Cytotoxic Steroidal Saponins from *Polygonatum punctatum*

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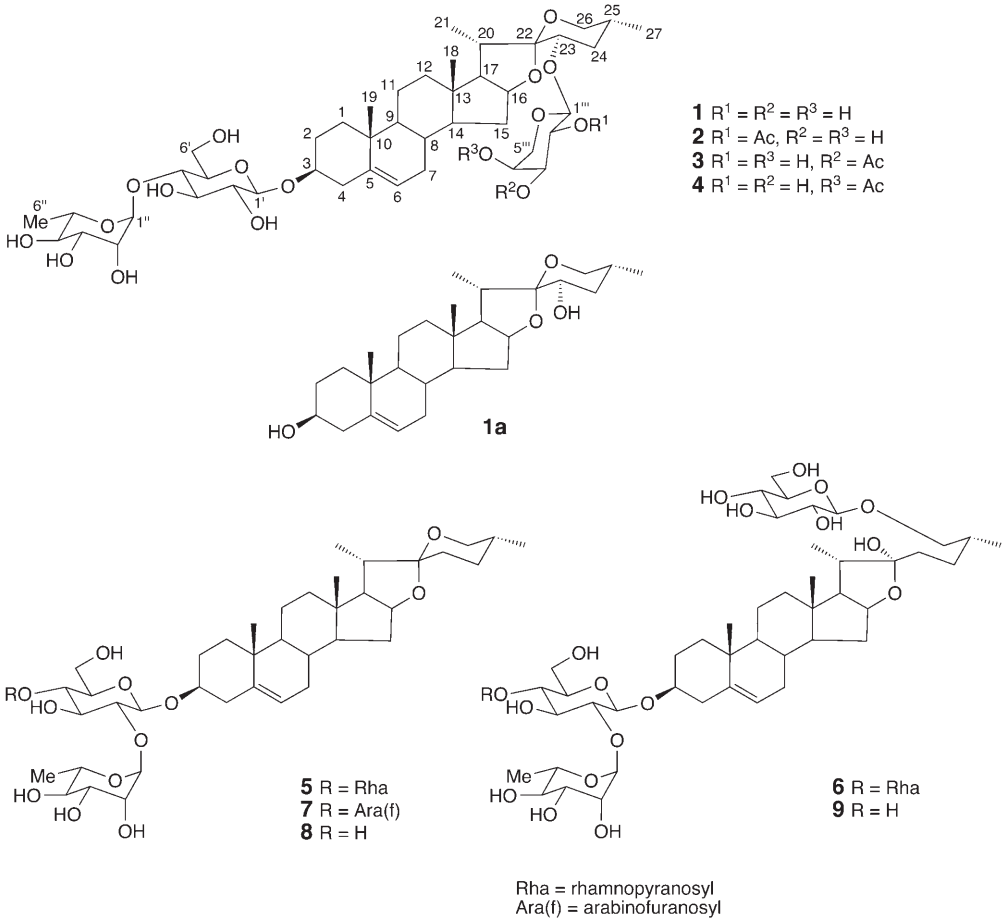
Four new steroidal saponins, polypunctosides A–D (**1**–**4**, resp.), were isolated from the rhizomes of *Polygonatum punctatum*, together with five known steroidal saponins. On the basis of chemical and spectral evidence, the structures of the new saponins were established as (3*β*,23*S*,25*R*)-23-(*α*-L-arabinopyranosyloxy)spirost-5-en-3-yl 4-*O*-(6-deoxy-*α*-L-mannopyranosyl)-D-glucopyranoside (**1**), (3*β*,23*S*,25*R*)-23-[(2-*O*-acetyl-*α*-L-arabinopyranosyl)oxy]spirost-5-en-3-yl 4-*O*-(6-deoxy-*α*-L-mannopyranosyl)-D-glucopyranoside (**2**), (3*β*,23*S*,25*R*)-23-[(3-*O*-acetyl-*α*-L-arabinopyranosyl)oxy]spirost-5-en-3-yl 4-*O*-(6-deoxy-*α*-L-mannopyranosyl)-D-glucopyranoside (**3**), and (3*β*,23*S*,25*R*)-23-[(4-*O*-acetyl-*α*-L-arabinopyranosyl)oxy]spirost-5-en-3-yl 4-*O*-(6-deoxy-*α*-L-mannopyranosyl)-D-glucopyranoside (**4**). The cytotoxic activity of the isolated saponins was evaluated towards HeLa cells.

