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Steroidal and pregnane glycosides from *Ypsilandra thibetica*

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Abstract: The whole plants of *Ypsilandra thibetica* have been analyzed as part of a systematic study on saponin constituents of medicinal plants. This has resulted in the isolation of two new bisdesmosidic furostanol saponins, named ypsilandroside P (**1**) and ypsilandroside Q (**2**), and one new pregnane glycoside, named ypsilandroside R (**3**), together with nine known steroidal glycosides. Their structures were elucidated on the basis of extensive spectroscopic analysis, including that of 2D NMR data, and the results of acidic hydrolysis. Ypsilandroside P (**1**) was cytotoxicity against two human tumor cell lines.

Keywords: Ypsilandra thibetica, Liliaceae, furostanol glycoside, pregnane glycoside, ypsilandroside

Introduction

In a continuation of our study on saponin constituents of medicinal plants, we have examined the saponin riched fraction prepared from the EtOH extract of the air-dried whole plants of *Ypsilandra thibetica* (Liliaceae). This perennial plant distributes in southwestern China and has been used as hemostatic agent in Chinese folk medicine.[1,2] In our recent study, we found that this species was a rich source of steroidal saponins. Two sapogenin, 22 spirostanol saponins, and two C-22 steroidal lactone glycosides were obtained from the title plants.[3–6] Further phytochemical investigation has been carried out on this species, with particular attention to the steroidal glycoside constituents, and has resulted in the isolation of two new bisdesmosidic furostanol saponins (**1** and **2**) and one new pregnane glycoside (**3**), together with nine known steroidal glycosides: protoprogenin II (**4**),[7] proto-Pb (**5**),[8] saponin Th (**6**),[9] pseudoproto Pb (**7**),[10] pregnane 5,16-dien-3*β*-ol-20-oxo 3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (**8**),[11] smilaxchinoside B (**9**),[12] parispseudoside C (**10**),[13] parispseudoside A (**11**),[13] and 26-*O*-*β*-D-glucopyranosyl-3*β*,26-dihydroxy-20,22-*seco*-25(*R*)-furost-5-en-20,22-dione 3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (**12**).[14] This paper reports the isolation, structural determination, and cytotoxic activity of these glycosides.

Results and Discussion

Compound **1**, obtained as a white amorphous powder, gave a pseudo-molecular ion peak $[M - H]^-$ at *m/z* 1207.5736 (calcd. 1207.5747) in its HRESIMS. Combined with ^{13}C NMR spectroscopic data (Table 2), its molecular formula was

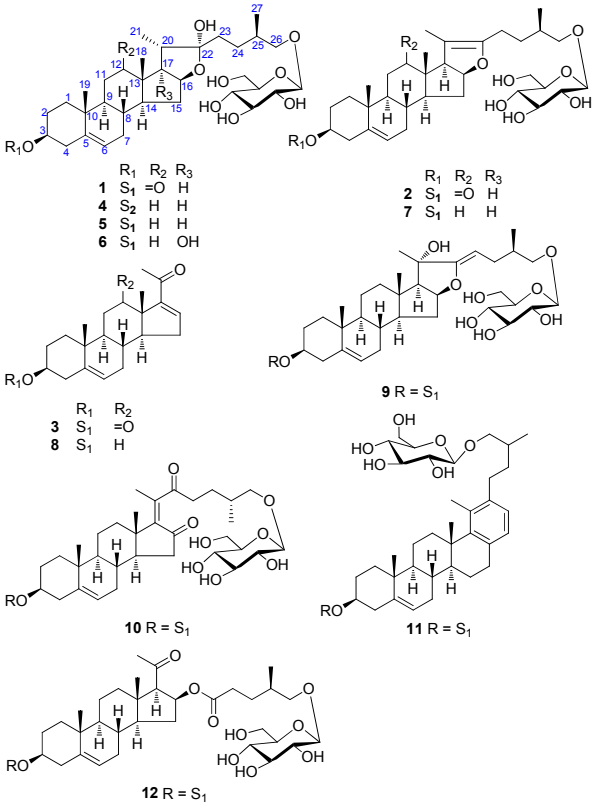

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Table 1. ^{1}H NMR spectral data for compounds 1–3 (δ in ppm, J in Hz, C_5D_5N)[a]

