Five New Steroidal Compounds from *Ypsilandra thibetica*

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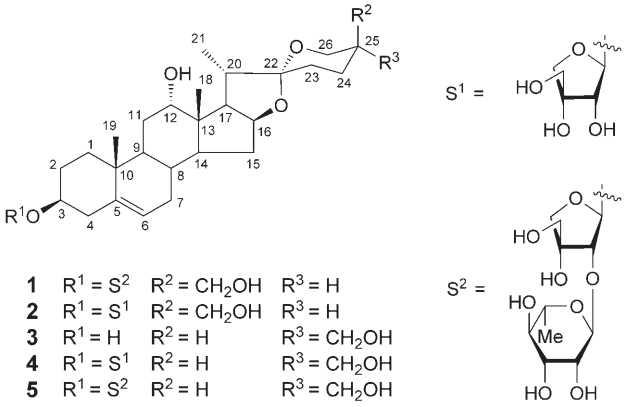
Five new steroids, including the four new steroidal saponins ypsilandroside B (**1**), ypsilandroside A (**2**), isoypsilandroside A (**4**), and isoypsilandroside B (**5**), as well as the new steroidal sapogenin isoypsilandrogenin (**3**), were isolated from the whole plant of *Ypsilandra thibetica* FRANCH. Their structures were determined on the basis of spectroscopic analyses, including extensive 2D-NMR techniques. The structure and relative configuration of ypsilandroside B (=(3*β*,12*α*,25*R*)-3-[(*α*-L-rhamnopyranosyl-(1→2)-*β*-D-apiofuranosyl)oxy]spirost-5-ene-12,27-diol; **1**) was unequivocally determined by single-crystal X-ray diffraction. The antifungal and antibacterial activities of **1**–**5** towards various types of fungi and bacteria are reported.

Introduction. – *Ypsilandra thibetica* (Melanthiaceae), mainly distributed in Southwest China [1], has been used in traditional Chinese medicine (TCM) as a haemostatic, especially in Sichuan and Yunnan Provinces [2]. The anticancer activities of furostane saponins from the whole plants of *Y. thibetica* were reported [3].

In continuation of our search for new secondary metabolites of *Y. thibetica*, we herein report five new compounds, ypsilandroside B (**1**), ypsilandroside A (**2**), ypsilandrogenin (**3**), isoypsilandroside A (**4**), and isoypsilandroside B (**5**). Compounds **1** and **5** are rare steroidal saponins containing a disaccharide in which an apiofuranosyl (Api) group is directly attached to C(3) of the aglycone.

Results and Discussion. – 1. *Structure Elucidation.* The whole plant of *Y. thibetica* was extracted with 70% aq. EtOH at reflux. The EtOH extract was evaporated, and the residue was taken up in H_2O and re-extracted with petroleum ether (PE) and AcOEt successively. The AcOEt extract was repeatedly chromatographed over silica gel, *RP-18* gel, and further purified by semipreparative HPLC on C_{18} silica gel to afford **1**–**5**.

Ypsilandroside B (**1**) was obtained as colorless needles. HR-ESI-MS showed the $[M-H]^-$ ion peak at *m/z* 723.3937 (calc. 723.3955), in accord with the molecular formula $C_{38}H_{60}O_{13}$. Negative-ion FAB-MS of **1** gave the quasi-molecular-ion at *m/z* 723, and other significant peaks at 577 ($[M-H-C_6H_{10}O_4)]^-$), 445 ($[M-H-C_6H_{10}O_4-C_5H_8O_4]^-$), suggesting the presence of hexosyl and pentosyl units. Acid



