Five New Steroidal Alkaloid Glycosides from *Solanum tuberosum*

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Five new steroidal alkaloid glycosides, **1**–**5**, along with the known analog **6**, have been isolated from the aerial parts of *Solanum tuberosum*. The structures of the new compounds were elucidated by spectroscopic methods, including 1D- and 2D-NMR and HR-ESI-MS techniques, as well as by comparison of the spectral data with those of related compounds. Only compound **6** showed significant cytotoxicity.

Introduction. – Steroidal alkaloid glycosides are the main chemical components of *Solanum* species, and they exhibit various pharmacological activities such as cytotoxic [1], antiviral [2], anti-inflammatory [3], and antifungal properties [4], making *Solanum tuberosum* an attractive target for the search of health-promoting phytochemicals. With this aim, we investigated the aerial parts of *S. tuberosum*, and isolated six steroidal alkaloid glycosides, **1**–**6**, including a rare 14α-hydroxy steroidal alkaloid glycoside, **1**, two new ones, **2** and **4**, two novel 7-oxo derivatives, **3** and **5**, and the known metabolite **6** (*Fig. 1*). The cytotoxicities of **1**–**4** and **6** against human cancer cell lines SMMC-7721, NCI-H460, and A-549 were evaluated. In this article, we reported the isolation, structure elucidation, and the cytotoxicity evaluation of the compounds isolated from the aerial parts of this plant.

Results and Discussion. – *Structure Elucidation*. All compounds, **1**–**6**, showed positive *Liebermann–Burchard*, *Dragendorff*, and *Molish* reactions, indicating the steroidal alkaloid glycoside nature of these compounds. The presence of glucose, rhamnose, and/or galactose in the hydrolysates of each compound was confirmed by co-TLC with authentic samples. Glucose, rhamnose, and galactose were assigned D-, L-, and D-forms, respectively, by GC/MS analysis of their silyl derivatives. The β-anomeric configurations of the D-glucopyranosyl and D-galactosyl moieties were determined by the coupling constants ($^3J(1,2) > 7$ Hz), respectively. The α-anomeric configuration of the L-rhamnopyranosyl group was deduced from the small coupling constant of the anomeric H-atom and the chemical shifts of C(3) and C(5) [5].

Compound **1** was obtained as a white amorphous powder. Its positive-ion-mode HR-ESI-MS displayed a quasimolecular-ion peak at m/z 722.4481 ($[M+H]^+$; calc. 722.4474) indicating the molecular formula $C_{39}H_{63}NO_{11}$, with nine degrees of unsaturation. Its IR spectrum revealed the presence of OH groups (3423 cm^{-1}) and of an olefinic bond (1641 cm^{-1}). Upon acid hydrolysis, compound **1** afforded an aglycone, along with D-glucose and L-rhamnose, which were identified by GC/MS

