Steroidal Saponins from *Solanum nigrum*

Xinlan Zhou,[†] Xiangjiu He,[‡] Guanghui Wang,[†] Hao Gao,[†] Guangxiong Zhou,[‡] Wencai Ye,[‡] and Xinsheng Yao*[,†,‡]

Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, 110016, People's Republic of China, and Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou, 510632, People's Republic of China

Received March 3, 2006

Six new steroidal saponins, solanigrosides C−H (**2**−**7**), and one known saponin, degalactotigonin (**1**), were isolated from the whole plant of *Solanum nigrum*. Their chemical structures were elucidated using spectroscopic analysis, chemical degradation, and derivatization. All seven compounds were tested for their cytotoxicity using four human tumor cell lines (HepG2, NCI-H460, MCF-7, SF-268). Only compound **1** was cytotoxic, with IC_{50} values of 0.25−4.49 μM.

The plant *Solanum nigrum* L., a popular medicinal herb in China, is believed to have various therapeutic properties, especially against certain types of cancer. Its therapeutic mechanism remains unknown. In a previous study, we have identified three steroidal glycosides (β_2-solamargine, solamargine, and degalactotigonin) from this plant, all of which exhibited cytotoxicity in six cultured human solid tumor cell lines: HT-29, HCT-15, LNCaP, PC-3, T47D, and MDA-MB-231.[1] The current study was conducted to extend our search for cytotoxic saponins from this plant. This paper describes the structure elucidation of six new saponins, solanigrosides C−H (**2**−**7**), isolated from the extract of the whole plant and their cytotoxicity against four human tumor cell lines.

Results and Discussion

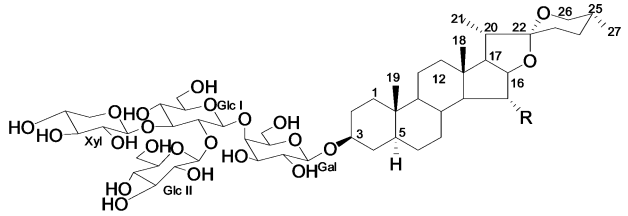
Compound **1** was obtained as a white, amorphous power. It showed a quasi-molecular ion peak of $[M + Na]^+$ at *m*/*z* 1057. The ^1H and ^{13}C NMR data of this compound were identical to those of degalactotigonin, namely, (3β,5α,25*R*)-spirostan-3-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-galactopyranoside.[1]

The molecular formula of compound **2**, solanigroside C, was deduced as $C_{51}H_{82}O_{26}$ by HRESIMS (*m*/*z* 1133.4938 $[M + Na]^+$, calcd 1133.4992). Its IR spectrum showed an absorption band at 1757 cm^{-1}, suggesting the presence of a δ-lactone carbonyl group. The ^1H NMR spectrum displayed signals for two tertiary methyl groups at δ 0.64 (3H, s) and 0.96 (3H, s), two secondary methyls at δ 1.13 (3H, d, J = 6.4 Hz) and 1.26 (3H, d, J = 6.9 Hz), attributable to a steroidal aglycone moiety, and four anomeric signals appearing at δ 5.56 (d, J = 7.8 Hz), 5.28 (d, J = 7.8 Hz), 5.13 (d, J = 7.9 Hz), and 4.84 (d, J = 7.9 Hz). Additionally, the ^{13}C NMR spectrum showed an ester carbonyl resonance at δ 180.4, four oxygenated methines at δ 77.3, 77.5, 78.8, and 91.9, and a spiroketal carbon at δ 109.7, which was closely analogous to tigogenin glycoside.[3] In the $^1H-^1H$ COSY spectrum, there were correlations in a sequence of H_3-21 [δ 1.26 (3H, d)], H-20 [δ 2.75 (1H, m)], H-17 [δ 2.11 1H, (m)], H-16 [δ 4.96 (1H, dd, J = 3.9, 9.1 Hz)], and H-15 [δ 4.24 (1H, m)] and in a sequence of H_3-27 [δ 1.13 (3H, d, J = 6.4 Hz), H-25 [δ 2.96 (1H, overlapped)], H-24 [δ 2.97 (1H, overlapped) and 1.92 (1H, m)], and H-23 [4.67 (1H, br d, J = 8.3 Hz)]. This indicated that two hydroxyl groups were attached to C-15 (δ 78.8) and C-23 (δ 77.5) of tigogenin. The location of the lactone ring was determined from the HMBC spectrum. The cross-peaks observed for H_3-27/C-26 (δ 180.4) and H-24, H-25/C-26 were consistent with the presence of the F-ring lactone moiety. ROESY correlations of H-18/H-15, H-20; H-23/H_3-21, H-20; and H-27/H_2-24 indicated that the orientations of the hydroxyl groups at C-15 and C-23 and the methyl group at C-25 were α. The chemical shift for C-20 (δ 37.1) supports an *R*-configuration at C-22, since C-20 for a 22*S*-isomer showed a 6 ppm downfield shift, compared to the corresponding value in a 22*R*-isomer. On the basis of these data, the structure of the aglycone was identified as (22*R*,25*R*)-3β,15α,23α-trihydroxy-5α-spirostan-26-one. The monosaccharides obtained after acid hydrolysis of **2** were derivatized into aldononitrile peracetate derivatives and analyzed by GC-MS using authentic samples as references. Glucose and galactose in the relative proportions of 3:1 were detected. The absolute configurations of the sugar residues were assumed to be D-galactose and D-glucose. These assumptions were based on the usual configuration of naturally occurring monosaccharides. NMR coupling constants ($^3J_{1,2}$ > 7 Hz) for anomeric protons indicated that the anomeric carbon configuration was β for the D-galactopyranosyl and D-glucopyranosyl moieties. The attachment points of the sugar chain and interglycosidic linkage were established by an HMBC experiment. Long-range correlations were observed between H-1 (δ 4.84) of galactosyl and C-3 (δ 77.3) of the aglycone, H-1 (δ 5.13) of glucosyl I and C-4 (δ 80.0) of galactosyl, H-1 (δ 5.56) of glucosyl II and C-2 (δ 81.2) of glucosyl I, and H-1 (δ 5.28) of glucosyl III and C-3 (δ 88.3) of glucosyl I. The ^{13}C NMR chemical shifts of the sugar chain were in good agreement with reported data.[5] On the basis of the above data, the structure of **2** was established as (22*R*,25*R*)-3β,15α,23α-trihydroxy-5α-spirostan-26-one 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-galactopyranoside.