1 $R^1 = S^2$ $R^2 = CH_2OH$ $R^3 = H$
2 $R^1 = S^1$ $R^2 = CH_2OH$ $R^3 = H$
3 $R^1 = H$ $R^2 = H$ $R^3 = CH_2OH$
4 $R^1 = S^1$ $R^2 = H$ $R^3 = CH_2OH$
5 $R^1 = S^2$ $R^2 = H$ $R^3 = CH_2OH$

hydrolysis of **1** on TLC plates produced L-rhamnose (L-Rha) and D-apiose (D-Api)[1]), as deduced by comparison with authentic samples. The ^{1}H-NMR spectrum of the aglycone of **1** revealed three Me groups [δ(H) 1.16 (*d*, $J = 7$ Hz), 0.94 (*s*), 0.99 (*s*)], two oxymethylenes [δ(H) 3.97–4.00 (*m*), 4.14–4.16 (*m*), 3.95–3.97 (*m*), 4.12 (*d*, $J =$ 5.0 Hz)], three oxymethines [δ(H) 3.69–3.71 (*m*), 3.97 (br. *s*), 4.47–4.49 (*m*)], and one olefinic H-atom [δ(H) 5.30 (br. *s*)]. The ^{13}C-NMR data (*Table*) showed a characteristic acetal signal at δ(C) 110.0 (*s*), suggesting a hydroxylated spirostane skeleton. The DEPT spectrum of the aglycone moiety of **1** also exhibited olefinic signals at δ(C) 141.0 (*s*) and 122.1 (*d*), characteristic of an unsaturation at C(5) [4].

By careful analysis of the COSY correlations of the aglycone of **1**, the following three spin systems were established: the first only contained *A*-ring resonances; the second was a large segment starting from ring *B* and extending to ring *E*; and the last included *F*-ring H-atoms. These three substructures were connected by analysis of the following HMBC correlations (see *Fig. 1*): H–C(6) (δ(H) 5.30) with C(4) (δ(C) 39.4), C(8) (δ(C) 32.1), and C(10) (δ(C) 36.9); Me(18) (δ(H) 0.94) with C(1) (δ(C) 37.4), C(5) (δ(C) 141.0), C(9) (δ(C) 44.4), and C(10) (δ(C) 36.9); Me(21) (δ(H) 1.16) with C(17) (δ(C) 53.8) and C(22) (δ(C) 110.0); and CH_2(23) (δ(H) 1.53, 1.94) with C(20) (δ(C) 42.8) and C(22) (δ(C) 110.0).

The ^{1}H- and ^{13}C-NMR chemical shifts arising from rings *A* – *E* of the aglycone of **1** were in good agreement with those of heloniogenin [5], suggesting the same partial structure, with β- and α-orientation of the 3- and 12-OH groups, respectively, as in heloniogenin. A HMBC correlation between Me(18) (δ(H) 0.94) and C(12) (δ(C) 71.5), and NOE correlations (*Fig. 1*) of H–C(12) (δ(H) 3.97) with Me(18), and of H–C(3) (δ(H) 3.69–3.71) with H_α–C(1) (δ(H) 1.03) confirmed this assumption. With respect to the *F*-ring, the presence of an oxymethylene signal [δ(H) 3.95–3.97, 4.12; δ(C) 61.6] and the disappearance of the characteristic Me(27) *doublet* strongly implied hydroxylation at C(27). This was confirmed by the HMBC correlations between CH_2(27) (δ(H) 3.95–3.97, 4.12) and C(24) (δ(C) 21.8), C(25) (δ(C) 36.4), and C(26) (δ(C) 60.8).

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[1]) *IUPAC* Name for Apiofuranose: 3-*C*-(hydroxymethyl)-*erythro*-furanose.

Table. *^{13}C-NMR Data of* **1**–**5**. In C_5D_5N solution at 125 MHz (**1** and **4**) or at 100 MHz (**2**, **3**, **5**); δ in ppm. Assignments based on DEPT, HMQC, and HMBC correlations.