Introduction. – *Polygonatum punctatum* ROYLE ex KUNTH is a liliaceous plant growing in the southeast of Yunnan province, China. As one of crude materials of 'Huang-Jing', a Chinese traditional medicine herb, its rhizomes are used for treatment of lung illness, palpitations, and diabetes. As a part of our continuous research for discovering novel secondary metabolites from *liliaceous* plants [1–7], we investigated the rhizomes of *P. punctatum* and isolated nine steroidal saponins; among them, polypunctoside A–D (**1**–**4**) are unknown natural products. In the present paper, we report the structure elucidation and cytotoxic activity of these compounds.

Results and Discussion. – The MeOH extracts of the *P. polygonatum* were suspended in H_2O and extracted with BuOH. The BuOH fraction was chromatographed (reversed-phase silica-gel column chromatography and prep. HPLC) to afford nine compounds, **1**–**9**. Among them, compounds **5**–**9** were identified as dioscin (**5**) [8], protodioscin (**6**) [9][10], saponin Pa (**7**) [11], prosapogenin A of dioscin (**8**) and its prototype (3*β*,22*ξ*,25*R*)-3-{[2-*O*-(6-deoxy-*α*-L-mannopyranosyl)-*β*-D-glucopyranosyl]-oxy}-22-hydroxyfurost-5-en-26-yl *β*-D-glucopyranoside (**9**) [12], on the basis of their spectroscopic data and by comparison with literature data.

Compounds **1**–**4**, obtained as white amorphous powders, belong to steroidal saponins, as indicated by four characteristic Me signals at δ(H) 0.67–1.33 in the ^{1}H-NMR spectrum, and a quaternary C-atom signal at *ca.* δ(C) 110 in the ^{13}C-NMR spectrum. Their molecular formulae were assigned to be $C_{44}H_{70}O_{17}$ (**1**), $C_{46}H_{72}O_{18}$ (**2**),



$C_{46}H_{72}O_{18}$ (**3**), and $C_{46}H_{72}O_{18}$ (**4**) on the basis of the negative-ion HR-FAB mass spectra.

The ^{1}H- and ^{13}C-NMR spectra of **1** (*Table 1*) exhibited four characteristic Me signals at δ(H) 0.68 (*d*, J = 6.1 Hz), 0.84 (*s*), 1.03 (*s*), and 1.19 (*d*, J = 6.8), and a quaternary C-atom resonance at δ(C) 110.58 (C(22)), indicating the presence of a steroidal skeleton. The molecular ion peak at *m/z* 870 ($[M]^-$) and the fragment ions at *m/z* 738 ($[M-$pentose$]^-$) and 724 ($[M-$deoxyhexose$]^-$) indicated the existence of one deoxyhexose and one pentose in the sugar moiety, as well as the appearance of three sets of sugar units (three anomeric H-atoms at δ(H) 5.79 (br. *s*), 4.92 (*d*, J = 7.3 Hz), 4.88 (*d*, J = 5.9 Hz)). Complete acid hydrolysis with HCl yielded D-glucose, L-rhamnose, and L-arabinose, and a sapogenin, **1a**, which exhibited a molecular ion peak at *m/z* 430 in its EI mass spectrum. On comparison with diosgenin, an additional tertiary C-atom and one less secondary C-atom were observed in **1a** from the ^{13}C-NMR spectrum by DEPT measurements. The chemical shift of C(22) was downfield to δ 110.54, and the signal at δ(C) 67.04 could be assigned to C(23) according to its long-range coupled cross-peak

Table 1. *^{13}C-NMR Data of Compounds* **1**–**4** (at 500 MHz in (D_5)pyridine; δ in ppm)