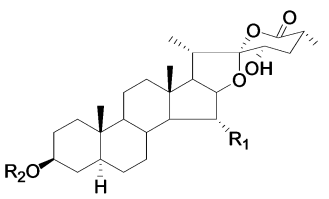
The molecular formula of compound **3** was deduced as $C_{55}H_{88}O_{27}$ by HRESIMS (*m*/*z* 1203.5400 $[M + Na]^+$, calcd. 1203.5411). The IR spectrum showed an absorption band at 1757 cm^{-1}, suggesting the presence of a δ-lactone carbonyl group. The ^1H NMR spectrum of **3** displayed two tertiary methyl groups at δ 0.85 (3H, s) and 0.90 (3H, s), three secondary methyls at δ 1.19 (3H, d, J = 7.2 Hz), 1.29 (3H, d, J = 6.9 Hz), and 1.73 (3H, d, J = 6.1 Hz), and five anomeric protons at δ 4.87 (1H, d, J = 7.7 Hz), 4.97 (1H, d, J = 7.9 Hz), 5.21 (1H, d, J = 7.8 Hz), 5.34 (1H, d, J = 7.6 Hz), and 6.31 (1H, br s). In the ^{13}C NMR spectrum of **3**, the chemical shifts of the aglycone moiety and signals due to C-1−13 and C-19−27 were similar to those of **2**, whereas signals due to C-14−18 were similar in chemical shifts to those of **1**. These data suggest that the aglycone in **3** should be a 15-deoxy derivative of **2**. Acid hydrolysis of **3** with 2 M HCl was followed by GC-MS analysis of its aldononitrile peracetate derivatives using authentic samples as references. This resulted in D-glucose, D-galactose, L-rhamnose, D-xylose, and L-arabinose in a ratio of 1:1:1:1:1. In the HMBC spectrum, the anomeric protons at δ 4.87 (H-1 of the galactosyl), 4.97 (H-1 of the glucosyl), 6.31 (H-1 of the rhamnosyl), 5.34 (H-1 of the arabinosyl), and 5.21 (H-1 of the xylosyl) showed long-range correlations with carbon signals at δ 77.2 (C-3 of the

* To whom correspondence should be addressed. Tel: +86-20-85225849. Fax: +86-20-85225849. E-mail: yaoxinsheng@vip.tom.com.
[†] Shenyang Pharmaceutical University.
[‡] Jinan University.

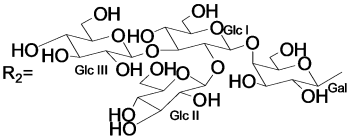
10.1021/np060091z CCC: $33.50
Published on Web 07/26/2006



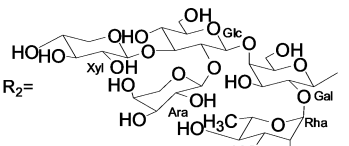
1 R=H
6 R=OH



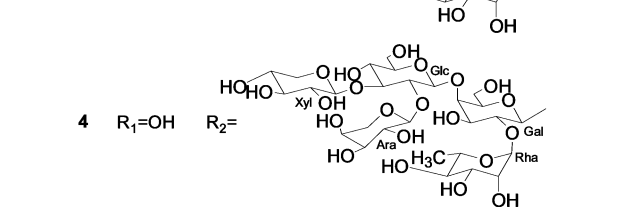
2 R_1=OH R_2=



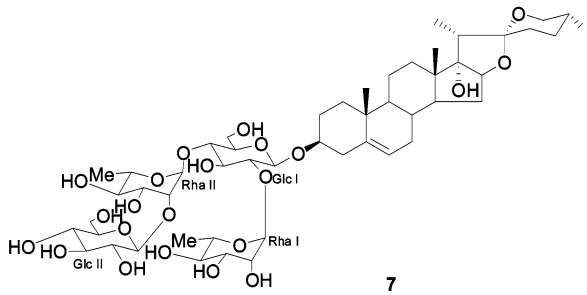
3 R_1=H R_2=



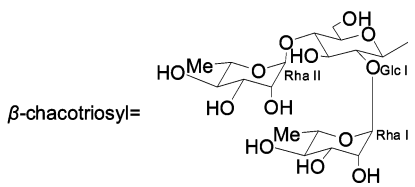
4 R_1=OH R_2=



5



7



β-chacotriosyl=

aglycone), 81.5 (C-4 of the galactosyl), 77.4 (C-2 of the galactosyl), 81.5 (C-2 of the glucosyl), and 87.9 (C-3 of the glucosyl), respectively. The sugar portion of **3** was identical to that of nigrumnin I[6] by comparison of the ^{13}C NMR chemical shifts in the same solvent. Consequently, the structure of **3** was determined as (22*R*,25*R*)-3*β*,23α-dihydroxy-5α-spirostan-26-one 3-*O*-α-L-arabinopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-O-[α-L-rhamnopyranosyl-(1→2)]-O-*β*-D-galactopyranoside and named solanigroside D.

