Full Papers

Spirostanol and Furostanol Glycosides from the Fresh Tubers of *Polianthes tuberosa*

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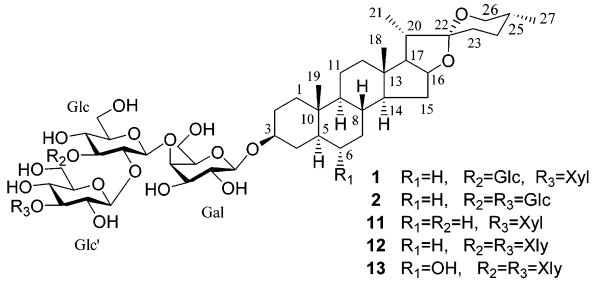
Received September 24, 2003

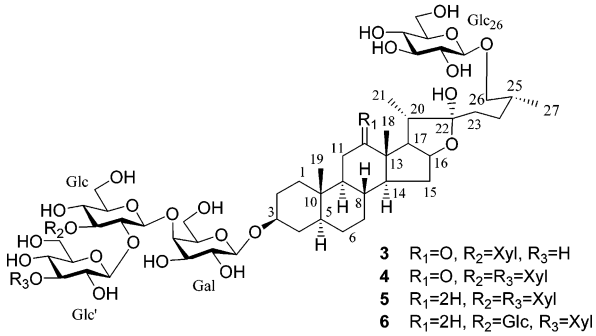
Six new steroid glycosides—two spirostanols, polianthosides B and C (**1**, **2**), and four furostanols, polianthosides D−G (**3**−**6**)—were isolated from the fresh tubers of *Polianthes tuberosa*, together with seven known spirostanols (**7**−**13**) and a known furostanol (**14**) saponins. Their structures were elucidated on the basis of spectroscopic analysis and the results of acidic and enzymatic hydrolysis. The cytotoxic activities of **1**−**14** against HeLa cells are reported.

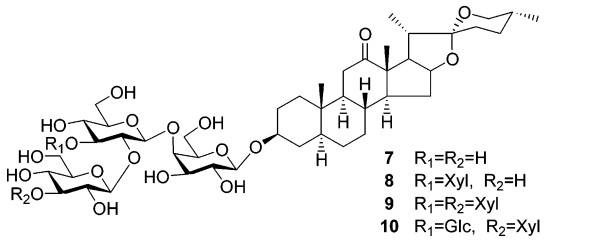
The genus *Polianthes*, comprising about 12 species, is native to Mexico. *Polianthes tuberosa* L. (Agavaceae), a well-known ornamental plant, is widely cultivated in the south of the People's Republic of China. Its flowers are used as high-class flavor and the tubers as a Chinese folk medicine used for the treatment of acute infectious diseases and pyrogenic inflammations.[1] Several steroid sapogenins, such as hecogenin, 9-dehydroxyhecogenin, and tigogenin,[2] as well as glycosides, 29-hydroxystigmast-5-en-3*β*-yl *β*-D-glucoside,[3] (22*S*)-2*β*,3*β*,22-trihydroxycholest-5-en-16*β*-yl *β*-D-glucoside,[4] and diribofuranosyl ethyleneglycol,[5] and spirostanol pentaglycosides[6] were identified from the underground parts of *P. tuberosa*. Our search for bioactive saponins from *P. tuberosa* has led to the isolation of six new steroid glycosides—two spirostanols, polianthosides B and C (**1**, **2**), and four furostanols, polianthosides D−G (**3**−**6**)—together with eight known saponins (**7**−**14**) from the fresh tubers. We describe herein the structure determination of **1**−**6** on the basis of spectroscopic and chemical methods. The cytotoxic activities of **1**−**14** against HeLa cells are also reported.

Results and Discussion

The fresh tubers of *P. tuberosa* (24 kg) were extracted with 80% EtOH under reflux. The EtOH extract was partitioned between *n*-butanol and H_2O. The *n*-butanol layer was concentrated and then chromatographed repeatedly over silica gel and RP-8 columns to give compounds **1**−**6** and eight known steroid saponins, identified as hecogenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**7**),[7] hecogenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xyloyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**8**),[8] hecogenin 3-*O*-*β*-D-xyloyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xyloyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**9**),[9] agamenoside F (**10**),[10] tigogenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xyloyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**11**),[11] tigogenin 3-*O*-*β*-D-xyloyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xyloyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**12**),[9] chlorogenin 3-*O*-*β*-D-xyloyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xyloyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**13**),[9] and 26-*O*-*β*-D-glucopyranosyl-(25*R*)-5α-furost-3*β*,22α,26-triol 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (uttroside B) (**14**),[12] respectively.