Pos.	1[b]	2[c]	3[c]	Pos.	1[b]	2[c]	3[c]
1a	1.96, m	1.53, m	1.47, m	Glc-1′	4.93, d (7.2)	4.91, d (7.9)	4.93, d (7.4)
1b	0.89, m	0.89, m	0.87, m	2′	4.18, m	4.20, m	4.21, m
2a	2.00, m	2.00, m	1.63, m	3′	4.20, m	4.23, m	4.20, m
2b	1.78, m	1.88, m	1.41, m	4′	4.39, m	4.41, m	4.40, m
3	3.81, m	3.82, m	3.81, m	5′	3.60, br. d (9.1)	3.61, m	3.59, br. s
4a	2.80, m	2.81, dd (11.0, 2.4)	2.82, dd (13.3, 1.8)	6′a	4.17, d (14.2)	4.17, d (12.3)	4.18, m
4b	2.68, m	2.68, t (11.0)	2.68, dd (12.3, 5.9)	6′b	4.02, m	4.04, m	4.03, d (11.1)
6	5.22, br. s	5.28, br. s	5.30, br. s	Rha-1″	6.41, br. s	6.41, br. s	6.41, br. s
7a	1.87, m	1.89, m (2H)	1.90, m	2″	4.86, m	4.85, m	4.85, m
7b	1.43, m		1.52, m	3″	4.62, m	4.63, m	4.63, m
8	1.85, m	1.79, m	1.86, m	4″	4.35, m	4.37, m	4.36, m
9	1.30, m	1.32, m	1.38, m	5″	4.93, m	4.95, m	4.94, m
11a	2.56, t (13.5)	2.53, t (14.0)	2.64, t (13.5)	6″	1.58, d (5.2)	1.59, d (5.9)	1.59, d (5.6)
11b	2.30, dd (14.4, 5.6)	2.31, dd (14.5, 5.7)	2.25, dd (13.5, 6.3)	Rha-1‴	5.84, br. s	5.84, br. s	5.83, br. s
14	1.41, m	1.23, m	1.58, m	2‴	4.51, m	4.51, m	4.53, m
15a	2.09, m	2.20, m	2.25, m	3‴	4.55, m	4.56, m	4.55, m
15b	1.64, m	1.67, m	2.07, m	4‴	4.44, m	4.45, m	4.43, m
16	4.88, m	4.84, m	6.54, s	5‴	4.92, m	4.93, m	4.93, m
17	2.94, t (7.5)	3.45, d (10.2)		6‴	1.58, d (5.2)	1.59, d (5.9)	1.59, d (5.6)
18	1.15, s	0.95, s	1.33, s	Rha-1⁗	6.29, br. s	6.30, br. s	6.29, br. s
19	1.06, s	1.07, s	1.07, s	2⁗	4.90, m	4.90, m	4.90, m
20	2.20, dd (13.2, 6.5)			3⁗	4.52, m	4.53, m	4.53, m
21	1.53, d (6.7)	1.74, s	2.32, s	4⁗	4.30, m	4.31, m	4.31, m
23a	2.05, m	2.20, m (2H)		5⁗	4.36, m	4.38, m	4.36, m
23b	1.52, m			6⁗	1.75, d (6.1)	1.76, d (6.2)	1.76, d (6.2)
24a	2.04, m	1.89, m		Glc-1⁗′	4.80, d (7.7)	4.84, d (7.8)	
24b	1.66, m	1.45, m		2⁗′	3.60, br. d (9.1)	3.60, dd (9.4, 5.4)	
25	1.92, m	1.93, m		3⁗′	4.24, m	4.26, m	
26a	3.94, dd (9.1, 7.1)	3.95, dd (9.4, 7.1)		4⁗′	4.21, m	4.25, m	
26b	3.60, dd (9.1, 5.8)	3.59, dd (9.4, 5.4)		5⁗′	3.94, m	3.96, m	
27	0.97, d (6.5)	1.01, d (6.6)		6⁗′a	4.54, m	4.58, m	
				6⁗′b	4.38, m	4.40, m	