Position	**1**	**2**	**3**	**4**	**5**	Position	**1**	**2**	**4**	**5**
1	37.4	37.4	37.7	37.4	37.4	Api:				
2	30.3	30.3	32.4	30.3	30.3	1′	107.0	108.4	108.4	107.0
3	77.7	78.0	71.3	78.0	77.7	2′	82.5	77.5	77.5	82.4
4	39.4	39.3	43.6	39.4	39.4	3′	80.5	80.2	80.2	80.6
5	141.0	141.0	142.1	141.0	140.9	4′	74.9	74.8	74.8	74.9
6	122.1	121.9	121.3	121.9	122.1	5′	65.9	65.4	65.4	65.9
7	32.4	32.4	32.6	32.3	32.4	Rha:				
8	32.1	32.0	32.1	32.0	32.0	1″	102.0			102.0
9	44.4	44.4	44.5	44.3	44.4	2″	72.0			72.0
10	36.9	36.9	36.8	36.9	36.9	3″	72.7			72.7
11	29.4	29.3	29.5	29.3	29.4	4″	74.1			74.1
12	71.5	71.4	71.5	71.4	71.4	5″	70.3			70.3
13	45.1	45.1	45.1	45.1	45.1	6″	18.8			18.8
14	48.3	48.2	48.4	48.3	48.3					
15	32.4	32.4	32.4	32.4	32.4					
16	81.2	81.2	81.1	81.1	81.2					
17	53.8	53.8	53.9	53.9	53.9					
18	17.3	17.3	17.3	17.3	17.3					
19	19.4	19.4	19.5	19.4	19.4					
20	42.8	42.8	42.4	42.4	42.4					
21	14.9	14.9	15.0	15.0	15.1					
22	110.0	109.9	109.8	109.8	109.8					
23	27.4	27.4	31.7	31.6	31.7					
24	21.8	21.7	24.2	24.1	24.2					
25	36.4	36.3	39.3	39.3	39.3					
26	60.8	60.7	64.1	64.1	64.1					
27	61.6	61.5	64.5	64.5	64.5					

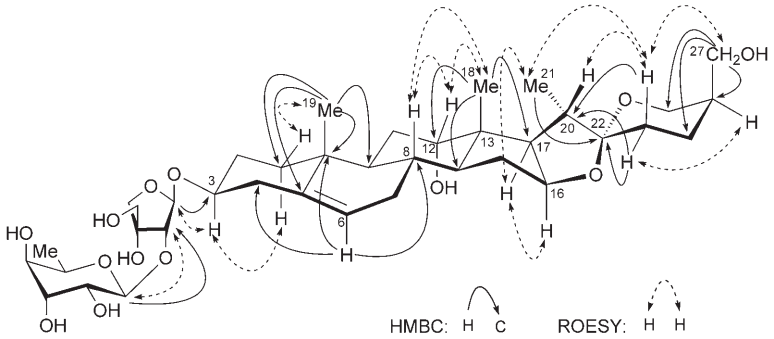

Fig. 1. *Key HMBC and NOE correlations of* **1**

The configuration at C(25) was determined by a ROESY experiment. The (*R*)-configuration was apparent from the NOE interactions of H_β–C(23) (δ(H) 1.94) with H–C(20) (δ(H) 1.97), Me(21) (δ(H) 1.16), and CH_2(27) (δ(H) 3.95–3.97, 4.12), and

of H_α–C(23) (δ(H) 1.53) with H–C(25) (δ(H) 1.92). Therefore, the aglycone of **1** was (3β,12α,25*R*)-spirost-5-ene-3,12,27-triol.

The sequence of sugars and binding sites to the aglycone of **1** were determined by HMBC experiments. The ^{1}H- and ^{13}C-NMR data indicated an α-configured L-Rha and a β-configured D-Api unit. In the HMBC spectra (*Fig. 1*), correlations of δ(H) 5.71 (H–C(1′)) with δ(C) 77.7 (C(3)), and of δ(H) 5.84 (H–C(1″)) with δ(C) 82.5 (C(2′)) were observed. Therefore, the structure of ypsilandroside B (**1**) was established as (3β,12α,25*R*)-3-[(α-L-rhamnopyranosyl-(1→2)-β-D-apiofuranosyl)oxy]spirost-5-ene-12,27-diol. Finally, the structure and relative configuration of **1** were unequivocally corroborated by single-crystal X-ray diffraction (*Fig. 2*).