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10.1021/np034028a CCC: $27.50
Published on Web 12/12/2003

Compound **1** was obtained as a white amorphous powder. Its molecular formula was assigned as $C_{56}H_{92}O_{27}$ on the basis of the ^{13}C NMR data and negative ion HRFABMS ($[M - H]^-$, *m/z* 1195.5709). The negative ion FABMS also showed fragment ion peaks at *m/z* 1033 [M − H − 162(hexosyl)]$^-$, 901 [M − H − 132(pentosyl) − 162(hexosyl)]$^-$, 739 [M − H − 132(pentosyl) − 162(hexosyl) − 162(hexosyl)]$^-$, and 577 [M − H − 132(pentosyl) − 162(hexosyl) − 162(hexosyl) − 162(hexosyl)]$^-$. The ^1H NMR spectrum of **1** showed two three-proton singlet signals at δ 0.81 and 0.63 and two three-proton doublet signals at δ 1.13 (J = 6.8 Hz) and 0.69 (J = 5.2 Hz), characteristic of the spirostanol skeleton, as well as signals for five anomeric protons at δ 4.86, 5.10, 5.19, 5.55, and 5.08. Acid hydrolysis of **1** with 1 M HCl produced tigogenin, which was identified by normal- and reversed-phase TLC comparison with an authentic sample, and D-xylose, D-glucose, and D-galactose as sugar residues determined by GC analysis. The ^{13}C NMR data of the sugar moiety were closely related to those of **10**, suggesting that both compounds had the same sugar linkages. In the HMBC spectrum of **1**, correlations of δ_H 4.86 (H-Gal-1) with δ_C 77.9 (C-3), δ_H 5.10 (H-Glc-1) with δ_C 80.0 (C-Gal-4), δ_H 5.19 (H-Glc″-1) with δ_C 88.3 (C-Glc-3), δ_H 5.55 (H-Glc′-1) with δ_C 81.0 (C-Glc-2), and δ_H 5.08 (H-Xyl-1) with δ_C 87.1 (C-Glc′-3) were observed. In addition, the IR spectrum showed absorptions at 982, 921, 898, and 865 cm^{-1}, among which the band at 898 cm^{-1} was stronger than the band at 921 cm^{-1}, indicating the *R* configuration at C-25.[13] On the basis of the above evidence, **1** was determined to be tigogenin 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosy-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside, named polianthoside B.

The molecular formula of **2** was established as $C_{57}H_{94}O_{28}$ on the basis of its negative ion HRFABMS ($[M]^-$ *m/z* 1226.5870). The negative ion FABMS also showed fragment ion peaks at *m/z* 1063 [M − H − 162(hexosyl)]$^-$ and 901 [M − H − 162(hexosyl) − 162(hexosyl)]$^-$ and suggested a hexosyl unit as the terminal sugar moiety. The ^1H NMR spectrum of **2** exhibited four characteristic methyl signals for the spirostanol skeleton at δ 0.80, 0.62, 1.12, and 0.68, as well as five anomeric proton signals. Acid hydrolysis of **2** yielded tigogenin as the aglycone by TLC comparison and D-glucose and D-galactose as sugar residues by GC analysis.

The HMQC-TOCSY spectrum assigned the^{13}C NMR chemical shifts for each sugar unit. The HMBC, $^1H-^1H$ COSY, and ROESY spectra determined the sugar linkage sequence. In the HMBC spectrum, correlations of δ_H 4.86 (H-Gal-1) with δ_C 77.5 (C-3 of aglycone), δ_H 5.11 (H-Glc-1) with δ_C 80.0 (C-Gal-4), δ_H 5.51 (H-Glc′-1) with δ_C 81.0 (C-Glc-2), δ_H 5.19 (H-Glc″-1) with δ_C 88.5 (C-Glc-3), and δ_H 5.13 (H-Glc‴-1) with δ_C 87.5 (C-Glc′-3) were observed. Therefore, polianthoside C (**2**) was characterized as tigogenin 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside.

Compounds **3**−**6** were obtained as white amorphous powders. The DEPT spectrum showed a characteristic quaternary carbon signal around δ 110. These observations suggested that **3**−**6** were furostanol glycosides.

The molecular formulas of **3** and **4** were determined to be $C_{56}H_{92}O_{29}$ and $C_{61}H_{100}O_{33}$, respectively, by HRFABMS. Acid hydrolysis of **3** and **4** yielded hecogenin, which was confirmed by direct comparison of the ^{13}C chemical shifts with those of the reference data,[14] and D-glucose, D-galactose, and D-xylose as sugar residues determined by GC analysis. The ^{13}C NMR data of the aglycone moiety of **3** and **4** were similar to each other and to those of terrestrosin I (26-*O*-*β*-D-glucopyranosyl-(25*R,S*)-5α-furost-3*β*,22α,26-triol-12-one 3-*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside),[15] which was isolated from *Tribulus terrestris*. In the ^1H NMR spectrum, **3** showed the presence of five sugar units and **4** showed six anomeric proton signals. These observations suggested that **3** was a furostanol pentaglycoside, and **4**, a hexaglycoside with the same aglycone as **3**. The *J* values (>5 Hz) of the anomeric protons indicated the *β*-orientation for each anomeric center of the sugar units.