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

determined as $C_{57}H_{92}O_{27}$. The ^{1}H NMR spectrum of **1** (Table 1) showed signals of four steroid methyl groups at δ_H 0.97 (3H, d, J = 6.5 Hz, Me-27), 1.06 (3H, s, Me-19), 1.15 (3H, s, Me-18), and 1.53 (3H, d, J = 6.7 Hz, Me-21), an olefinic proton at δ_H 5.22 (1H, br. s), as well as signals for five anomeric proton signals at δ_H 4.80 (1H, d, J = 7.7 Hz, H-1⁗′), 4.93 (1H, d, J = 7.2 Hz, H-1′), 5.84 (1H, br. s, H-1‴), 6.29 (1H, br. s, H-1⁗), and 6.41 (1H, br. s, H-1″). The three methyl carbon signals at δ_C 18.7, 18.9, and 18.5 and their corresponding proton signals at δ_H 1.58 (3H, d, J = 5.2 Hz, H-6″), 1.58 (3H, d, J = 5.2 Hz, H-6‴), and 1.75 (3H, d, J = 6.1 Hz, H-6⁗) indicated that **1** had three deoxy sugars. The monosaccharides of the acidic hydrolysate of **1** were identified as D-glucose and L-rhamnose on the basis of GC analysis and comparison with authentic standards. The above ^{1}H NMR and chemical data, together with an acetalic carbon signal at δ_C 110.9 in the ^{13}C NMR spectrum[15] and a positive coloration with Ehrlich reagent,[16,17] indicated **1** to be a furostanol saponin with up to five monosaccharides. A comparison of the ^{1}H and ^{13}C spectroscopic signals of the aglycone moiety of **1** with Proto-Pb (**5**)[8] indicated that the signals were similar except for the presence of a carbonyl group (δ_C 213.1). In the HMBC spectrum (Figure 1), the long-range correlations from δ_H 1.15 (Me-18, s) to δ_C 213.1 (C-12, s), 55.4 (C-13, s), 56.0 (C-14, d), and 54.9 (C-17, d) indicated that the carbonyl group was attached at C-12 of the aglycone of **1**. The configuration of the methyl group at C-25 is *R* on the basis of the proton signals of C-26 at δ_H 3.94 (1H, 26-H$_a$) and 3.60 (1H, 26-H$_b$), and the difference (Δ_{ab}) of the proton signals at C-26 was 0.34.[18,19] From the above evidence, the aglycone of **1** was identified as (25*R*)-furost-5-en-3β,22α,26-triol-12-one.

Comparison of the carbon chemical shift thus assigned with

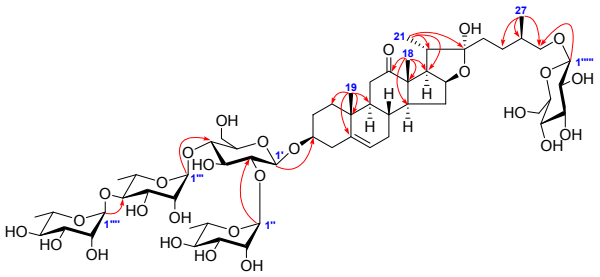

Figure 1. Selected HMBC correlations of conpound **1**.

those of the reference methyl glycosides,[15,19] taking into account the known effects of *O*-glycosylation, indicated that **1** contained a terminal β-D-glucopyranosyl units, two terminal α-L-rhamnopyranosyl unit, a C-4 substituted α-L-rhamnopyranosyl unit, and a C-2 and C-4 disubstituted β-D-glucopyranosyl unit. The β-configuration of the anomeric protons of the glucopyranosyl residue were assigned based on its $J_{1H\text{-}2H}$ value (J = 7.2~7.7 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.[20,21] In the HMBC spectrum, a correlation peak between the signals at δ_H 4.80 (H-1 of terminal glucosyl) and δ_C 75.4 (C-26 of aglycon) implied that one glucose unit was attached at C-26 of the aglycon, which is a structural feature most frequently encountered in the plant furostanol saponins.[15] Consequently, a tetraglycoside was assumed to be located at C-3 of the aglycon. The sequence of the tetrasaccharide, which was the same as the known compounds **5**–**12**, was established from the further HMBC correlations: H-1′ (δ_H 4.93) of Glc with C-3 (δ_C 77.8) of the aglycone, H-1″ (δ_H 6.41) of 2′-Rha