	1	**1a**[a])	**2**	**3**	**4**
Aglycone:					
C(1)	37.01	37.19	37.55	37.42	37.36
C(2)	31.82	31.76	32.07	32.05	32.05
C(3)	78.32	71.69	78.35	78.65	78.41
C(4)	39.24	42.26	39.39	39.41	39.40
C(5)	140.84	140.87	140.98	141.03	141.00
C(6)	121.75	121.22	121.85	121.84	121.82
C(7)	32.26	32.03	32.37	32.41	32.45
C(8)	31.46	31.29	31.43	31.64	31.59
C(9)	50.20	50.08	50.49	50.39	50.43
C(10)	37.39	36.62	37.15	37.17	37.18
C(11)	21.07	20.80	21.25	21.13	21.06
C(12)	40.86	39.85	40.12	40.37	40.24
C(13)	40.86	40.69	40.93	41.02	40.99
C(14)	56.56	56.43	56.73	56.71	56.64
C(15)	31.46	31.61	30.29	30.01	30.11
C(16)	81.35	81.51	81.36	81.43	81.49
C(17)	61.98	61.58	62.08	62.19	62.13
Me(18)	19.32	19.34	19.47	19.42	19.39
Me(19)	16.96	16.30	16.96	17.13	17.00
C(20)	35.76	35.57	35.21	35.88	35.75
Me(21)	14.62	13.98	14.55	14.67	14.60
C(22)	110.58	110.54	110.10	110.59	110.25
C(23)	75.75	67.04	76.77	76.00	76.55
C(24)	35.75	38.52	36.59	37.54	37.54
C(25)	31.46	30.77	31.58	31.64	31.64
C(26)	65.71	65.89	65.79	65.82	65.82
Me(27)	16.74	16.48	16.83	16.82	16.82
β-D-Glucopyranose:					
C(1′)	102.35		102.49	102.51	102.43
C(2′)	75.35		75.55	75.70	75.60
C(3′)	76.58		76.77	76.00	76.12
C(4′)	78.32		78.42	78.48	78.41
C(5′)	76.92		77.13	76.78	77.09
C(6′)	61.46		61.66	61.70	61.53
α-L-Rhamnopyranose:					
C(1″)	102.55		102.73	102.74	102.65
C(2″)	72.64		72.84	72.83	72.70
C(3″)	72.17		72.63	72.60	72.32
C(4″)	73.82		74.04	74.02	73.98
C(5″)	70.28		70.42	70.43	70.22
Me(6″)	18.45		18.58	18.54	18.34
α-L-Arabinopyranose:					
C(1‴)	106.09		102.10	105.89	106.49
C(2‴)	72.64		74.04	69.17	72.60
C(3‴)	74.22		72.05	76.38	72.06
C(4‴)	68.03		68.40	66.20	72.83
C(5‴)	65.76		65.12	65.82	63.77
Me*C*O			170.11	171.04	170.86
*Me*CO			21.35	21.13	21.10

[a]) In $CDCl_3$.

between δ(H) 3.42 (H–C(23)) and C(22), and H–C(23) and δ(C) 38.52 (C(24)) in the HMBC experiment. The downfield shift of C(23) indicated that it was substituted by a OH group. The coupling constants between H–C(23) and H–C(24) (*dd*, *J*(ax,ax) = 11.0, *J*(ax,eq) = 4.3 Hz) were consistent with a (23*S*)-configuration. The γ-gauche effect caused by the α-OH–C(23) group led to an upfield shift for C(20) (6.8 ppm) [13]. The coupling pattern of the signal for H–C(26) (δ(H) 3.38 (*dd*, *J* = 11.0, 3.0, H_{eq}; δ(H) 3.22, *t*, *J* = 11.2, H_{ax})) evidenced the presence of an equatorial Me(27) group. From these data, the structure of the sapogenin **1a** was assigned as (3β,23*S*,25*R*)-spirost-5-ene-3,23-diol, which has been previously reported and named as isoplexigenin B, the NMR spectrum of which was not discussed in detail [14].

Comparing the ^{13}C-NMR spectrum of **1** with those of **1a**, the downfield shifts of C(3) and C(2) and the upfield shift of C(4) suggested the presence of a sugar moiety linked to C(3) of the steroidal aglycone, which was confirmed by the long-range correlation of the anomeric H-atom at δ(H) 4.92 (H–C(1′)) with the aglycone C(3) resonance at δ(C) 78.32 in the HMBC spectrum. At the same time, the chemical shift of C(23) changed from δ(C) 67.04 in **1a** to 75.75 in **1**, and the signal correlated with δ(C) 35.75 (C(24)), 31.46 (C(25)), 65.71 (C(26)), and 16.74 (C(27)) in the HMQC-TOCSY spectrum, indicating that C(23) was glycosylated, and **1** was a bisdesmoside. The ^{13}C-NMR resonances of the sugar units were assigned by HMQC and HMQC/TOCSY experiments (see *Table 1*). The long-range correlation of the anomeric H-atoms at δ(H) 4.88 (H–C(1‴)) and 5.79 (H–C(1″)) with the ^{13}C-NMR resonances at δ(C) 75.75 (C(23)) and 78.32 (C(4′)) established the sequence of the sugar moieties. Thus, the structure of compound **1** was elucidated as (3β,23*S*,25*R*)-23-(α-L-arabinopyranosyloxy)spirost-5-en-3-yl 4-*O*-(6-deoxy-α-L-mannopyranosyl)-D-glucopyranoside and named as polypunctoside A.