Comparing the ^{13}C NMR data of the sugar moieties of **3** with those of **14**, it was indicated that **3** had the same sugar moieties as **14**; that is, a *β*-D-glucopyranosyl unit was attached at C-26 of the aglycone and the same sugar chain as **14** was linked to C-3 of the aglycone. This was further confirmed by the three-bond $^1H-^{13}C$ long-range correlations. In the HMBC spectrum of **3**, correlations of δ_H 4.84 (H-Gal-1) with δ_C 77.8 (C-3 of aglycone), δ_H 5.14 (H-Glc-1) with δ_C 80.0 (C-Gal-4), δ_H 5.51 (H-Glc′-1) with δ_C 81.4 (C-Glc-2), and δ_H 5.18 (H-Xyl-1) with δ_C 87.0 (C-Glc-3), as well as δ_H 4.77 (H-Glc$_{26}$-1) with δ_C 75.3 (C-26 of aglycone), were observed. Moreover, enzymatic hydrolysis of **3** with *β*-glucosidase yielded hecogenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xyloyranosyl (1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**8**). On the basis of the above evidence, **3** was determined to be 26-*O*-*β*-D-glucopyranosyl-(25*R*)-5α-furost-3*β*,22α,26-triol-12-one 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside, named polianthoside D.

The sequence of the sugars and binding sites at the aglycone of **4** were determined by 2D NMR experiments. The ^{13}C chemical shifts due to each sugar unit were assigned by the HMQC-TOCSY spectrum. In the HMBC spectrum of **4**, the following correlations were observed: δ_H 4.83 (H-Gal-1) with δ_C 77.8 (C-3 of aglycone), δ_H 5.14 (H-Glc-1) with δ_C 79.8 (C-Gal-4), δ_H 5.53 (H-Glc′-1) with δ_C 80.8 (C-Glc-2), δ_H 5.11 (H-Xyl-1) with δ_C 86.8 (C-Glc-3), δ_H 5.05 (H-Xyl′-1) with δ_C 86.8 (C-Glc′-3), and δ_H 4.77 (H-Glc$_{26}$-1) with δ_C 75.2 (C-26 of aglycone). In addition, enzymatic hydrolysis of **4** with *β*-glucosidase yielded hecogenin 3-*O*-*β*-D-xyloyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xyloyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**9**). Therefore, polianthoside E (**4**) was elucidated as 26-*O*-*β*-D-glucopyranosyl-(25*R*)-5α-furost-3*β*,22α,-26-triol-12-one3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside.

The negative ion HRFABMS assigned the molecular formulas of **5** and **6** as $C_{61}H_{102}O_{32}$ and $C_{62}H_{104}O_{33}$ (**5**: *m/z* 1345.6194 $[M - H]^-$, **6**: *m/z* 1375.6420 $[M - H]^-$), respectively. Acid hydrolysis of **5** and **6** gave D-glucose, D-galactose, and D-xylose as sugar residues determined by GC analysis, and tigogenin, which was confirmed by ^{13}C NMR data.[14] The ^{13}C chemical shifts of the aglycone of **5** and **6** were identical to those of **14** and terrestrosin H (26-*O*-*β*-D-glucopyranosyl-(25*R,S*)-5α-furost-3*β*,22α,26-triol 3-*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-galactopyranoside) isolated from *Tribulus terrestris*.[15] Comparing the ^{13}C chemical shifts of the sugar moieties of **5** with those of **4** indicated that **5** had the same sequence of sugar linkage as **4**. The sugar sequence and linkage position to the aglycone of **6** were determined by the HMBC spectrum, which showed correlations of δ_H 4.86 (H-Gal-1) with δ_C 77.8 (C-3 of aglycone), δ_H 5.11 (H-Glc-1) with δ_C 80.1 (C-Gal-4), δ_H 5.54 (H-Glc′-1) with δ_C 81.0 (C-Glc-2), δ_H 5.08 (H-Glc″-1) with δ_C 88.5 (C-Glc-3), δ_H 5.07 (H-Xyl-