The positive-ion HRESIMS of compound **4** displayed a pseudomolecular ion at *m*/*z* 1219.5392 ([M + Na]$^+$, calcd. 1219.5360), indicating the molecular formula $C_{55}H_{88}O_{28}$. The ^{1}H NMR spectrum of **4** showed signals for two tertiary methyl groups at *δ* 0.89 (3H, s) and 0.98 (3H, s), three secondary methyls at *δ* 1.16 (3H, d, *J* = 6.4 Hz), 1.28 (3H, d, *J* = 6.9 Hz), and 1.71 (3H, d, *J* = 6.2 Hz), and five anomeric protons at *δ* 4.84 (1H, d, *J* = 7.7 Hz), 4.99 (1H, d, *J* = 7.9 Hz), 5.21 (1H, d, *J* = 7.8 Hz), 5.33 (1H, d, *J* = 7.7 Hz), and 6.29 (1H, br s). Analysis of the ^{13}C NMR spectrum of **4** and comparison with those of **2** and **3** revealed that it possessed the aglycone moiety identical to that of **2** and the sugar chain identical to that of **3**. The structure of **4** was thus determined as (22*R*,25*R*)-3*β*,15α,23α-trihydroxy-5α-spirostan-26-one 3-*O*-α-L-arabinopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-*O*-*β*-D-galactopyranoside and named solanigroside E.

The positive-ion HRESIMS of compound **5** showed an accurate [M + Na]$^+$ ion at *m*/*z* 1235.5642 (calcd 1235.5673), corresponding to the molecular formula $C_{56}H_{92}O_{28}$. The ^{1}H NMR spectrum of **5** displayed two tertiary methyl groups at *δ* 0. 57 (3H, s) and 1.11 (3H, s), two secondary methyls at *δ* 0.60 (3H, d, *J* = 6.4 Hz) and 1.25 (3H, d, *J* = 6.9 Hz), and five anomeric protons at *δ* 4.87 (1H, d, *J* = 7.6 Hz), 4.99 (1H, overlapped), 5.20 (1H, d, *J* = 7.9 Hz), 5.24 (1H, d, *J* = 7.7 Hz), and 5.57 (1H, d, *J* = 7.3 Hz). Acid hydrolysis of **5** with 2 M HCl was followed by GC-MS analysis of its aldononitrile peracetate derivatives using authentic samples as references. The results indicated D-glucose, D-galactose, and D-xylose in a ratio of 3:1:1. In the ^{13}C NMR spectrum of **5**, the chemical shifts of the aglycone moiety, except for the signals arising from C-20, C-23, and C-24, and the suger moiety linked to C-3 were similar to those of **1**. In the ^{1}H−^{1}H COSY spectrum, there were correlations in a sequence of H_3-27 [*δ* 0.60], H-25 [*δ* 1.80 (m)], H-24 [*δ* 1.90 (m) and 2.42 (m)], and H-23 [*δ* 4.08]. The evidence indicated one more hydroxyl group attached to C-23 (*δ* 76.2) on tigogenin. ROESY correlations from H-23 to H-25 suggested the hydroxyl group at C-23 in an α-orientation. The attachment points of the sugar chain and interglycosidic linkage were established by an HMBC experiment. Long-range correlations were observed between H-1 (*δ* 4.87) of galactosyl and C-3 (*δ* 77.5) of the aglycone, H-1 (*δ* 5.20) of glucosyl I and C-4 (*δ* 79.9) of galactosyl, H-1 (*δ* 5.57) of glucosyl II and C-2 (*δ* 81.4) of glucosyl I, H-1 (*δ* 5.24) of xylosyl and C-3 (*δ* 86.8) of glucosyl I, and H-1 (*δ* 4.99) of glucosyl III and C-23 (*δ* 76.2), respectively. From the above evidence, the structure of **5** was concluded as 23-*O*-*β*-D-glucopyranosyl-(25*R*)-3*β*,23α-dihydroxy-5α-spirostan-3-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranoside.

The molecular formula of compound **6** was deduced as $C_{50}H_{82}O_{23}$ by HRESIMS (*m*/*z* 1073.5151 [M + Na]$^+$, calcd 1073.5145). The ^{1}H NMR spectrum of **6** displayed two tertiary methyl groups at *δ* 0.68 (3H, s) and 0.91 (3H, s), two secondary methyls at *δ* 0.66 (3H, d, *J* = 5.6 Hz) and 1.14 (3H, d, *J* = 6.6 Hz), and four anomeric protons at *δ* 4.89 (1H, d, *J* = 7.6 Hz), 5.21 (1H, d, *J* = 7.9 Hz), 5.26 (1H, d, *J* = 7.7 Hz), and 5.59 (1H, d, *J* = 7.3 Hz). Analysis of the ^{13}C NMR spectrum of **6** and comparison with those of **1** and **2** revealed that it possessed a sugar chain identical to that of **1**. The signals due to C-1−13 and C-19−27 of the aglycone moiety showed chemical shifts similar to those of **1**, but signals due to C-14−18 were similar to those of **2**, suggesting that the aglycone in **6** should be a 15-hydroxy derivative of **1**. Consequently, the structure of **6** was determined as (25*R*)-3*β*,15α-dihydroxy-5α-