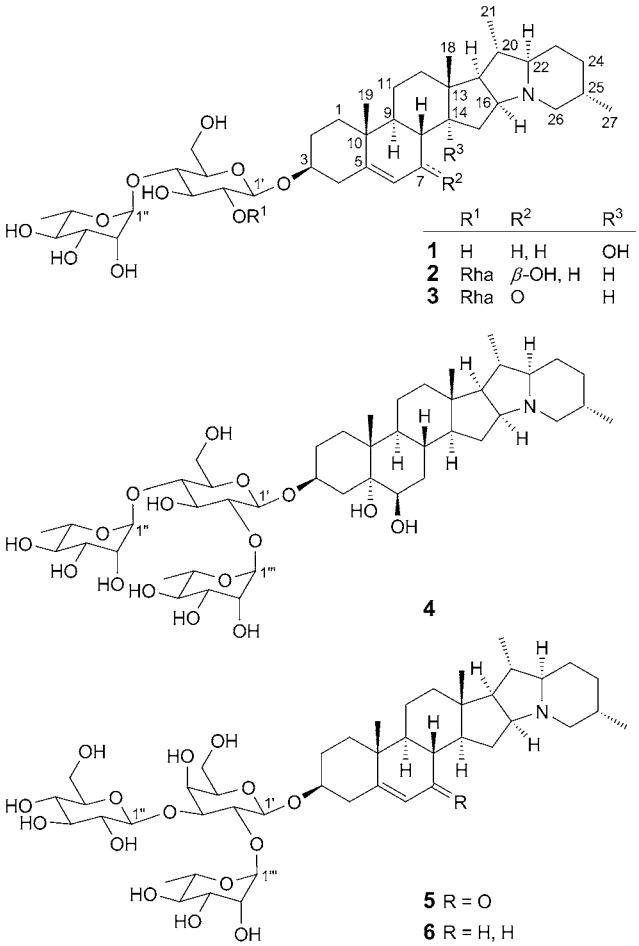

Fig. 1. *Structures of* **1**–**6**

comparison with authentic samples. In the ^{1}H-NMR spectrum of the aglycone of **1** (*Table 1*), there were signals of two tertiary Me groups at δ(H) 0.96 (*s*, Me(19)) and 1.20 (*s*, Me(18)), two secondary Me groups at δ(H) 0.79 (*d*, $J = 6.4$, Me(27)) and 0.96 (*d*, $J = 4.8$, Me(21)), and an olefinic H-atom at δ(H) 5.42 (br. *s*, H–C(6)). The ^{13}C-NMR spectrum of the aglycone (*Table 1*) displayed 27 C-atom signals, which were classified into those of four Me, ten CH_2, nine CH, and four quaternary C-atoms (one O-bearing and one olefinic C-atoms) on the basis of DEPT and HSQC spectra. These spectral features were characteristic for alkaloids of the solanidine group [6]. The HMBCs (*Fig. 2*) Me(19)/C(1), C(5), and C(9); H–C(4)/C(2), C(3), C(5), and C(6); H–C(6)/C(7), C(8), and C(10); Me(18)/C(12), C(13), C(14), and C(17); H–C(17)/C(13) and C(16); Me(21)/C(17), C(20), and C(22); and Me(27)/C(24), C(25), and C(26) confirmed this assumption and also located the OH group signal at δ(C) 87.0 (C(14)). In the ROESY spectrum (*Fig. 2*), H–C(3) (δ(H) 3.84) was assigned α-