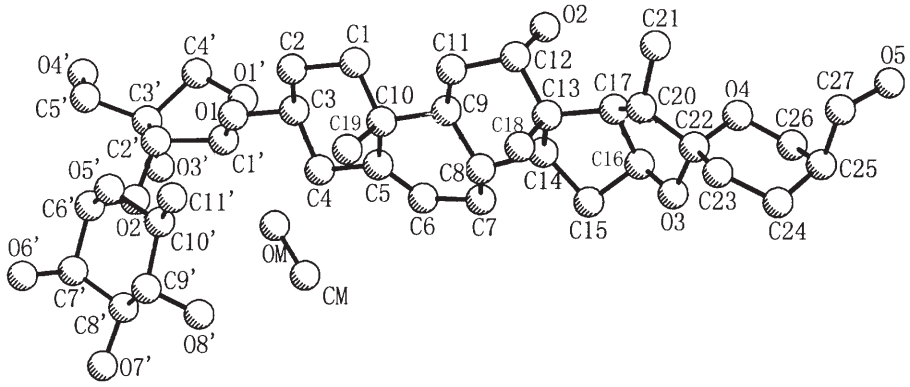

Fig. 2. *X-Ray Crystal Structure of* **1**

Ypsilandroside A (**2**) was obtained as colorless plates. HR-ESI-MS Analysis revealed the molecular formula $C_{32}H_{50}O_9$ (*m*/*z* 577.3367 ($[M-H]^-$); calc. 577.3376), which is 146 mass units lower than that of **1**. The ^{1}H- and ^{13}C-NMR data of **2** were almost identical with those of **1**, except that there was only one sugar unit in compound **2**. This was also confirmed by the resonance for C(2′) of the D-Api unit, which was shifted upfield to δ(C) 77.5. On the basis of this evidence, ypsilandroside A (**2**) was identified as (3β,12α,25*R*)-3-[(β-D-apiofuranosyl)oxy]spirost-5-ene-12,27-diol.

Isoypsilandrogenin (**3**) was obtained as colorless plates. HR-ESI-MS of **3** showed the $[M+H]^+$ ion peak at *m*/*z* 447.3104 (calc. 447.3110), corresponding to the molecular formula $C_{27}H_{42}O_5$. The ^{13}C-NMR data of **3** showed characteristic signals of a spirost-5-ene derivative [6] at δ(C) 142.1 (*s*, C(5)), 121.3 (*d*, C(6)), and 109.8 (*s*, C(22)). There was no sugar present in compound **3**. The EI mass spectrum showed the M^+ signal at *m*/*z* 446, which is 32 mass units above that for diosgenin [7]. Thus, compound **3** had two OH groups more than diosgenin. The base peak at *m*/*z* 155 ($[C_9H_{15}O_2]^+$) in the EI mass spectrum suggested the presence of an OH group in the *F*-ring. The ^{1}H- and ^{13}C-NMR chemical shifts of **3** and of the aglycone of **1** were very similar regarding rings *A*–*E*. However, the ^{13}C-NMR resonances of the *F*-ring of **3** were shifted downfield. Thus, it was reasonable to assume a different configuration at C(25). In a ROESY experiment, NOE correlations of H_β–C(23) with H–C(20) and H–C(25) were observed, which

confirmed the above assumption. Thus, isoypsilandrogenin (**3**) was identified as (3*β*,12*α*,25*S*)-spirost-5-ene-3,12,27-triol.