Table 1. ^{1}H NMR (δ) Data of Compounds **2**−**7** (400 MHz, in pyridine-d_5)

position	**2**	**3**	**4**	**5**	**6**	**7**
1	1.50	1.56 m	1.55	1.46	1.55	1.75
	0.75 m	0.79 m	0.78 m	0.76	not observed	0.95
2	1.98 m	2.03 m	2.01 m	2.00	2.05 m	2.00 m
	1.57	1.78 m	1.59	1.56	1.57	1.86 m
3	3.83	3.91 m	3.83	3.89	3.90	3.82 m
4	1.70	1.91	1.72	1.75	1.76	2.74 br s
	1.32	1.67	1.35	1.29	1.36 m	
5	0.90	0.90	0.94	0.88 m	0.96 m	
6	1.19	1.18	1.20	1.04	1.21 m	5.26
				0.91		
7	2.52 m	1.51 m	2.54 m	1.46	2.60 m	1.93 m
	1.29	0.77 m	1.31	0.76	not observed	1.50
8	1.69	1.40	1.71	1.35	1.78	1.50
9	0.59 m	0.48 m	0.63 m	0.48 m	0.66	0.98
10						
11	1.34	1.38	1.36	1.35	1.43 m	1.59
	1.11	1.21	1.13	1.14		1.50
12	1.60	1.67	1.63	1.69	1.62	1.68
	1.07 m	1.01	1.09	1.05	1.18	1.62
13						
14	1.42	1.00	1.44	1.04	1.48 m	2.07
15	4.24	1.96	4.27	2.00	4.38	2.16 m
		1.29		1.46		1.50
16	4.96 dd (9.1, 3.9)	4.87 m	4.99	4.61	4.67 m	4.46 m
17	2.11 m	1.89 m	2.13 m	1.90	2.04 m	
18	0.96 s	0.90 s	0.98 s	1.11 s	0.91 s	0.94 s
19	0.64 s	0.85 s	0.89 s	0.57 s	0.68 s	1.07 s
20	2.75 m	2.71 m	2.76 m	3.24 m	2.01 m	2.26 d (7.1)
21	1.26 d (6.9)	1.29 d (6.9)	1.28 d (6.9)	1.25 d (6.9)	1.14 d (6.6)	1.21 d (7.2)
22						
23	4.67 br d (8.3)	4.67 m	4.69	4.08	1.68 m	2.22 m
						1.68
24	2.97	2.98 m	2.97	2.42 m	1.57	1.58
	1.92 m	1.94	1.94 m	1.90		
25	2.96	3.09 m	2.97	1.80 m	1.57	1.58
26				3.50 m	3.48 m	3.49 d (7.3)
					3.37 m	
27	1.13 d (6.4)	1.19 d (7.2)	1.16 d (6.4)	0.60 d (6.4)	0.66 d (5.6)	0.66 d (5.7)
	Gal	Gal	Gal	Gal	Gal	Glc I
1	4.84 d (7.9)	4.87 d (7.7)	4.84 d (7.7)	4.87 d (7.6)	4.89 d (7.6)	4.91
2	4.40	4.41 m	4.42 m	4.41	4.43	4.19
3	4.09	4.15	4.18	4.11	4.02	4.19
4	4.57	4.50 m	4.49	4.57	4.63	4.40 m
5	4.00	3.96	3.95	4.09	4.10	3.64 br d (10.1)
6	4.67	4.73 m	4.72	4.70 m	4.71 m	4.28
	4.20	4.20	4.22	4.22	4.23	
	Glc I	Glc	Glc	Glc I	Glc I	Rha I
1	5.13 d (7.9)	4.97 d (7.9)	4.99 d (7.9)	5.20 d (7.9)	5.21 d (7.9)	6.44 br s
2	4.36	4.25 m	4.29	4.43	4.45	4.84 m
3	4.18	4.07	4.08	4.20	4.19 m	4.61 m
4	3.82	3.81	3.81	3.84	3.86 m	4.36 m
5	3.82	3.82	3.83	3.90	3.90	4.94
6	4.54	4.52	4.51	4.55	4.59	1.75 d (6.2)
	4.24	4.03	4.04	4.39	4.38	
	Glc II	Ara	Ara	Glc II	Glc II	Rha II
1	5.56 d (7.8)	5.34 d (7.6)	5.33 d (7.7)	5.57 d (7.3)	5.59 d (7.3)	6.08 br s
2	4.02	4.42	4.44	4.09	4.11	4.64 m
3	4.16	4.02	4.03	4.08	4.11	4.50 m
4	4.15	4.17	4.20	4.21	4.22	4.18
5	3.82	4.67	4.69	4.21	4.11	4.94
		3.58 br d (12.2)	3.58 m			
6	4.54			4.52	4.53 m	1.59 d (6.1)
	4.35			3.92	4.07	
	Glc III	Xyl	Xyl	Xyl	Xyl	Glc II
1	5.28 d (7.8)	5.21 d (7.8)	5.21 d (7.8)	5.24 d (7.7)	5.26 d (7.7)	5.25 d (7.6)
2	4.02	3.96	3.98	3.96	3.98	4.08 m
3	4.11	4.07	4.09	4.09	4.10	3.93 m
4	4.23	4.11 m	4.12	4.10	4.11	4.07 m
5	4.02	4.21	4.25	4.22	4.25	4.19
		3.65 m	3.67 m	3.68 t (10.2)	3.69 m	

Table 1 (Continued)

position	2	3	4	5	6	7
6	4.50 4.00					4.55 4.27
		Rha	Rha	Glc III		
		6.31 br s	6.29 br s	4.99		
		4.79 m	4.77 m	4.02		
		4.55 m	4.57 m	3.97		
		4.32 m	4.36 m	4.25		
		4.93 m	4.93 m	4.25		
		1.73 d (6.1)	1.71 d (6.2)	4.52		
				4.39		

spirostan-3-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-galactopyranoside.