Table 2. ^{13}C NMR spectral data for compounds 1–3 (C_5D_5N)

Pos.	1[a]	2[b]	3[b]	Pos.	1[a]	2[b]	3[b]
1	37.2, CH_2	37.1, CH_2	37.0, CH_2	Glc-1′	100.4, CH	100.4, CH	100.4, CH
2	30.0, CH_2	30.0, CH_2	29.9, CH_2	2′	78.0, CH	77.9, CH	78.0, CH
3	77.8, CH	77.9, CH	77.9, CH	3′	77.7, CH	77.7, CH	77.8, CH
4	38.8, CH_2	38.8, CH_2	38.8, CH_2	4′	77.8, CH	77.8, CH	77.9, CH
5	140.6, C	140.7, C	141.1, C	5′	77.1, CH	77.1, CH	77.1, CH
6	121.7, CH	121.6, CH	121.5, CH	6′	61.2, CH_2	61.3, CH_2	61.3, CH_2
7	31.9, CH_2	31.9, CH_2	31.4, CH_2	Rha-1″	102.3, CH	102.2, CH	102.2, CH
8	31.0, CH	30.8, CH	30.2, CH	2″	72.6, CH	72.5, CH	72.5, CH
9	52.4, CH	52.4, CH	53.8, CH	3″	72.7, CH	72.6, CH	72.7, CH
10	37.7, C	37.8, C	38.0, C	4″	74.2, CH	74.1, CH	74.2, CH
11	37.6, CH_2	37.7, CH_2	37.8, CH_2	5″	69.5, CH	69.6, CH	69.6, CH
12	213.1, C	212.8, C	209.2, C	6″	18.7, CH_3	18.7, CH_3	18.7, CH_3
13	55.4, C	57.2, C	61.3, C	Rha-1‴	102.3, CH	102.3, CH	102.3, CH
14	56.0, CH	54.4, CH	56.2, CH	2‴	72.9, CH	72.9, CH	72.9, CH
15	31.9, CH_2	34.0, CH_2	31.8, CH_2	3‴	73.4, CH	73.3, CH	73.3, CH
16	79.8, CH	83.1, CH	142.8, CH	4‴	80.5, CH	80.4 , CH	80.4, CH
17	54.9, CH	56.1, CH	150.7, C	5‴	68.4, CH	68.4, CH	68.4, CH
18	16.1, CH_3	14.0, CH_3	16.4, CH_3	6‴	18.9, CH_3	18.9, CH_3	18.8, CH_3
19	19.0, CH_3	18.9, CH_3	18.9, CH_3	Rha-1⁗	103.4, CH	103.3, CH	103.3, CH
20	41.4, CH	103.1, C	196.0, C	2⁗	72.7, CH	72.6, CH	72.5, CH
21	15.3, CH_3	11.6, CH_3	27.7, CH_3	3⁗	72.9, CH	72.9, CH	72.9, CH
22	110.9, C	153.2, C		4⁗	74.0, CH	74.0, CH	74.1, CH
23	37.1, CH_2	23.8, CH_2		5⁗	70.5, CH	70.4, CH	70.5, CH
24	28.4, CH_2	31.4, CH_2		6⁗	18.5, CH_3	18.5, CH_3	18.5, CH_3
25	34.3, CH	33.6, CH		Glc-1⁗′	105.0, CH	105.0, CH	
26	75.4, CH_2	75.2, CH_2		2⁗′	75.3, CH	75.0, CH	
27	17.5, CH_3	17.3, CH_3		3⁗′	78.7, CH	78.6, CH	
				4⁗′	71.7, CH	71.7, CH	
				5⁗′	78.6, CH	78.5, CH	
				6⁗′	62.9, CH_2	62.9, CH_2	

[a]Recorded at 100 MHz; [b]Recorded at 125 MHz.

with C-2′ (δ_C 78.0) of Glc, H-1‴ (δ_H 5.84) of 4′-Rha with C-4′ (δ_C 77.8) of Glc, and H-1⁗ (δ_H 6.29) of 4″-Rha with C-4‴ (δ_C 80.5) of 4′-Rha. Therefore, **1** was determined to be 26-*O*-*β*-D-glucopyranosyl-(25*R*)-3*β*,22*α*,26-trihydroxyfurost-5-en-12-one 3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside, and named ypsilandroside P.