Table 1. ^{13}C NMR Data of Compounds **1** and **2**

aglycone	**1**	**2**	sugar	**1**	**2**
1	37.4 (t)	37.2 (t)	Gal 1	102.6(d)	102.5(d)
2	30.6 (t)	30.0 (t)	2	73.2 (d)	73.2 (d)
3	77.9 (d)	77.5 (d)	3	75.5 (d)	75.4 (d)
4	35.0 (t)	34.9 (t)	4	80.0 (d)	80.0 (d)
5	44.8 (d)	44.7 (d)	5	75.6 (d)	75.6 (d)
6	29.1 (t)	29.0 (t)	6	60.9 (t)	60.7 (t)
7	32.6 (t)	32.5 (t)	Glc 1	104.8 (d)	104.8 (d)
8	35.4 (d)	35.3 (d)	2	81.0 (d)	81.0 (d)
9	54.6 (d)	54.5 (d)	3	88.3 (d)	88.5 (d)
10	36.0 (s)	35.9 (s)	4	70.9 (d)	70.8 (d)
11	21.5 (t)	21.3 (t)	5	77.6 (d)	77.5 (d)
12	40.3 (t)	40.2 (t)	6	63.2 (t)	63.1 (t)
13	41.0 (s)	40.8 (s)	Glc′ 1	104.1 (d)	104.1 (d)
14	56.6 (d)	56.5 (d)	2	75.2 (d)	74.8 (d)
15	32.3 (t)	32.2 (t)	3	87.1 (d)	87.5 (d)
16	81.3 (d)	80.2 (d)	4	69.2 (d)	69.3 (d)
17	63.2 (d)	63.1 (d)	5	78.2 (d)	78.0 (d)
18	16.8 (q)	16.7 (q)	6	62.2 (t)	62.1 (t)
19	12.5 (q)	12.4 (q)	Xyl 1	106.1 (d)	
20	42.5 (d)	42.1 (d)	2	75.5 (d)	
21	15.2 (q)	15.1 (q)	3	77.7 (d)	
22	109.5 (s)	109.3 (s)	4	70.9 (d)	
23	32.0 (t)	31.9 (t)	5	67.2 (t)	
24	29.4 (t)	29.3 (t)	Glc″ 1	104.5 (d)	104.5 (d)
25	30.8 (d)	30.7 (d)	2	75.5 (d)	75.5 (d)
26	67.1 (t)	67.0 (t)	3	78.7 (d)	78.6 (d)
27	17.5 (q)	17.4 (q)	4	71.7 (d)	71.6 (d)
			5	78.5 (d)	78.5 (d)
			6	62.5 (t)	62.4 (t)
			Glc‴ 1		105.4 (d)
			2		75.6 (d)
			3		78.6 (d)
			4		71.6 (d)
			5		78.5 (d)
			6		62.6 (t)

1) with δ_C 86.9 (C-Glc′-3), and δ_H 4.77 (H-Glc$_{26}$-1) with δ_C 75.2 (C-26 of aglycone). Furthermore, enzymatic hydrolysis of **5** and **6** with β-glucosidase yielded **12** and tigogenin 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, identified by its ^1H and ^{13}C NMR data, respectively. On the basis of the above evidence, polianthosides F (**5**) and G (**6**) were deduced to be 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furost-3β,22α,26-triol 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, and 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furost-3β,22α,26-triol 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, respectively.

Compounds **1**−**14** were tested for in vitro cytotoxicity against HeLa cells. The IC_{50} values are listed in Table 3. It is noticed that most of the saponins (**3**, **4**, and **7**−**10**) with a carbonyl group at C-12 of the aglycone showed stronger cytotoxicities (IC_{50} 4.02−8.61 μg/mL) against HeLa cells than saponins **1**, **2**, **5**, and **14**, with no carbonyl group attached at the aglycone (IC_{50} > 18.83 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter. IR (KBr) spectra were measured on a Bio-Rad FTS-135 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 instrument (500 MHz for ^1H NMR, and 125 MHz for ^{13}C NMR) at 25 °C, using TMS as an internal standard. The negative ion and high-resolution FAB mass spectra were recorded on a VG AutoSpec-3000 mass spectrometer using glycerol as matrix. Precoated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC. Detection was done by spraying the plates with 5% anisaldehyde−sulfric acid, followed by heating.

Plant Material. The fresh tubers of *P. tuberosa* L. cv Double were obtained from Kunming Qianhui Seed and Seedling Limited Company during June 2001.

Extract and Isolation. The fresh tubers of *P. tuberosa* (24 kg) were extracted with hot 80% EtOH three times for 4 h, and the combined extract was concentrated under reduced pressure. Then concentrated extract was partitioned between *n*-butanol and H_2O. Half the *n*-butanol layer (450 g) was chromatographed on silica gel with $CHCl_3$−MeOH−H_2O (7:3:0.5) and gave four fractions (I−IV). Fraction 3 (60 g) was subjected to silica gel ($CHCl_3$−MeOH−H_2O) and RP-8 (MeOH−H_2O) column chromatography to afford **7** (570 mg), **8** (4.9 g), **9** (420 mg), **10** (210 mg), and **11** (2 g). Fraction 4 (150 g) was repeatedly chromatographed on silica gel with $CHCl_3$−MeOH−H_2O and RP-8 with MeOH−H_2O to yield **1** (70 mg), **2** (34 mg), **3** (740 mg), **4** (2.5 g), **5** (3.6 g), **6** (90 mg), **12** (482 mg), **13** (300 mg), and **14** (520 mg). Since the furostanol glycoside generally exists as an equilibrium mixture of 22-methoxy and 22-hydroxy forms with the presence of MeOH, furostanol glycosides **3**−**6** and **14** afforded their 22-hydroxy forms after being refluxed with 70% aqueous acetone for 10 h.[16]