Solanigroside H, **7**, had the molecular formula $C_{51}H_{82}O_{22}$, as determined by HRESIMS in the positive-ion mode (HRESIMS *m*/*z* $[M + Na]^+$ 1069.5226, calcd for $C_{51}H_{82}O_{22}Na$, 1069.5195). The ^{1}H NMR spectrum of **7** displayed two tertiary methyl groups at *δ* 0.94 (3H, s) and 1.07 (3H, s), four secondary methyls at *δ* 0.66 (3H, d, *J* = 5.7 Hz), 1.21 (3H, d, *J* = 7.2 Hz), 1.59 (3H, d, *J* = 6.1 Hz), and 1.75 (3H, d, *J* = 6.2 Hz), an olefinic proton at *δ* 5.26, and four anomeric protons at *δ* 4.91 (1H, overlapped), 5.25 (1H, d, *J* = 7.6 Hz), 6.08 (1H, br s), and 6.44 (1H, br s). In the ^{13}C NMR spectrum of **7**, the chemical shifts of the aglycone moiety showed two olefinic carbons at *δ* 140.9 and 121.8, two oxygenated methines at *δ* 78.0 and 90.1, one oxygenated quaternary carbon at *δ* 90.0, and a spiroketal carbon at *δ* 109.7. In the HMBC experiment, the methyl proton at *δ* 1.07 (Me-19) showed long-range correlations with the carbon at *δ* 140.9, and the olefinic proton at *δ* 5.26 with the carbon at *δ* 37.1 (C-10). This evidence indicated the presence of a double bond ($\Delta^{5,6}$) in the B ring. Moreover, a signal due to C-17 usually at around *δ* 63 was observed at *δ* 90.0, indicating the presence of a hydroxy group at C-17. Since the signals due to C-1−21 were almost identical to those of SNF-10,[7] the configuration at C-17 was suggested to be *S*. Comparison of ^{13}C NMR data of the sugar moieties in **7** with those of *β*-chacotriosyl[8] indicated that C-2 of the rhamnosyl II in **7** has shifted downfield to *δ* 82.2 from *δ* 72.6 in *β*-chacotriosyl. This significant glycosylation shift clearly showed that glucosyl II was linked to the C-2 of rhamnosyl II. This linkage was further confirmed by long-range correlations observed between H-1 (*δ* 6.44) of rhamnosyl I and C-2 (*δ* 77.7) of glucosyl I, H-1 (*δ* 6.08) of rhamnosyl II and C-4 (*δ* 78.2) of glucosyl I, and H-1 (*δ* 5.25) of glucosyl II and C-2 (*δ* 82.2) of rhamnosyl II, respectively. Although the correlation between H-1 (*δ* 4.91) of glucosyl I and C-3 (*δ* 78.0) of the aglycone was not observed, the suger moiety was suggested to connect to C-3 of the aglycone due to the glycosylation shift of C-3 (*δ* 78.0 from around *δ* 70.6 of tigogenin), Therefore, the structure of **7** was determined as (25*R*)-3*β*,17α-dihydroxyspirostan-5-ene-3-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-α-L-rhamnnosyl-(1→4)-*O*-[α-L-rhamnnosyl-(1→2)]-*O*-*β*-D-glucopyranoside.

Steroidal glycosides **1**−**7** were evaluated for their cytotoxicity in vitro against human tumor cell lines HepG2 (human liver carcinoma), NCI-H460 (human lung carcinoma), MCF-7 (human breast carcinoma), and SF-268 (human glioma) with 10-hydroxycamptothecine as positive control. **1** showed considerable cytotoxicity, with IC_{50} values of 0.25 ± 0.59 *μ*M to HepG2, 4.49 ± 1.69 *μ*M to NCI-H460, 1.57 ± 0.85 *μ*M to MCF-7, and 3.19 ± 2.03 *μ*M to SF-268, whereas 10-hydroxycamptothecine showed cytotoxicity with IC_{50} values of 6.49 ± 0.86 *μ*M to HepG2, 38.55 ± 2.35 *μ*M to NCI-H460, 19.12 ± 0.98 *μ*M to MCF-7, and 29.88 ± 1.23 *μ*M to SF-268. **5** and **6**, the corresponding 23-O-Glc and 15-OH derivatives of **1**, did not show any cell growth inhibitory activity, suggesting that the structure of the aglycone moiety contributed to the cytotoxicity. Compounds **2**−**4** and **7** did not show any cell growth inhibitory activity either. Since their aglycone and suger moieties varied in comparison with **1**, there was no evidence to speculate whether the suger moieties contributed to the cytotoxicity.