Comparison of the spectral data of compound **1**, with those of saponin **2** showed an additional Me-group signal at δ(H) 2.13 (*s*) in the ^{1}H-NMR spectrum of **2** and signals due to an AcO group at δ(C) 170.11 and 21.35 in the ^{13}C-NMR spectrum, while the chemical shifts due to the aglycone and sugar moieties were in good agreement with those of **1**. Alkaline hydrolysis of **2** gave **1**, confirming that **2** was an *O*-Ac derivative of **1**. Long-range cross-peaks between the Ac H-atoms, and α-L-arabinopyranosyl C(2‴) (δ 74.04), and α-L-arabinopyranosyl H–C(2‴) (δ 5.76 (*dd*, *J* = 6.2, 7.9 Hz)) and Ac C=O C-atom (δ 170.11) established that the AcO group was attached to C(2‴) of the α-L-arabinopyranosyl moiety. The ^{13}C-NMR resonances of the sugar units were assigned by HMQC and HMQC/TOCSY experiments (see *Table 1*). Thus, the structure of **2** was determined as (3β,23*S*,25*R*)-23-[(2-*O*-acetyl-α-L-arabinopyranosyl)oxy]spirost-5-en-3-yl 4-*O*-(6-deoxy-α-L-mannopyranosyl)-D-glucopyranoside and named as polypunctoside B.

Compounds **3** and **4** have the same molecular formula as **2**. Comparison of their spectral data with those of **2** indicated that they are very similar to each other, differing only in the position of the Ac group, which led the C(3‴) shift to δ(C) 76.38 in compound **3** and C(4‴) to δ(C) 72.83 in compound **4**. In compound **3**, the HMBC correlation between H–C(3‴) (δ(H) 5.76 (*dd*, *J* = 6.2, 7.9 Hz) and the C=O C-atom (δ(C) 171.04) indicated that the AcO group should be positioned at C(3‴), and the HMBC correlation of H–C(4‴) (δ(H) 5.58) with the C=O C-atom (δ(C) 170.86) in compound **4** showed the linkage of the AcO group to C(4‴). The ^{13}C-NMR resonances

of the sugar units were assigned by HMQC and HMQC/TOCSY experiments (see *Table 1*). Thus, the structures of compounds **3** and **4** were assigned as (3*β*,23*S*,25*R*)-23-[(3-*O*-acetyl-*α*-L-arabinopyranosyl)oxy]spirost-5-en-3-yl 4-*O*-(6-deoxy-*α*-L-mannopyranosyl)-D-glucopyranoside and (3*β*,23*S*,25*R*)-23-[(4-*O*-acetyl-*α*-L-arabinopyranosyl)oxy]spirost-5-en-3-yl 4-*O*-(6-deoxy-*α*-L-mannopyranosyl)-D-glucopyranoside, and were named as polypunctoside C and D, respectively.

The isolated steroidal saponins were evaluated for their cytotoxic activity towards Hela cell line, *in vitro*. The results indicated that compounds **1**–**5** showed potent activity (*Table 2*), and the Ac groups in compounds **2**–**4** influenced their cytotoxic activity, compared with compound **1**.