Compound 1: white amorphous powder; $[\alpha]_D^{18.3}$ −52.04° (*c* 0.0221, pyridine); IR (KBr) ν_{max} 3412, 2929, 1455, 1373, 1158, 1073, 982, 921, 898, 865 cm^{-1} (absorption: 898 > 921); ^1H NMR (pyridine-d_5) δ 3.58 (1H, H-3), 4.46 (1H, q-like, J = 8.3 Hz, H-16), 0.81 (3H, s, H-18), 0.63 (3H, s, H-19), 1.13 (3H, d, J = 6.8 Hz, H-21), 3.53, 3.50 (1H each, H-26), 0.69 (3H, d, J = 5.2 Hz, H-27), 4.86 (d, J = 7.4 Hz, H-Gal-1), 5.10 (1H, d, J = 10.3 Hz, H-Glc-1), 5.55 (1H, br, H-Glc′-1), 5.19 (1H, d, J = 7.8 Hz, H-Glc″-1), 5.08 (1H, d, J = 7.6 Hz, H-Xyl-1); ^{13}C NMR (pyridine-d_5), see Table 1; FABMS (negative mode) *m*/*z* 1195 [M − H]$^-$, 1033 [M − H − 162(hexosyl)]$^-$, 901 [M − H − 132-(pentosyl) − 162(hexosyl)]$^-$, 739 [M − H − 132(pentosyl) − 162(hexosyl) − 162(hexosyl)]$^-$, 577 [M − H − 132(pentosyl) − 162(hexosyl) − 162(hexosyl) − 162(hexosyl)]$^-$; HRFABMS *m*/*z* 1195.5709 [M − H]$^-$ (calcd for $C_{56}H_{91}O_{27}$, 1195.5748).

Acid Hydrolysis of 1. Compound of **1** (2 mg) was refluxed with 1 mol/L HCl−dioxane (1:1, v/v, 4 mL) on a water bath for 6 h. The reaction mixture was evaporated to dryness. The dry reaction mixture was partitioned between $CHCl_3$ and H_2O four times. The $CHCl_3$ extract was concentrated and identified as tigogenin by normal- and reversed-phase TLC comparison with authentic sample. The sugar residues were diluted in 5 mL of pyridine without water and treated with 0.5 mL of trimethyl-chlorsilan (TMCS, Fluka) at room temperature for 30 min. The reaction mixture was evaporated to dryness under reduced pressure. The mixture of trimethylsilylated derivatives of the monosaccharides was diluted in 0.5 mL of ether without water and then analyzed by GC. GC condition: AC-5 capillary column (30 m × 0.25 mm i.d.); detector FID (270 °C); column temperature 180−260 °C, rate 5° C/min. t_R (second): 692 (D-glucose), 653 (D-galactose), and 510 (D-xylose).

Compound 2: white amorphous powder; $[\alpha]_D^{19.8}$ −32.79° (*c* 0.0183, pyridine); IR (KBr) ν_{max} 3402, 2923, 1606, 1451, 1383, 1158, 1074, 983, 922, 899, 867 cm^{-1} (absorption: 899 > 922); ^1H NMR (pyridine-d_5) δ 3.58 (1H, H-3), 4.46 (1H, q-like, J = 10.2 Hz, H-16), 0.80 (3H, s, H-18), 0.62 (3H, s, H-19), 1.12 (3H, d, J = 6.9 Hz, H-21), 3.56, 3.48 (1H each, H-26), 0.68 (3H, d, J = 4.3 Hz, H-27), 4.86 (1H, d, J = 7.3 Hz, H-Gal-1), 5.11 (1H, d, J = 7.7 Hz, H-Glc-1), 5.51 (1H, br, H-Glc′-1), 5.19 (1H, d, J = 7.7 Hz, H-Glc″-1), 5.13 (1H, d, J = 9.8 Hz, H-Glc‴-1); ^{13}C NMR (pyridine-d_5), see Table 1; FABMS (negative mode) *m*/*z* 1225 [M − H]$^-$, 1063 [M − H − 162(hexosyl)]$^-$, 901 [M − H − 162(hexosyl) − 162(hexosyl)]$^-$; HRFABMS *m*/*z* 1226.5870 [M − H]$^-$ (calcd for $C_{57}H_{94}O_{27}$, 1226.5932).

Acid Hydrolysis of 2. Compound of **2** (2 mg) was subjected to acid hydrolysis as described for **1** to give tigogenin, by TLC comparison with authentic sample, and D-glucose and D-galactose as sugar moieties by GC analysis.