Experimental Section

General Experimental Procedures. Melting points were determined with an X-5 hot stage microscope melting point apparatus (uncorrected). Optical rotations were obtained on a P-1020 digital polarimeter (JASCO corporation). IR spectra were measured on a JASCO FT/IR-480 plus instrument. 1D and 2D NMR spectra were recorded on a Bruker AV-400 spectrometer in C_5D_5N solution. ESIMS spectra were acquired using a Bruker Esquire 2000 mass spectrometer. HRESIMS spectra were recorded using a Micromass Q-TOF mass spectrometer. Column chromatography was done on Diaion D-101 (Mitsubishi Kasei), silica gel (200−300 mesh, Qingdao Factory of Marine Chemical Industry, Qingdao, China), and ODS (40−63 *μ*m, Merck). TLC was performed using Merck TLC plates precoated silica gel 60 F_{254}, and the spots were detected by spraying with 10% H_2SO_4−EtOH and heating. Preparative HPLC was performed using an ODS column (19 mm × 300 mm, 10 *μ*m, XTerra Prep. Rp18, detector: RID). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO). RPMI-1640 medium, fetal bovine serum (FBS), and trypsin-EDTA solution (1×) were obtained from GIBCO-BRL (Grand Island, NY).

Plant Material. The herb *S. nigrum* L. was collected in the suburb of Shenyang (Liaoning Province) in June 2003 and identified by Prof. Qishi Sun (Division of Pharmacognosy, Shenyang Pharmaceutical University, China). A voucher specimen (No. 250) is available at the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang (110016), China.

Extraction and Isolation. The dried, whole plant of *S. nigrum* (19.8 kg) was extracted with 60% EtOH (200 L × 2). The solvent was removed under vacuum to yield the crude extract (3800 g). A suspension of the extract in H_2O was centrifuged and then applied to a D-101 macroresin column (120 mm × 1500 mm) and eluted with H_2O (40 L), 30% EtOH (40 L), 60% EtOH (40 L), and 95% EtOH (40 L) successively. The 60% EtOH elute (130 g) was dried and then extracted with MeOH. The MeOH extract was separated by silica gel (3000 g) using $CHCl_3$−MeOH gradient mixtures (10:0−6:4) to give 10 fractions (1−10). Compound **1** (880.5 mg, 0.0445‰ yield) was separated from fraction 7, eluted with $CHCl_3$−MeOH (7:3). Fraction 9 (57 g), eluted with $CHCl_3$−MeOH (6:4), was further separated by silica gel column chromatography eluting with $CHCl_3$−MeOH−H_2O (90:10:0.5; 80:20:1; 75:25:2; 70:30:5; 65:35:5) and then purified by ODS column chromatography eluting with MeOH−H_2O (3:7; 5:5; 7:3) and repeated RP-18 HPLC preparation to yield compounds **2** (9.5 mg, 0.0005% yield), **3** (145.1 mg, 0.0073% yield), **4** (225.1 mg, 0.0114% yield), and **5** (363.9 mg, 0.0184% yield). Fraction 8 was also further purified by silica gel column chromatography eluting with $CHCl_3$−MeOH−H_2O (80:20:1; 75:25:2; 70:30:5; 65:35:4; 60:40:5), ODS column chromatography eluting with MeOH−H_2O (6:4; 7:3), and repeated RP-18 HPLC preparation to yield compounds **6** (2.0 mg, 0.0001% yield) and **7** (5.4 mg, 0.0003% yield).

Degalactotigonin (1): white, amorphous powder, identified by comparing its ^{1}H and ^{13}C data with those in ref 1; ^{13}C NMR (pyridine-d_5, 100 MHz) spectral data, see Table 2.

Solanigroside C (2): white, amorphous powder; $[\alpha]^{25}_D$ −21.1 (*c* 0.54, MeOH); IR (KBr) ν_{max} 3419 (OH), 2932 (CH), 1757 (−C=O) cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz) data, see Table 1; ^{13}C NMR