Isoypsilandroside A (**4**) was obtained as colorless plates. The molecular formula $C_{32}H_{50}O_9$ was deduced on the basis of HR-ESI-MS (*m*/*z* 577.3367 ($[M-H]^-$); calc. 577.3376). Negative-ion FAB-MS showed a significant fragment-ion peak at *m*/*z* 445 ($[M-H-C_5H_8O_4]^-$), suggesting the presence of a pentosyl unit. Comparing the ^{1}H- and ^{13}C-NMR data of the aglycone of **4** with those of **3**, it was evident that the aglycone of **4** was (3*β*,12*α*,25*S*)-spirost-5-ene-3,12,27-triol. Acid hydrolysis of **4** on a TLC plate produced D-Api, as confirmed by comparison with an authentic sample; and the ^{1}H- and ^{13}C-NMR data indicated a *β*-D-pyranose. In the HMBC spectrum of **4**, a correlation between *δ*(H) 5.73 (H–C(1′)) and *δ*(C) 78.0 (C(3)) was observed, which indicated that the D-Api residue was linked at C(3) of the aglycone. Accordingly, isoypsilandroside A (**4**) was identified as (3*β*,12*α*,25*S*)-3-[(*β*-D-apiofuranosyl)oxy]spirost-5-ene-12,27-diol.

Isoypsilandroside B (**5**), obtained as colorless needles, had the molecular formula $C_{38}H_{60}O_{13}$, as determined by HR-ESI-MS (*m*/*z* 723.3946 ($[M-H]^-$); calc. 723.3955). Negative-ion FAB-MS showed the $[M-H]^-$ peak at *m*/*z* 723, which is 146 mass units higher than in the case of **4**. The NMR data indicated that **5** had the same aglycone as **4**, but different sugar moieties. Acid hydrolysis of **5** on a TLC plate produced L-Rha and D-Api, as confirmed by comparison with authentic samples. By ^{1}H- and ^{13}C-NMR analyses, the sugar moieties of **5** and **1** were found to be identical. Consequently, isoypsilandroside B (**5**) was identified as the 25-epimer of **1**.

2. *Biological Activity.* The biological activities of **1**–**5** towards seven types of pathogenic fungi and four animal-pathogenic bacteria were determined. The fungi included *Candida tropicalis*, *Exserohilum turcicum*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Paecilomyce lilacinus*, *Saccharomyces cerevisiae*, and *Verticillium chlamydosporium*, and the bacteria tested were *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus cereus*. All stock cultures were grown on tryptic soy agar plates. The test strains were transferred to fresh tryptic soy broth before use, and a disk containing only DMSO was used as negative control. The activities were determined with the paper-disk method.

Only compound **3** showed a significant antifungal activity; compounds **1**, **3**, and **5** exhibited antibacterial activities at concentrations of up to 50 μg/disk. Isoypsilandrogenin (**3**), at a concentration of 50 μg/disk, afforded inhibitory-zone sizes of 9.5 mm against *F. moniliforme*, and of 16 mm against *S. cerevisiae*. Ypsilandroside B (**1**), at 12 μg/disk, showed moderate antibacterial activity in standard *Petri*-plate assays, affording an inhibitory zone of 13 mm against *B. cereus*. Isoypsilandrogenin (**3**), at 50 μg/disk, and isoypsilandroside B (**5**), at 4 μg/disk, gave inhibitory zones of 17 and 8 mm, respecitvely, against *M. luteus*. In addition, compounds **1** and **5** showed no cytotoxic activity against tumor-cell lines of AGS, BGC-823, Eca-109, K562, and SPC-A-1 by the microculture-tetrazolium method (MTT), and no effect on the inflammatory factors of TNF-*α* and IL-1*β*.

Experimental Part

General. Thin-layer chromatography (TLC) was performed on silica-gel plates; visualization by spraying with 10% H_2SO_4 in EtOH, followed by heating. Column chromatography (CC) was performed on silica gel (200–300 mesh, 10–40 μm; *Qingdao Marine Chemical Inc.*), *Lichroprep RP-18* (43–63 μm; *Merck*), and *Sephadex LH-20* (*Pharmacia*). Semiprep. HPLC: *Agilent 1100* liquid chromatograph with diode array detector (set at 200 nm) and *Zorbax SB-C18* (5 μm) column (25 cm × 9.4 mm i.d.). M.p.: *XRC-1* apparatus; uncorrected. Optical rotations: *JASCO DIP-370* digital polarimeter. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AM-400* (400/100 MHz) and *Bruker DRX-500* (500/125 MHz) instruments, in (D_5)pyridine at 25°; δ in ppm rel. to Me_4Si, *J* in Hz. FAB- and EI-MS: *VG AutoSpec-3000* mass spectrometer; in *m/z*. ESI- and HR-ESI-MS: *API Qstar Pulsar* instrument. X-Ray analysis: *MAC DIP-2030K* diffractometer.