Compound 3: white amorphous powder; $[\alpha]_D^{18.1}$ −23.21 (*c* 0.0474, pyridine); IR (KBr) ν_{max} 3407, 2927,1704, 1373, 1160, 1071, 1040, 894 cm^{-1}; ^1H NMR (pyridine-d_5) δ 3.77 (1H, H-3),

Table 2. ^{13}C NMR Data of Compounds **3**−**6**

aglycone	**3**	**4**	**5**	**6**	sugar	**3**	**4**	**5**	**6**
1	36.8 (t)	36.7 (t)	37.3 (t)	37.3 (t)	Gal 1	102.5 (d)	102.5 (d)	102.6 (d)	102.5 (d)
2	29.8 (t)	29.7 (t)	30.0 (t)	30.0 (t)	2	73.3 (d)	73.2 (d)	73.2 (d)	73.2 (d)
3	77.8 (d)	77.8 (d)	77.8 (d)	77.8 (d)	3	75.6 (d)	75.2 (d)	75.2 (d)	75.2 (d)
4	34.8 (t)	34.7 (t)	34.9 (t)	34.9 (t)	4	80.0 (d)	79.8 (d)	79.8 (d)	80.1 (d)
5	44.6 (d)	44.6 (d)	44.8 (d)	44.8 (d)	5	75.4 (d)	75.2 (d)	75.3 (d)	75.5 (d)
6	28.7 (t)	28.7 (t)	29.1 (t)	29.1 (t)	6	60.8 (t)	60.8 (t)	60.8 (t)	60.8 (t)
7	31.9 (t)	31.8 (t)	32.6 (t)	32.5 (t)	Glc 1	105.2 (d)	105.0 (d)	104.9 (d)	104.9 (d)
8	34.5 (d)	34.3 (d)	35.4 (d)	35.4 (d)	2	81.4 (d)	80.8 (d)	80.8 (d)	81.0 (d)
9	55.8 (d)	55.7 (d)	54.6 (d)	54.6 (d)	3	87.0 (d)	86.8 (d)	86.9 (d)	88.5 (d)
10	36.4 (s)	36.4 (s)	35.9 (s)	35.9 (s)	4	70.6 (d)	70.5 (d)	70.5 (d)	70.8 (d)
11	38.2 (t)	38.1 (t)	21.4 (t)	21.4 (t)	5	77.6 (d)	77.6 (d)	77.6 (d)	77.6 (d)
12	213.3 (s)	213.2 (s)	40.8 (t)	40.8 (t)	6	63.1 (t)	63.0 (t)	63.0 (t)	62.9 (t)
13	55.9 (s)	55.8 (s)	40.3 (s)	40.3 (s)	Glc′ 1	104.9 (d)	104.0 (d)	104.0 (d)	104.0 (d)
14	56.0 (d)	56.0 (d)	56.5 (d)	56.5 (d)	2	76.2 (d)	75.2 (d)	75.2 (d)	75.2 (d)
15	31.9 (t)	31.8 (t)	32.6 (t)	32.5 (t)	3	77.8 (d)	86.8 (d)	86.9 (d)	86.9 (d)
16	79.8 (d)	79.8 (d)	81.3 (d)	81.2 (d)	4	71.1 (d)	69.2 (d)	69.2 (d)	69.2 (d)
17	55.2 (d)	55.1 (d)	64.0 (d)	64.0 (d)	5	78.6 (d)	78.5 (d)	78.5 (d)	78.5 (d)
18	16.4 (q)	16.4 (q)	16.9 (q)	16.9 (q)	6	62.5 (t)	62.2 (t)	62.2 (t)	62.2 (t)
19	11.9 (q)	11.8 (q)	12.4 (q)	12.4 (q)	Xyl 1	105.0 (d)	105.0 (d)	104.9 (d)	
20	41.3 (d)	41.3 (d)	41.2 (d)	41.2 (d)	2	75.2 (d)	75.2 (d)	75.3 (d)	
21	15.4 (q)	15.4 (q)	16.6 (q)	16.6 (q)	3	78.6 (d)	78.6 (d)	78.6 (d)	
22	111.0 (s)	110.8 (s)	110.8 (s)	110.8 (s)	4	70.8 (d)	70.8 (d)	70.8 (d)	
23	37.2 (t)	37.2 (t)	37.3 (t)	37.3 (t)	5	67.4 (t)	67.2 (t)	67.3 (t)	
24	28.5 (t)	28.5 (t)	28.5 (t)	28.5 (t)	Xyl′ 1		106.2 (d)	106.2 (d)	106.1 (d)
25	34.4 (d)	34.3 (d)	34.4 (d)	34.3 (d)	2		75.4 (d)	75.5 (d)	75.2 (d)
26	75.3 (t)	75.2 (t)	75.3 (t)	75.2 (t)	3		77.3 (d)	77.6 (d)	77.6 (d)
27	17.6 (q)	17.5 (q)	17.6 (q)	17.6 (q)	4		70.8 (d)	70.8 (d)	70.8 (d)
					5		67.2 (t)	67.2 (t)	67.2 (t)
					Glc″1				104.6 (d)
					2				75.5 (d)
					3				78.6 (d)
					4				71.8 (d)
					5				78.5 (d)
					6				62.5 (t)
					Glc_{26} 1	105.0 (d)	105.0 (d)	104.9 (d)	104.9 (d)
					2	75.5 (d)	75.4 (d)	75.5 (d)	75.5 (d)
					3	78.5 (d)	78.6 (d)	78.6 (d)	78.6 (d)
					4	71.8 (d)	71.8 (d)	71.8 (d)	71.7 (d)
					5	78.6 (d)	78.6 (d)	78.5 (d)	78.5 (d)
					6	62.9 (t)	62.9 (t)	62.9 (t)	62.9 (t)