Table 2. ^{13}C NMR (δ) Data for Compounds **1**−**7** (100 MHz, in pyridine-d_5)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	β-chacotriosyl[8]
1	37.0	37.1	37.3	37.2	37.2	37.4	37.5	
2	29.0	29.7	30.0	29.7	29.9	29.9	30.1	
3	77.2	77.3	77.2	77.6	77.5	77.4	78.0	
4	34.6	34.6	34.4	34.2	34.9	34.9	38.9	
5	44.4	44.4	44.7	44.5	44.7	44.7	140.9	
6	28.7	28.9	29.0	29.0	28.9	29.1	121.8	
7	32.2	32.6	32.4	32.6	32.4	32.9	32.4	
8	35.0	35.7	35.1	35.7	35.1	36.0	32.3	
9	54.2	54.2	54.4	54.4	54.4	54.6	50.2	
10	35.6	35.7	35.9	35.9	35.8	36.1	37.1	
11	21.1	21.0	21.2	21.0	21.3	21.3	20.9	
12	39.9	40.7	40.2	40.7	40.5	40.8	32.1	
13	40.5	41.1	41.4	41.2	41.3	40.8	45.1	
14	56.2	60.3	56.3	60.5	56.4	61.0	53.0	
15	31.9	78.8	32.4	78.8	32.0	78.8	32.1	
16	81.1	91.9	82.1	91.9	81.3	91.4	90.1	
17	62.8	60.0	63.0	60.1	62.3	60.6	90.0	
18	16.4	17.9	16.7	17.8	16.7	17.9	17.1	
19	12.1	12.2	12.4	12.3	12.2	12.5	19.4	
20	41.8	37.1	37.3	37.1	35.7	42.0	44.8	
21	14.8	15.4	15.6	15.3	14.7	15.1	9.7	
22	109.0	109.7	110.1	109.6	110.6	108.9	109.8	
23	31.6	77.5	77.9	77.5	76.2	31.8	31.8	
24	29.7	30.9	31.1	30.9	37.2	29.3	28.8	
25	30.4	33.8	34.1	33.8	31.5	30.5	30.4	
26	66.6	180.4	180.6	180.3	65.8	66.9	66.7	
27	17.1	16.0	16.3	16.0	17.3	17.3	17.3	
	Gal	Gal	Gal	Gal	Gal	Gal	GlcI	Glc (inner)
1	102.2	102.1	100.2	99.9	102.5	102.4	100.3	100.3
2	73.0	73.0	77.4	77.5	73.2	73.2	77.7	77.8
3	75.1	75.3	76.6	76.7	75.1	75.4	77.8	78.0
4	79.7	80.0	81.5	81.2	79.9	79.9	78.2	78.6
5	76.0	75.1	75.1	74.8	76.3	76.3	76.8	77.0
6	60.4	60.3	60.4	60.2	60.6	60.6	61.3	61.3
	Glc I	Glc I	Glc	Glc	Glc I	Glc I	Rha I	Rha (1→2)
1	104.6	104.8	105.5	105.1	105.2	105.2	101.9	102.0
2	80.9	81.2	81.5	81.2	81.4	81.4	72.5	72.6
3	86.6	88.3	87.9	87.4	86.8	86.8	72.8	72.9
4	70.5	70.6	70.4	70.2	70.5	70.5	74.1	74.1
5	78.5	77.1	77.7	77.6	78.7	78.7	69.5	69.5
6	62.3	62.1	62.9	62.7	62.5	62.5	18.6	18.6
	Glc II	Glc II	Ara	Ara	Glc II	Glc II	Rha II	Rha (1→4)
1	104.7	104.7	105.8	105.5	104.8	105.0	101.7	102.9
2	75.4	75.1	73.3	73.0	75.6	75.6	82.2	72.6
3	78.4	78.4	74.7	74.5	77.6	77.6	72.6	72.7
4	70.8	71.3	69.7	69.5	71.1	71.1	74.5	73.9
5	77.5	78.4	67.3	67.1	77.8	77.8	69.7	70.4
6	62.8	62.0			63.0	63.0	18.3	18.5
	Xyl	Glc III	Xyl	Xyl	Xyl	Xyl	Glc II	
1	104.9	104.3	105.0	104.8	105.0	104.9	107.3	
2	74.8	75.9	75.1	74.8	75.4	75.1	75.7	
3	77.4	77.6	78.7	78.5	78.7	78.8	78.8	
4	70.2	70.6	70.7	70.5	70.7	70.8	71.5	
5	67.1	78.4	67.3	67.1	67.3	67.4	78.3	
6		62.8					62.9	
			Rha	Rha	Glc III			
1			101.7	101.7	106.2			
2			72.4	72.2	75.4			
3			72.7	72.5	78.5			
4			74.1	73.8	71.7			
5			69.6	69.4	78.9			
6			18.5	18.2	62.8			

(pyridine-d_5, 100 MHz) data, see Table 2; ESIMS (in negative-ion mode) *m*/*z* 1109 $[M - H]^-$, 947 $[(M - H) - 162]^-$, 785 $[(M - H) - 162 - 162]^-$, 623 $[(M - H) - 162 - 162 - 162]^-$, 461 $[(M - H) - 162 - 162 - 162 - 162]^-$; HRESIMS *m*/*z* 1133.4938 $[M + Na]^+$ (calcd for $C_{51}H_{82}O_{26}Na$, 1133.4992).

Solanigroside D (3): white, amorphous powder; $[\alpha]^{25}_D$ −45.4 (*c* 0.84, MeOH); IR (KBr) ν_{max} 3420 (OH), 2933 (CH), 1757 (−C═O) cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) data, see Table 1; ^{13}C NMR (pyridine-d_5, 100 MHz) data, see Table 2; ESIMS (in positive-ion mode) *m*/*z* 1203 $[M + Na]^+$, 1071 $[(M + Na) - 132]^+$, 939 $[(M + Na) - 132 - 132]^+$, 793 $[(M + Na) - 132 - 132 - 146]^+$; HRESIMS *m*/*z* 1203.5400 $[M + Na]^+$ (calcd for $C_{55}H_{88}O_{27}Na$, 1203.5411).

Solanigroside E (4): white, amorphous powder; $[\alpha]^{25}_D$ −36.1 (*c* 1.07, MeOH); IR (KBr) ν_{max} 3407 (OH), 2934 (CH), 1758 (−C═O)

cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) data, see Table 1; ^{13}C NMR (pyridine-d_5, 100 MHz) data, see Table 2; ESIMS (in positive-ion mode) *m/z* 1219 $[M + Na]^+$, 1073 $[(M + Na) - 146]^+$, 941 $[(M + Na) - 146 - 132]^+$, 809 $[(M + Na) - 146 - 132 - 132]^+$; HRESIMS *m/z* 1219.5392 $[M + Na]^+$ (calcd for $C_{55}H_{88}O_{28}Na$, 1219.5360).

Solanigroside F (5): white, amorphous powder; $[\alpha]^{25}_D$ −37.6 (*c* 0.98, MeOH); IR (KBr) ν_{max} 3407 (OH), 2929 (CH) cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) data, see Table 1; ^{13}C NMR (pyridine-d_5, 100 MHz) data, see Table 2; ESIMS (in negative-ion mode) *m/z* 1211 $[M - H]^-$, 1079 $[(M - H) - 132]^-$, 917 $[(M - H) - 132 - 162]^-$, 755 $[(M - H) - 132 - 162 - 162]^-$, 593 $[(M - H) - 132 - 162 - 162 - 162]^-$; HRESIMS *m/z* 1235.5642 $[M + Na]^+$ (calcd for $C_{56}H_{92}O_{28}Na$, 1235.5673).