Table 2. *Cytotoxic Activities of* **1**–**9** *against HeLa Cells*

Compound	IC_{50} [μg/ml]	Compound	IC_{50} [μg/ml]
1	39.8 ± 0.6	**6**	> 200
2	3.2 ± 0.6	**7**	> 200
3	2.5 ± 0.4	**8**	> 200
4	2.6 ± 0.5	**9**	> 200
5	20.4 ± 0.7	Cisplatin	0.75 ± 0.03

Experimental Part

General. TLC: Precoated silica-gel plates (*Qingdao Haiyang Chemical Co.*); detection by spraying with 5% anisaldehyde in H_2SO_4, followed by heating. HPLC: *Waters 501* pump and *Waters Preppak* 25 × 10 cartridge (μ*Bondapak* C_{18}); at the wavelength of 205 nm. M.p.: *Boetius* micromelting point apparatus; uncorrected. Optical rotations: *HORIBA SEPA-300* high-sensitive polarimeter. IR Spectra: *Bio-Rad FTS-135* spectrophotometer; KBr disks; in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: in (D_5)pyridine unless otherwise noted, and with a Bruker *DRX-500* spectrometer, at 25°; *δ* in ppm rel. to Me_4Si as internal standard, *J* in Hz. FAB-MS: *VG AutoSpec-3000* mass spectrometer, glycerol matrix; in *m*/*z*.

Plant Material. The rhizomes of *P. punctatum* Royle ex Kunth were collected in Simao, southeast of Yunnan province, China. The plants were identified by Prof. *H. Li* of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen is deposited in the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Fresh rhizomes of *P. punctatum* (10.4 kg) were extracted with hot MeOH. The extract was concentrated to almost dryness under reduced pressure, and the residue was diluted with H_2O and extracted with BuOH. The BuOH phase was fractionated on a silica gel column with $CHCl_3$/MeOH/H_2O 7 : 3 : 1 (lower layer) and gave three fractions. *Fr. 1* was submitted to silica-gel CC with a gradient solvent system of $CHCl_3$/EtOH/H_2O 7 : 4 : 1 and 8 : 3 : 1 (lower layer) and $CHCl_3$/MeOH/AcOEt/H_2O 6 : 3 : 4 : 0.5, and gave compound *prosapogenin A of dioscin* (**8**; 100 mg) and a mixture. The mixture was submitted to *RP-8* CC with 70% MeOH, and finally purified by HPLC with MeOH/H_2O 6 : 4 to yield **2** (130 mg), **3** (60 mg), and **4** (40 mg). *Fr. 2* was separated by silica-gel CC with $CHCl_3$/MeOH/H_2O 7 : 2 : 1 (lower layer) and yielded **1** (500 mg), *dioscin* (**5**; 5.1 g), *saponin Pa* (**7**; 800 mg), and a mixture. The mixture was refluxed in 30% aq. acetone and yielded *(3β,22ξ,25*R*)-3-{[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy}-22-hydroxyfurost-5-en-26-yl β-D-glucopyranoside* (**9**; 300 mg). *Fr. 3* was separated by silica-gel CC with $CHCl_3$/MeOH/H_2O 7 : 3 : 0.5 and then refluxed in 30% aq. acetone to yield *protodioscin* (**6**; 6.5 g).

*(3β,23*S*,25*R*)-23-(α-L-Arabinopyranosyloxy)spirost-5-en-3-yl 4-*O*-(6-Deoxy-α-L-mannopyranosyl)-D-glucopyranoside* (= *Polypunctoside A*; **1**). White amorphous powder. M.p. 262–267°. $[\alpha]^{20}_{D} = -61.79$ ($c = 0.4$, MeOH). IR: 3434, 2935, 1645, 1457, 1383, 947, 903, 812. ^{1}H-NMR ((D_5)pyridine): 5.79 (br. *s*,

H–C(1″)); 5.28 (br. *s*, H–C(6)); 4.92 (*d*, *J*=7.3, H–C(1′)); 4.88 (*d*, *J*=5.9, H–C(1‴)); 4.62 (*m*, H–C(16)); 2.98 (*m*, H–C(20)); (1.66 (*d*, *J*=6.2, Me(6″)); 1.19 (*d*, *J*=6.8, Me(21)); 1.03 (*s*, Me(19)); 0.84 (*s*, Me(18)); 0.68 (*d*, *J*=6.1, Me(27)). ^{13}C-NMR: see *Table 1*. FAB-MS: 870 ([*M*]$^-$), 738 ([*M* – Ara]$^-$), 724 ([*M* – Rha]$^-$). HR-FAB-MS: 869.4478 ([*M*($C_{44}H_{70}O_{17}$) – H]$^-$; calc. 869.4535).