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

Extraction and Isolation. The powdered, air-dried whole plants of *Y. thibetica* (10.0 kg) were extracted three times with 70% aq. EtOH (50 l) at reflux for a total of 6 h. After removal of the solvent *in vacuo*, the residue was suspended in H_2O and extracted with petroleum ether (PE; 2 × 10 l) and AcOEt (3 × 10 l). The AcOEt-soluble material (700 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 1:0 → 0:1): five fractions (Fr.). *Fr. 2* (100 g) was further subjected to repeated CC (SiO_2; 1. PE/AcOEt 4:1 → 0:1; 2. $CHCl_3$/MeOH 9:1) to afford pure **3** (80 mg) and a mixture (120 mg) of **2** and **4**, which was further separated by semiprep. HPLC (*RP-18*, MeOH/H_2O 57:43; 3 ml/min) to afford pure **4** (15 mg) and **2** (11 mg). *Fr. 3* was purified by CC (SiO_2; $CHCl_3$/MeOH/H_2O 9:1:0 → 8:2:0.1): *Fr. 3.1–3.4*. *Fr. 3.2* was repeatedly chromatographed (*RP-18*, MeOH/H_2O 6:4 → 9:1) to afford a mixture (300 mg) of **1** and **5**, which was separated by semiprep. HPLC (*RP-18*, MeOH/H_2O 60:40; 3 ml/min) to afford pure **1** (11 mg) and **5** (14 mg).

Ypsilandroside B (= *(3β,12α,25*R*)-3-[(α-*L*-Rhamnopyranosyl-(1 → 2)-β-*D*-apiofuranosyl)oxy]spiro-st-5-ene-12,27-diol*; **1**)[1]. Colorless needles. M.p. 276–279° (MeOH). $[\alpha]_D^{22} = -10.0$ (*c* = 0.60, pyridine). IR (KBr): 3443, 2935, 1637, 1378, 1056. ^{1}H-NMR (500 MHz, (D_5)pyridine): 5.84 (*d*, *J* = 1.0, H–C(1″)); 5.71 (*d*, *J* = 2.9, H–C(1′)); 5.30 (br. *s*, H–C(6)); 4.47–4.49 (*m*, H–C(16)); 4.14–4.16 (*m*, H_α–C(26)); 4.12 (*d*, *J* = 5.0, H_b–C(27)); 3.97–4.00 (*m*, H_β–C(26)); 3.97 (br. *s*, H–C(12)); 3.95–3.97 (*m*, H_a–C(27)); 3.69–3.71 (*m*, H–C(3)); 3.05 (*dd*, *J* = 6.8, 8.6, H–C(17)); 1.72 (*d*, *J* = 6.1, Me(6″)); 1.16 (*d*, *J* = 7.0, Me(21)); 0.99 (*s*, Me(19)); 0.94 (*s*, Me(18)). ^{13}C-NMR: see *Table*. FAB-MS (neg.): 723 ([*M*–H]$^-$), 577 ([*M* – H – $C_6H_{10}O_4$]$^-$), 445 ([*M* – H – $C_6H_{10}O_4$ – $C_5H_8O_4$]$^-$). HR-ESI-MS: 723.3937 ([*M* – H]$^-$, $C_{38}H_{59}O_{13}^-$; calc. 723.3955).