Table 3. Cytotoxic Activities of **1**−**14** against Hela Cells

compound	IC_{50} (μg/mL)	compound	IC_{50} (μg/mL)	compound	IC_{50} (μg/mL)
1	>20	**6**	5.36	**11**	3.54
2	>20	**7**	8.61	**12**	7.20
3	7.86	**8**	8.17	**13**	7.50
4	5.21	**9**	4.02	**14**	18.83
5	20.00	**10**	5.10	cisplatin	0.75

4.47 (1H, m, H-16), 0.65 (3H, s, H-18), 1.11 (3H, s, H-19), 1.51 (3H, d, J = 6.0 Hz, H-21), 4.21, 3.82, (1H each, m, H-26), 0.96 (3H, d, J = 6.3 Hz, H-27), 4.84 (1H, d, J = 6.6 Hz, H-Gal-1), 5.14 (1H, d, J = 6.9 Hz, H-Glc-1), 5.51 (1H, d, J = 6.3 Hz, H-Glc′-1), 5.18 (1H, d, J = 7.4 Hz, H-Xyl-1), 4.77 (1H, d, J = 7.7 Hz, H-Glc_{26}-1); ^{13}C NMR (pyridine-d_5), see Table 2; FABMS (negative mode) m/z 1228 $[M]^-$, 1066 $[M - 162(hexosyl)]^-$, 771 $[M - H - 132(pentosyl) - 162(hexosyl) - 162(hexosyl)]^-$; HRFABMS m/z 1227.5735 $[M - H]^-$ (calcd for $C_{56}H_{91}O_{29}$, 1227.5646).

Acid Hydrolysis of 3. Compound **3** (35 mg) was refluxed with 1 mol/L HCl−dioxane (1:1, v/v, 4 mL) on a water bath for 6 h. The reaction mixture was evaporated to dryness. The dry reaction mixture was partitioned between $CHCl_3$ and H_2O four times. The $CHCl_3$ extract was concentrated and chromatographed on silica gel to give hecogenin (23 mg), identified by ^{13}C NMR comparison with the reference data, and D-glucose, D-galactose, and D-xylose as sugar moieties by GC analysis.

Enzymatic Hydrolysis of 3. Compound **3** (28 mg) and β-glucosidase (Sigma) in a NaOAc−HOAc buffer (pH 5.0) were incubated at 25 °C for 16 h. The resulting precipitate was treated with *n*-butanol. The *n*-butanol extract was purified on silica gel to give **8** (6 mg).

Compound 4: white amorphous powder; $[\alpha]_D^{18.1}$ −23.53 (c 0.0340, pyridine); IR (KBr) ν_{max} 3435, 2928, 1704, 1424, 1375, 1161, 1075, 1040, 894 cm^{-1}; ^1H NMR (pyridine-d_5) δ 3.77 (1H, H-3), 4.52 (1H, m, H-16), 0.63 (3H, s, H-18), 1.11 (3H, s, H-19), 1.52 (3H, d, J = 6.7 Hz, H-21), 4.21, 3.84, (1H each, m, H-26), 0.96 (3H, d, J = 6.5 Hz, H-27), 4.83 (1H, d, J = 7.0 Hz, H-Gal-1), 5.14 (1H, d, J = 7.0 Hz, H-Glc-1), 5.53 (1H, d, J = 6.2 Hz, H-Glc′-1), 5.05 (1H, d, J = 7.0 Hz, H-Xyl′-1), 5.11 (1H, d, J = 7.7 Hz, H-Xyl-1), 4.77 (1H, d, J = 7.7 Hz, H-Glc_{26}-1); ^{13}C NMR (pyridine-d_5), see Table 2; FABMS (negative mode) m/z 1360 $[M]^-$, 1228 $[M - 132(pentosyl)]^-$, 1066 $[M - 132(pentosyl) - 162(hexosyl)]^-$, 934 $[M - 132(pentosyl) - 162(hexosyl) - 132(pentosyl)]^-$, 771 $[M - H - 132(pentosyl) - 162(hexosyl) - 132(pentosyl) - 162(hexosyl)]^-$; HRFABMS m/z 1359.6039 $[M - H]^-$ (calcd for $C_{61}H_{99}O_{33}$, 1359.6069).

Acid Hydrolysis of 4. Compound **4** (206 mg) was subjected to acid hydrolysis as described for **3** to afford hecogenin (163 mg), and D-glucose, D-galactose, and D-xylose as sugar moieties by GC analysis.

Enzymatic Hydrolysis of 4. Compound **4** (75 mg) was subjected to enzymatic hydrolysis with β-glucosidase as described for **3** to give **9** (18 mg).