Compound **2** displayed a $[M - H]^-$ ion at *m/z* 1189.5645 (calcd. for $C_{57}H_{89}O_{26}$, 1189.5642) in the HRESIMS and gave a red color with Ehrlich's reagent. The NMR spectral data suggested **2** is a furostanol saponin closely related to **1**. It differed from **1** in the presence of one more olefinic functionality [δ_C 153.2 (s) and 103.1 (s)] in addition to the 5(6)-en group. Furthermore, the Me-21 methyl doublet signal observed at δ_H 1.53 (J = 6.7 Hz) in the ^{1}H NMR spectrum of **1** was absent from **2**, but was replaced by a methyl singlet at δ_H 1.74. These data were suggestive of **2** being the corresponding $\Delta^{20(22)}$-furostanol saponin of **1,** which was confirmed by the mass difference of *m/z* = 18 and HMBC correlations. In the HMBC spectrum of **2**, the correlations of Me-21 (δ_H 1.74) with C-17 (δ_C 56.1), C-20 (δ_C 103.1), and C-22 (δ_C 153.2) were observed. Thus, the structure of **2** was established as 26-*O*-*β*-D-*β*-D-glucopyranosyl-(25*R*)-3*β*,26-dihydroxyfurost-5,20(22)-diene-12-one 3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside, and named ypsilandroside Q.

Compound **3** had a molecular formula of $C_{45}H_{68}O_{20}$, established by analysis of HRESIMS (*m/z* 963.3953 $[M + Cl]^-$, calcd. 963.3992) and ^{13}C NMR spectrum (45 signals). The ^{1}H NMR spectrum of **3** displayed two three-proton singlet signals at δ_H 1.07 (s) and 1.33 (s), indicating the presence of two angular methyl groups, and a methyl singlet at δ_H 2.32 (s) attached to a deshielding moiety, as well as four anomeric proton signals at δ_H 4.93 (1H, d, J = 7.4 Hz), 5.83 (1H, br. s), 6.29 (1H, br. s), and 6.41 (1H, br. s). The existence of an *α*,*β*-unsaturated carbonyl group was verified by the IR (1657 cm^{-1}), UV [227 nm (log*ε* 2.8)], and ^{13}C NMR [δ_C 196.0 (C=O), 150.7 (C), and 142.8 (CH)] spectra. These spectral data and comparison with those of the known compound **8**[11] indicated that **3** differed from **8** by the presence of a carbonyl group (δ_C 209.2) instead of a methylene moiety at C-12 in the latter. The HMBC correlations of δ_H 1.33 (Me-18) with δ_C 209.2 (C-12, s), 61.3 (C-13, s), 56.2 (C-14, d), 150.7 (C-17, s) indicated that the location of the carbonyl group at C-12. Thus, the aglycone of **3** was identified as 3*α*-hydroxypregna-5,16-dien-12,20-dione. The ^{1}H and ^{13}C NMR shifts of the tetraglycoside moiety linked to C-3 of the pregnane were superimposable on those of **1**, **2**, and **5**–**12**. On the basis of all the information above, the structure of **3** was characterized as pregnane 5,16-dien-3*β*-ol-12,20-dione 3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside, and named ypsilandroside R.

The cytotoxic activities of saponins **1**, **2**, and **12** against the growth of human tumor cell lines (A549 and HL-60) were evaluated. The results indicated that only compound **1** showed 86.4% inhibition to A549 cell lines and 75.9% inhibition to HL-60 cell lines at the tested concentration (10 μM).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco P-1020 automatic digital polarimeter. UV spectra were measured using a Shimadzu UV-2401PC spectrophotometer. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were run on Bruker AM-400 and DRX-500 instruments with TMS as

internal standard. 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 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 200nm 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.