Ypsilandroside A (= *(3β,12α,25*R*)-3-[(β-*D*-Apiofuranosyl)oxy]spirost-5-ene-12,27-diol*; **2**). Colorless plates (MeOH). M.p. 215–218°. $[\alpha]_D^{22} = 25.0$ (*c* = 0.20, pyridine). IR (KBr): 3447, 2933, 1648, 1378, 1058. ^{1}H-NMR (400 MHz, (D_5)pyridine): 5.73 (*d*, *J* = 2.9, H–C(1′)); 5.27 (br. *s*, H–C(6)); 4.63 (*d*, *J* = 8.3, H–C(16)); 4.18–4.21 (*m*, H_α–C(26)); 4.13 (*d*, *J* = 5.4, H–C(27)); 3.97 (br. *s*, H–C(12)); 3.95–3.98 (*m*, H_β–C(26)); 3.96–3.97 (*m*, H_a–C(27)); 3.69–3.71 (*m*, H–C(3)); 3.07 (*dd*, *J* = 7.1, 8.3, H–C(17)); 1.15 (*d*, *J* = 6.9, Me(21)); 0.96 (*s*, Me(19)); 0.93 (*s*, Me(18)). ^{13}C-NMR: see *Table*. FAB-MS (neg.): 577 ([*M* – H]$^-$), 445 ([*M* – H – $C_5H_8O_4$]$^-$). HR-ESI-MS: 577.3367 ([*M* – H]$^-$, $C_{32}H_{49}O_9^-$; calc. 577.3376).

Isoypsilandrogenin (= *(3β,12α,25*S*)-Spirost-5-ene-3,12,27-triol*; **3**). Colorless plates. M.p. 158–160° (MeOH). $[\alpha]_D^{25} = -63.0$ (*c* = 1.19, pyridine). IR (KBr): 3452, 2934, 1643, 1378. ^{1}H-NMR (400 MHz, (D_5)pyridine): 5.39 (br. *s*, H–C(6)); 4.65 (*q*, *J* = 7.2, H–C(16)); 4.00 (br. *s*, H–C(12)); 3.90–3.92 (*m*, H_β–C(26)); 3.70–3.74 (*m*, H_b–C(27)); 3.68–3.70 (*m*, H_α–C(26)); 3.59–3.63 (*m*, H_a–C(27)); 3.09–3.11 (*m*, H–C(3)); 3.06 (*dd*, *J* = 6.7, 8.0, H–C(17)); 1.21 (*d*, *J* = 7.0, Me(21)); 1.07 (*s*, Me(19)); 0.98 (*s*, Me(18)). ^{13}C-NMR: see *Table*. EI-MS: 446 (1, M^+), 428 (1), 298 (22), 280 (8), 269 (7), 251 (4), 155 (100), 142 (13), 131 (24). HR-ESI-MS: 447.3104 ([*M* + H]$^+$, $C_{27}H_{43}O_5^+$; calc. 447.3110).

Isoypsilandroside A (= *(3β,12α,25*S*)-3-[(β-*D*-Apiofuranosyl)oxy]spirost-5-ene-12,27-diol*; **4**). Colorless plates. M.p. 215–218° (MeOH). $[\alpha]_D^{22} = -15.6$ (*c* = 0.32, pyridine). IR (KBr): 3441, 2934, 1648, 1378,

1054. ^{1}H-NMR (500 MHz, (D_5)pyridine): 5.73 (*d*, *J* = 2.9, H–C(1′)); 5.27 (br. *s*, H–C(6)); 4.63–4.65 (*m*, H–C(16)); 3.96 (br. *s*, H–C(12)); 3.88–3.91 (*m*, H_β–C(26)); 3.70 (*d*, *J* = 5.1, H_b–C(27)); 3.68–3.70 (*m*, H_α–C(26)); 3.67–3.69 (*m*, H–C(3)); 3.58–3.60 (*m*, H_a–C(27)); 3.06 (*dd*, *J* = 7.0, 8.1, H–C(17)); 1.20 (*d*, *J* = 6.9, Me(21)); 0.96 (*s*, Me(19)); 0.95 (*s*, Me(18)). ^{13}C-NMR: see *Table*. FAB-MS (neg.): 577 ($[M-H]^-$), 445 ($[M-H-C_5H_8O_4]^-$). HR-ESI-MS: 577.3367 ($[M-H]^-$, $C_{32}H_{49}O_9^-$; calc. 577.3376).