Solanigroside G (6): white, amorphous powder; $[\alpha]^{25}_D$ −28.8 (*c* 0.41, MeOH); IR (KBr) ν_{max} 3435 (OH), 2930 (CH) cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) data, see Table 1; ^{13}C NMR (pyridine-d_5, 100 MHz) data, see Table 2; ESIMS (in negative-ion mode) *m/z* 1049 $[M - H]^-$, 917 $[(M - H) - 132]^-$, 755 $[(M - H) - 132 - 162]^-$, 593 $[(M - H) - 132 - 162 - 162]^-$; HRESIMS *m/z* 1073.5151 $[M + Na]^+$ (calcd for $C_{50}H_{82}O_{23}Na$, 1073.5145).

Solanigroside H (7): white, amorphous powder; $[\alpha]^{25}_D$ −63.9 (*c* 0.44, MeOH); IR (KBr) ν_{max} 3425 (OH), 2925 (CH) cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz) data, see Table 1; ^{13}C NMR (pyridine-d_5, 100 MHz) data, see Table 2; ESIMS (in negative-ion mode) *m/z* 1045 $[M - H]^-$, 883 $[(M - H) - 162]^-$, 737 $[(M - H) - 162 - 146]^-$; HRESIMS *m/z* 1069.5226 $[M + Na]^+$ (calcd for $C_{51}H_{82}O_{22}Na$, 1069.5195).

Acid Hydrolysis of Saponins 2−5. Each saponin (5 mg) was heated in an ampule with 5 mL of 2 M HCl at 100 °C for 2 h. The aglycone was extracted with CH_2Cl_2 (× 3) three times, and the aqueous residue was evaporated under reduced pressure. Pyridine (1 mL) and 2 mg of $NH_2OH{\cdot}HCl$ were added to the residue, and the mixture was heated at 100 °C for 1 h. After cooling, Ac_2O (1.5 mL) was added and the mixtures were heated at 100 °C for 1 h. The reaction mixtures were evaporated under reduced pressure, and the resulting aldononitrile peracetates were analyzed by GC-MS using standard aldononitrile peracetates as reference samples.

Bioassay. Cell Culture. HepG2, NCI-H460, MCF-7, and SF-268 cells were maintained in RPMI 1640 (Gibco BRL) containing 10% FBS (Gibco), 2 mg/mL $NaHCO_3$, 100 μg/mL penicillin sodium salt, and 100 μg/mL streptomycin sulfate. Cells were grown to 70% confluence, trypsinized with 0.05% trypsin−2 mM EDTA, and plated for experimental use. In all experiments, cells were grown in RPMI-1640 medium with 10% FBS for 24 h prior to treatment.

Cytotoxicity Assay. HepG2, MCF-7, NCI-H460, and SF-268 cells (1.0×10^4) were seeded in 96-well tissue culture plates and treated with the test compounds (100−3.125 μM) or vehicle (0.1% DMSO) at various concentrations and incubated for 48 h followed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay at 570 nm.[9] IC_{50} value is the concentration of test drug where $100 \times (C - T)/(C - B) = 50$, where *C* is the OD (optical density) of control; *T* is the OD of treated sample; *B* is blank. Briefly, the results of the seven compounds on different cell lines are the mean of the $IC_{50} \pm$ SD (standard deviation) of three independent experiments calculated with the LOGIT method.[10]

Acknowledgment. The authors are grateful to Professor Qishi Sun at Shenyang Pharmaceutical University (Liaoning Province, China) for identifying the plant materials and Ms. Qian Li and Mr. Yi Dai of Institute of Traditional Chinese Medicines and Natural Products, Jinan University (Guangzhou, China), for measuring all NMR and ESIMS spectra. All HRMS were measured at Shanghai Institute of Materia Medica, Chinese Academy of Science. We also thank Prof. Chan Ming Wai at Macau University of Science and Technology (Macau, China) for his help in preparation of the manuscript.

References and Notes

(1) Hu, K.; Kobayashi, H.; Dong, A. J.; Jing, Y. K.; Iwasaki, S. G.; Yao, X. S. *Planta Med.* **1999**, *65*, 35−38.
(2) Wang, W.; Lu, D. P. *J. Peking Univ. (Health Sci.)* **2005**, *37*, 240−244.
(3) Yamashita, T.; Matsumoto, T.; Yahara, S.; Yoshida, N.; Nohara, T. *Chem. Pharm. Bull.* **1991**, *39*, 1626−1628.
(4) Ferreira, F.; Soule, S.; Vazquez, A.; Moyna, P.; Kenne, L. *Phytochemistry* **1996**, *42*, 1409−1416.
(5) Mimaki, Y.; Kanmoto, T.; Sashida, Y.; Nishino, A.; Satomi, Y.; Nishino, H. *Phytochemistry* **1996**, *41*, 1405−1410.
(6) Ikeda, T.; Tsumagari, H.; Nohara, T. *Chem. Pharm. Bull.* **2000**, *48*, 1062−1064.
(7) Ando, J.; Miyazono, A.; Zhu, X. H.; Ikeda, T.; Nohara, T. *Chem. Pharm. Bull.* **1999**, *47*, 1794−1796.
(8) Hu, K.; Yao, X. S.; Dong, A. J.; Kobayashi, H.; Iwasaki, S.; Jing, Y. K. *J. Nat. Prod.* **1999**, *62*, 299−301.
(9) Carmichael, J.; DeGraff, W. C.; Gazdar, A. *Cancer Res.* **1987**, *47*, 936−942.
(10) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91−109.

NP060091Z