Plant Material. The plant material of *Y. thibetica* was collected in November 2006 from Luding County, Sichuan Province, 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.

Extraction and Isolation. The air-dried 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 loaded onto a macroporous resin column (YWD-3F) and eluted successively with H_2O, 40% EtOH (F1 fraction), 70% EtOH (F2 fraction), and 95% EtOH (F3 fraction), respectively. The 40% EtOH elutes were evaporated to dryness. Fraction F1 (33 g) was fractioned by silica gel column and eluted with a gradient of $CHCl_3$-MeOH-H_2O (8:2:0.2→7:3:0.5, v/v) to get two subfractions (F11 and F12). Fraction F11 was subjected to column chromatography on Rp-18 gel (MPLC, MeOH-H_2O 4:6→6.5:3.5) and semi-preparative HPLC (MeOH-H_2O 38:62 v/v; flow rate: 3 mL.min^{-1}) to obtain **3** (14 mg), **4** (28 mg), and **8** (19 mg). Fraction F12 was chromatographed over Rp-18 gel (MPLC, MeOH-H_2O 3:7→7:3) and semi-preparative HPLC (MeCN-H_2O 20:80→35:65 v/v; flow rate: 3 mL.min^{-1}) to yield **1** (17 mg), **2** (8 mg), **5** (43 mg), **6** (80 mg), **7** (23 mg), **9** (20 mg), **10** (17 mg), **11** (18 mg), and **12** (24 mg).

Ypsilandroside P (1): white amorphous powder; $[\alpha]_D^{24}$ −65.0 (*c* 0.26, MeOH); IR (KBr) ν_{max} 3431, 2934, 1706, 1640, 1453, 1381, 1130, 1044, 985, 911, 839, 804 cm^{-1} (intensity: 839 > 911); ^{1}H and ^{13}C NMR data see Tables 1 and 2; negative FABMS *m/z* 1208 $[M]^-$, 1062 $[M - 146]^-$, 915 $[M - H - 2 \times 146]^-$, 769 $[M - H - 3 \times 146]^-$; negative ion HRESIMS *m/z* 1207.5736 (calcd. for $C_{57}H_{91}O_{27}$ $[M - H]^-$, 1207.5747).

Ypsilandroside Q (2): white amorphous powder; $[\alpha]_D^{24}$ −66.8 (*c* 0.47, MeOH); IR (KBr) ν_{max} 3426, 2933, 1707, 1640, 1453, 1382, 1131, 1043, 984, 911, 841, 804 cm^{-1} (intensity: 841 > 911); ^{1}H and ^{13}C NMR data see Tables 1 and 2; negative FABMS *m/z* 1190 $[M]^-$, 1043 $[M - H - 146]^-$, 897 $[M - H - 2 \times 146]^-$; negative ion HRESIMS *m/z* 1189.5645 (calcd. for $C_{57}H_{89}O_{26}$ $[M - H]^-$, 1189.5642).

Ypsilandroside R (3): white amorphous powder; $[\alpha]_D^{22}$ −58.3 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 227 (2.8) nm; IR (KBr) ν_{max} 3418, 2934, 1713, 1657, 1376, 1132, 1053, 983 cm^{-1}; ^{1}H and ^{13}C NMR data see Tables 1 and 2; negative FABMS *m/z* 927 $[M - H]^-$, 781 $[M - H - 146]^-$, 635 $[M - H - 2 \times 146]^-$; negative ion HRESIMS *m/z* 963.3953 (calcd. for $C_{45}H_{68}O_{20}Cl$ $[M + Cl]^-$, 963.3992).

Acid Hydrolysis of Compounds 1–3 and GC Analysis. Compounds **1**–**3** (4 mg each) were refluxed with 4 M TFA-dioxane (1:1 v/v, 2 mL) on water bath for 4h. 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).

Cell-Growth Inhibition Assay. Growth inhibition of compounds on tumor cells was determined by microculture 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium hydrobromide (MTT) assay.[22]

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/ 10.1007/s13659-011-0039-z and is accessible for authorized users.

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