Isoypsilandroside B (= *(3β,12α,25*S*)-3-[(α-*L*-Rhamnopyranosyl-(1→2)-β-*D*-apiofuranosyl)oxy]-spirost-5-ene-12,27-diol*; **5**). Colorless needles. M.p. 276–279° (MeOH). $[\alpha]_D^{22} = -12.8$ (*c* = 0.70, pyridine). IR (KBr): 3440, 2937, 1642, 1378, 1053. ^{1}H-NMR (400 MHz, (D_5)pyridine): 5.80 (*s*, H–C(1″)); 5.66 (*d*, *J* = 2.60, H–C(1′)); 5.23 (br. *s*, H–C(6)); 4.51–4.53 (*m*, H–C(16)); 3.94 (br. *s*, H–C(12)); 3.84–3.86 (*m*, H_β–C(26)); 3.66 (*d*, *J* = 5.5, H_b–C(27)); 3.65–3.68 (*m*, H–C(3)); 3.62–3.66 (*m*, H_α–C(26)); 3.54–3.56 (*m*, H_a–C(27)); 3.04 (*dd*, *J* = 7.1, 8.2, H–C(17)); 1.72 (*d*, *J* = 5.70, H–C(6″)); 1.20 (*d*, *J* = 6.8, Me(21)); 0.99 (*s*, Me(19)); 0.96 (*s*, Me(18)). ^{13}C-NMR: see *Table*. FAB-MS (neg.): 723 ($[M-H]^-$), 577 ($[M-H-C_6H_{10}O_4]^-$), 445 ($[M-H-C_6H_{10}O_4-C_5H_8O_4]^-$). HR-ESI-MS: 723.3946 ($[M-H]^-$, $C_{38}H_{59}O_{13}^-$; calc. 723.3955).

Acid Hydrolysis. The appropriate compound (2 mg) was heated in 10% aq. HCl/1,4-dioxane (2 ml) at 80° for 4 h. The dioxane was removed, the residue was allowed to cool down, diluted fourfold with H_2O, and extracted with $CHCl_3$. The aq. layer was neutralized with aq. Ag_2CO_3 soln., and evaporated. Apiose (from **2** and **4**) or apiose/rhamnose (from **1** and **5**) were identified by co-TLC with authentic samples.

X-Ray Crystal Structure of Ypsilandroside B (**1**)[2]). Colorless, transparent needles (0.05 × 0.40 × 0.50 mm); $C_{39}H_{64}O_{14}\cdot$MeOH, M_r 756.93 g/mol; crystal system: orthorhombic, space group $P2_12_12_1$; unit-cell dimensions: *a* = 7.889(2), *b* = 11.145(2), *c* = 44.002(9) Å, *V* = 3868.8(13) Å^3, *Z* = 4; D_x = 1.300 g/cm^3.

Biological Assays. Antifungal and antibacterial activities were determined by the disk diffusion method, with minor modifications [8]. *E. coli*, *S. aureus*, *M. luteus*, and *B. cereus* were subcultured in tryptic soy broth (TSB), and incubated for 18 h at 37°. Then, the bacterial cells were suspended according to the *McFarland* protocol in saline soln. (suspension of *ca*. 10^{-5} CFU/ml). The suspension (15 μl) was mixed with sterile tryptic soy agar (TSA; 15 ml) at 40°, and poured onto an agar plate in a laminar-flow cabinet. Each test compound was dissolved in DMSO and added to a paper disk (6-mm diameter), which was dried and placed on the agar plate containing the bacterial cells (5 samples/disk, plus control). A disk containing only DMSO was used as neg. control. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone measured after 18 h of incubation at 37°. Experiments were run in triplicate ($n = 3$), and the results are presented as mean values.

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