* = 0.1, MeOH). IR (KBr): 3441, 2932, 2875, 1710, 1638, 1456, 1383, 1051, 981, 919, 899, 866 (intensity: 899 > 919). ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS (neg.): 1043 ($[M-H]^-$). HR-ESI-MS: 1043.5057 ($[M-H]^-$, $C_{51}H_{79}O_{22}^-$; calc. 1043.5063).

Ypsilandroside N (=*(3β,7β,25*R*)-7-Hydroxyspirost-5-en-3-yl* O*-6-Deoxy-α-*L*-mannopyranosyl-(1 → 4)*-O*-6-deoxy-α-*L*-mannopyranosyl-(1 → 4)*-O*-[6-deoxy-α-*L*-mannopyranosyl-(1 → 2)]-β-*D*-glucopyranoside*; **2**): Colorless amorphous powder. $[\alpha]_D^{25} = -79.5$ (*c* = 0.15, $CHCl_3$/MeOH 1:1). IR (KBr): 3423, 2935, 2874, 1648, 1457, 1385, 1051, 981, 919, 910, 898, 865 (intensity: 898 > 910). ^{1}H- and ^{13}C-NMR: *Table*. FAB-MS (neg.): 1029 ($[M-H]^-$), 883 ($[M-H-C_6H_{10}O_4]^-$), 737 ($[M-H-2C_6H_{10}O_4]^-$). HR-ESI-MS: 1029.5274 ($[M-H]^-$, $C_{51}H_{81}O_{21}^-$; calc. 1029.5270).

Ypsilandroside O (=*(3β,7α,25*R*)-7,17-Dihydroxyspirost-5-en-3-yl* O*-6-Deoxy-α-*L*-mannopyranosyl-(1 → 4)*-O*-6-deoxy-α-*L*-mannopyranosyl-(1 → 4)*-O*-[6-deoxy-α-*L*-mannopyranosyl-(1 → 2)]-β-*D*-glucopyranoside*; **3**): Colorless amorphous powder. $[\alpha]_D^{25} = -103.3$ (*c* = 0.30, $CHCl_3$/MeOH 1:1). IR (KBr): 3417, 2932, 2876, 1664, 1455, 1384, 1053, 980, 954, 913, 896, 867 (intensity: 896 > 913). ^{1}H- and ^{13}C-NMR: *Table*. FAB-MS (neg.): 1046 (M^-), 900 ($[M-C_6H_{10}O_4]^-$), 754 ($[M-2C_6H_{10}O_4]^-$). HR-ESI-MS: 1045.5199 ($[M-H]^-$, $C_{51}H_{81}O_{22}^-$; calc. 1045.5219).

Acid Hydrolysis and GC Analysis. Each compound **1**–**3** (2 mg) was refluxed in 4M CF_3COOH/dioxane 1:1 ((*v*/*v*), 2 ml) on a water bath for 4 h. After cooling, the mixture was extracted with $CHCl_3$

(3 × 5 ml). The aq. layer was concentrated with MeOH until neutral. The dried residue was dissolved in 1 ml anh. pyridine and treated with L-cysteine methyl ester hydrochloride (1.5 mg) under stirring at 60° for 1 h. Then 1-(trimethylsilyl)-1*H*-imidazole (1.0 ml) was added and the mixture kept at 60° for 30 min. The supernatant (4 µl) was analyzed by GC (H_2 flame ionization detector; *30QC2/AC-5* quartz capillary column (30 m × 0.32 mm); column temp. 180–280°, heating rate 3°/min; carrier gas N_2 (1 ml/min); injector temp. 250°; split ratio 1:50). The configurations of D-glucose and L-rhamnose (= 6-deoxy-L-mannose) for compounds **1**–**3** were determined by comparison of the retentions times of the corresponding derivatives with those of standard D-glucose and L-rhamnose, giving a single peak at 19.01 and 15.43 min, resp.

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