Compound 5: white amorphous powder; $[\alpha]_D^{19.8}$ −37.18 (c 0.0390, pyridine); IR (KBr) ν_{max} 3413, 2927, 1699, 1373, 1161, 1074, 1040, 894 cm^{-1}; ^1H NMR (pyridine-d_5) δ 3.77 (1H, H-3), 4.94 (1H, q-like, J = 7.3 Hz, H-16), 0.86 (3H, s, H-18), 0.61 (3H, s, H-19), 1.32 (3H, d, J = 6.9 Hz, H-21), 4.38, 3.93 (1H each, m, H-26), 0.96 (3H, d, J = 6.8 Hz, H-27), 4.91 (1H, d, J = 6.9 Hz, H-Gal-1), 5.17 (1H, d, J = 7.7 Hz, H-Glc-1), 5.56 (1H, d, J = 7.7 Hz, H-Glc′-1), 5.08 (1H, d, J = 7.3 Hz, H-Xyl′-1), 5.14 (1H, d, J = 7.7 Hz, H-Xyl-1), 4.87 (1H, d, J = 7.7 Hz, H-Glc_{26}-1); ^{13}C NMR (pyridine-d_5), see Table 2; FABMS (negative mode) m/z 1346 $[M]^-$, 1214 $[M - 132(pentosyl)]^-$, 1051

[M − H − 132(pentosyl) − 162(hexosyl)]$^-$, 919 [M − H − 132-(pentosyl) − 162(hexosyl) − 132(pentosyl)]$^-$, 757 [M − H − 132(pentosyl) − 162(hexosyl) − 132(pentosyl) − 162 (hexosyl)]$^-$; HRFABMS *m/z* 1345.6194 [M − H]$^-$ (calcd for $C_{61}H_{101}O_{32}$, 1345.6276).

Acid Hydrolysis of 5. Compound **5** (100 mg) was subjected to acid hydrolysis as described for **3** to afford tigogenin (72 mg), D-glucose, D-galactose, and D-xylose.

Enzymatic Hydrolysis of 5. Compound **5** (86 mg) was subjected to enzymatic hydrolysis with *β*-glucosidase as described for **3** to give **12** (20 mg).

Compound 6: white amorphous powder; $[\alpha]_D^{19.7}$ −35.26 (*c* 0.0390, pyridine); IR (KBr) ν_{max} 3412, 2927, 1704, 1649, 1453, 1425, 1376, 1160, 1075, 1040, 894 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 3.78 (1H, H-3), 4.92 (1H, m, H-16), 0.86 (3H, s, H-18), 0.63 (3H, s, H-19), 1.31 (3H, d, J = 6.3 Hz, H-21), 4.34, 3.81 (1H each, m, H-26), 0.96 (3H, d, J = 6.6 Hz, H-27), 4.86 (1H, d, J = 4.4 Hz, H−Gal-1), 5.11 (1H, d, J = 5.5 Hz, H-Glc-1), 5.54 (1H, H-Glc′-1), 5.18 (1H, d, J = 7.7 Hz, H-Glc″-1), 5.07 (1H, H-Xyl-1), 4.78 (1H, d, J = 7.7 Hz, H-Glc_{26}-1); ^{13}C NMR (pyridine-d_5), see Table 2; FABMS (negative mode) *m/z* 1376 [M]$^-$, 1214 [M − 162(hexosyl)]$^-$, 1082 [M − 132(pentosyl) − 162(hexosyl)]$^-$, 757 [M − H − 132(pentosyl) − 162(hexosyl) − 162(hexosyl) − 162(hexosyl)]$^-$; HRFABMS *m/z* 1375.6420 [M − H]$^-$ (calcd for $C_{62}H_{103}O_{33}$, 1375.6381).

Acid Hydrolysis of 6. Compound **6** (32 mg) was subjected to acid hydrolysis as described for **3** to afford tigogenin (22 mg), and D-glucose, D-galactose, and D-xylose.

Enzymatic Hydrolysis of 6. Compound **6** (21 mg) was subjected to enzymatic hydrolysis with *β*-glucosidase as described for **3** to gave tigogenin 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (4 mg), identified by ^{1}H and ^{13}C NMR data.

Cytotoxicity against HeLa Cells. The cytotoxicity against HeLa cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay on 96-well microplates.[17] Briefly, 196 *μ*L 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 °C. After incubation, 4 *μ*L of DMSO solution containing the sample was added to give the final concentration of the samples (25, 20, 15, 10, 5, 2.5, 1.25, 0.80 *μ*g/mL); 4 *μ*L of DMSO was added into control wells. The cells were further incubated for 72 h, and then 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 (*μ*g/mL), which was the mean of three determinations and reduced the viable cell number by 50%.

Acknowledgment. The authors are grateful to members of the Analytical Group in State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for measurements of all spectra. We are also grateful to Professor Xingcong Li (University of Mississippi) for his help.

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NP034028A