Acid Hydrolysis of Polypunctoside A (**1**). Compound **1** (10 mg) was dissolved in 1N HCl in MeOH/H_2O (1:1) and refluxed for 1 h. The mixture was neutralized with $NaHCO_3$ (1N) and extracted with $CHCl_3$ (3×). The $CHCl_3$ phase was submitted to the silica-gel CC with the mixture of petroleum ether acetone 8:2 and yielded **1a** (2 mg). The sugar residues were diluted in 5 ml of pyridine and treated with 0.5 ml of Me_3SiCl (*Fluka*) at r.t. for 30 min. The mixture was evaporated to dryness under reduced pressure. The mixture of trimethylsilylated derivatives of the monosaccharides was diluted in 0.5 ml of Et_2O and then analyzed by GC. GC: *AC-5* cap column (30 m × 0.25 mm i.d.); detector FID (270°); column temp. 180–260°, rate 5°/min. t_R [s]: 251 (L-arabinose), 258 (L-rhamnose), 411 (D-glucose).

*(3β,23*S*,25*R*)-Spirost-5-en-3,23-diol* (= *Isoplexigenin B*; **1a**). White crystals ($CHCl_3$). M.p. 243–245°. IR: 3505, 3375, 2955, 2931, 2905, 2873, 2850, 1661, 1460, 1065, 1052, 1010, 964, 948, 919. ^{1}H-NMR ($CDCl_3$): 5.46 (br. *s*, H–C(6)); 4.45 (*m*, H–C(16)); 3.42 (*dd*, *J*=11.0, 4.3, H–C(23)); 3.38 (*dd*, *J*=11.0, 3.0, H_{eq}–C(26)); 3.22 (*t*, *J*=11.1, H_{ax}–C(26)); 2.52 (*m*, H–C(20)); 0.94 (*s*, Me(19)); 0.93 (*d*, *J*=7.1, Me(21)); 0.81 (*s*, Me(18)); 0.79 (*d*, *J*=6.6, Me(27)). ^{13}C-NMR: see *Table 1*. EI-MS: 430 (55, M^+), 345 (100), 327 (65), 271(82), 253 (53).

*(3β,23*S*,25*R*)-23-[(2-*O*-acetyl-α-*L*-arabinopyranosyl)oxy]spirost-5-en-3-yl 4-*O*-(6-Deoxy-α-*L*-mannopyranosyl)-*D*-glucopyranoside* (= *Polypunctoside B*; **2**). White amorphous powder. M.p. 282–286°. $[\alpha]^{20}_D$ = –54.05 (*c*=0.4, MeOH). IR: 3435, 2935, 1732, 1640. ^{1}H-NMR ((D_5)pyridine): 5.87(br. *s*, H–C(1″)); 5.76 (*dd*, *J*=6.2, 7.9, H–C(2‴)); 5.28 (br. *s*, H–C(6)); 4.93 (*d*, *J*=7.3, H–C(1′)); 4.91 (*d*, *J*=6.2, H–C(1‴)); 4.54 (*m*, H–C(16)); 2.78 (*m*, H–C(20)); 2.13 (*s*, MeCO); 1.69 (*d*, *J*=6.2, Me(6″)); 1.19 (*d*, *J*=6.8, Me(21)); 1.03 (*s*, Me(19)); 0.84 (*s*, Me(18)); 0.68 (*d*, *J*=5.9, Me(27)). ^{13}C-NMR: see *Table 1*. FAB-MS: 912 ([*M*]$^-$), 870 ([*M* – Ac]$^-$), 852 ([*M* – Ac – H_2O]$^-$), 737 ([*M* – Ac – Ara]$^-$), 723 ([*M* – Ac – Rha]$^-$). HR-FAB-MS: ([*M*($C_{46}H_{72}O_{18}$) – H]$^-$; calc. 911.4640).

*(3β,23*S*,25*R*)-23-[(3-*O*-acetyl-α-*L*-arabinopyranosyl)oxy]spirost-5-en-3-yl 4-*O*-(6-Deoxy-α-*L*-mannopyranosyl)-*D*-glucopyranoside* (= *Polypunctoside C*; **3**). White amorphous powder. M.p. 270–275°. $[\alpha]^{20}_D$ = –52.18 (*c*=0.4, MeOH). IR: 3430, 2937, 1728, 1643, 1456, 1380, 1255, 1067, 1029, 963, 946, 837, 812. ^{1}H-NMR ((D_5)pyridine): 5.87 (br. *s*, H–C(1″)); 5.76 (*dd*, *J*=6.2, 7.9, H–C(3‴)); 5.27 (br. *s*, H–C(6)); 4.93 (*d*, *J*=6.7, H–C(1′)); 4.91 (*d*, *J*=6.2, H–C(1‴)); 4.58 (*m*, H–C(16)); 3.11 (*m*, H–C(20)); 2.02 (*s*, MeCO); 1.69 (*d*, *J*=6.2, Me(6″)); 1.17 (*d*, *J*=6.9, Me(21)); 1.03 (*s*, Me(19)); 0.82 (*s*, Me(18)); 0.70 (*d*, *J*=6.1, Me(27)). ^{13}C-NMR: see *Table 1*. FAB-MS: 912 ([*M*]$^-$), 870 ([*M* – Ac]$^-$), 851 ([*M* – Ac – H_2O – H]$^-$). HR-FAB-MS: 911.4555 ([*M*($C_{46}H_{72}O_{18}$) – H]$^-$; calc. 911.4640).

*(3β,23*S*,25*R*)-23-[(4-*O*-acetyl-α-*L*-arabinopyranosyl)oxy]spirost-5-en-3-yl 4-*O*-(6-Deoxy-α-*L*-mannopyranosyl)-*D*-glucopyranoside* (= *Polypunctoside D*, **4**). White amorphous powder. M.p. 289–293°. $[\alpha]^{20}_D$ = –53.20 (*c*=0.4, MeOH). IR: 3430, 2937, 1728, 1643, 1456, 1380, 1255, 1067, 1029, 963, 946, 837, 812. ^{1}H-NMR ((D_5)pyridine): 5.87 (br. *s*, H–C(1″)); 5.58 (br. *s*, H–C(4‴)); 5.28 (br. *s*, H–C(6)); 4.93 (*d*, *J*=6.9, H–C(1′)); 4.84 (*d*, *J*=6.2, H–C(1‴)); 4.57 (*m*, H–C(16)); 2.94 (*m*, H–C(20)); 1.93 (*s*, MeCO); 1.69 (*d*, *J*=6.2, Me(6″)); 1.22 (*d*, *J*=6.8, Me(21)); 1.11 (*s*, Me(19)); 0.84 (*s*, Me(18)); 0.68 (*d*, *J*=6.1, Me(27)). ^{13}C-NMR: see *Table 1*. FAB-MS: 912 ([*M*]$^-$), 870 ([*M* – Ac]$^-$), 851 ([*M* – Ac – H_2O – H]$^-$). HR-FAB-MS: 911.4555 ([*M*($C_{46}H_{72}O_{18}$) – H]$^-$; calc. 911.4640).

Alkaline Hydrolysis of **2**, **3**, *and* **4**. LiOH (10 mg) was added to a soln. of **2** (10 mg) in H_2O (5 ml). The mixture was heated with stirring at 40° for 5 h, then cooled to ambient temp., and the solvent was removed *in vacuo*, and the product was purified by *RP-8* CC (MeOH/H_2O 7:3) to afford **1** (5 mg). Compound **3** or **4** (10 mg) was hydrolyzed in alkaline medium according to the same procedure.

Cytotoxicity against HeLa Cells. The cytotoxicity against HeLa cells was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric assay on 96-well microplates [15]. Briefly, 196 µl of treated HeLa cell suspension (2×10^4 cells/ml in the RPMI 1640 medium) was placed in each well of a 96-well flat-bottom plate and incubated in 5% CO_2/air for 12 h at 37°. After incubation, 4 µl of DMSO soln. containing the sample was added to give the final concentration of the samples (25, 20, 15, 10, 5, 2.5, 1.25, 0.80 µg/ml); 4 µl of DMSO was added to control wells. The cells were further incubated for 72 h, and then the cell growth was evaluated by the MTT

method. The OD value was read on a plate reader at a wavelength of 570 nm. The cytotoxicity was expressed as IC_{50} value (μg/ml), which was the mean of three determinations and reduced the viable cell number by 50%.

We acknowledge the faculty of the instrument group at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, the Chinese Academy of Sciences for spectral analysis.

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Received July 17, 2006