Table 1. *^{1}H- and ^{13}C-NMR* (500 and 125 MHz, resp.) *Data the Aglycone Moieties of* **1** *and* **2** *in (D_5)Pyridine.* δ in ppm, J in Hz. Assignments are based on HSQC, HMBC, ROESY, and TOCSY experiments.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.04 (*dd*, *J* = 13.4, 3.4), 1.76–1.78 (*m*)	37.9 (*t*)	0.95–0.97 (*m*), 1.70–1.73 (*m*)	37.6 (*t*)
2	1.69–1.71 (*m*), 2.03–2.05 (*m*)	30.5 (*t*)	1.85–1.88 (*m*), 2.08–2.10 (*m*)	30.6 (*t*)
3	3.83–3.85 (*m*)	78.5 (*d*)	3.92–3.95 (*m*)	78.3 (*d*)
4	2.47–2.49 (*m*), 2.70 (*dd*, *J* = 13.1, 2.3)	39.6 (*t*)	2.77 (*d*, *J* = 11.5), 2.90 (*dd*, *J* = 11.5, 2.9)	38.9 (*t*)
5		140.7 (*s*)		142.0 (*s*)
6	5.42 (br. *s*)	122.7 (*d*)	5.74 (br. *s*)	128.9 (*d*)
7	1.90–1.93 (*m*), 2.51–2.54 (*m*)	26.9 (*t*)	4.03 (*d*, *J* = 8.0)	73.2 (*d*)
8	2.03–2.05 (*m*)	35.9 (*d*)	1.78–1.80 (*m*)	41.1 (*d*)
9	1.80–1.82 (*m*)	43.9 (*d*)	1.08–1.10 (*m*)	48.9 (*d*)
10		37.6 (*s*)		37.5 (*s*)
11	1.37–1.40 (*m*), 1.55 (br. *d*, *J* = 6.4)	20.7 (*t*)	1.41–1.43 (*m*), 1.47–1.50 (*m*)	21.5 (*t*)
12	1.42–1.45 (*m*), 2.25–2.27 (*m*)	32.4 (*t*)	1.13–1.15 (*m*), 1.67–1.70 (*m*)	40.4 (*t*)
13		45.3 (*s*)		41.4 (*s*)
14		87.0 (*s*)	1.38–1.40 (*m*)	57.6 (*d*)
15	1.72–1.74 (*m*), 2.08–2.11 (*m*)	38.9 (*t*)	1.46–1.48 (*m*), 2.69–2.71 (*m*)	33.4 (*t*)
16	3.25 (br. *s*)	69.9 (*d*)	3.04 (br. *s*)	70.6 (*d*)
17	2.47–2.50 (*m*)	59.9 (*d*)	1.62–1.64 (*m*)	62.4 (*d*)
18	1.20 (*s*)	20.5 (*q*)	0.97 (*s*)	18.0 (*q*)
19	0.96 (*s*)	19.6 (*q*)	1.05 (*s*)	17.0 (*q*)
20	1.90–1.93 (*m*)	37.2 (*d*)	1.95 (br. *s*)	37.2 (*d*)
21	0.96 (*d*, *J* = 4.8)	18.6 (*q*)	0.95 (*d*, *J* = 8.0)	19.1 (*q*)
22	1.76–1.78 (*m*)	75.4 (*d*)	1.95 (br. *s*)	75.5 (*d*)
23	1.45 (br. *d*, *J* = 12.1), 1.75–1.77 (*m*)	29.4 (*t*)	1.28–1.31 (*m*), 2.23–2.26 (*m*)	30.3 (*t*)
24	0.81–0.84 (*m*), 1.65–1.67 (*m*)	33.8 (*t*)	0.83–0.85 (*m*), 1.67–1.70 (*m*)	33.1 (*t*)
25	1.82–1.84 (*m*)	31.3 (*d*)	1.58–1.61 (*m*)	30.5 (*d*)
26	1.51–1.54 (*m*), 3.00 (br. *s*)	60.6 (*t*)	1.69–1.71 (*m*), 3.11 (br. *s*)	60.1 (*t*)
27	0.79 (*d*, *J* = 6.4)	19.8 (*q*)	0.76 (*d*, *J* = 7.0)	19.5 (*q*)

configuration due to its correlations with H_α–C(1) (δ(H) 1.04) and H_α–C(4) (δ(H) 2.70). To determine the configuration at C(14), 1D- and 2D-NMR spectra of compound **1** were recorded with (D_6)DMSO as solvent, and the cross-peak between δ(H) 3.21 (HO–C(14)), and 3.86 (H–C(16)) and 0.96 (H–C(21)) in the ROESY spectrum indicated α-configuration for HO–C(14). Two anomeric H-atom signals at δ(H) 4.93 (*d*, *J* = 7.7, 1 H) and 5.87 (br. *s*, 1 H) in the low-field region of the ^{1}H-NMR spectrum (*Table 2*) correlated with the corresponding anomeric C-atom signals at δ(C) 102.6 (C(1′)) and 102.9 (C(1″)), respectively (*Table 2*), in the HSQC spectrum. The HMBC features were observed (*Fig. 2*) between δ(H) 4.93 (H–C(1′)) and δ(C) 78.5 (C(3)), confirming that the β-D-Glc unit was attached at O–C(3) of the aglycone, as well as between δ(H) 5.87 (H–C(1″)) and δ(C) 78.7 (C(4′)), revealing that the disaccharide chain was of the type α-L-Rha-(1 → 4)-β-D-Glc. Therefore, the structure of **1** was established as (3β)-14-hydroxysolanid-5-en-3-yl 4-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside.

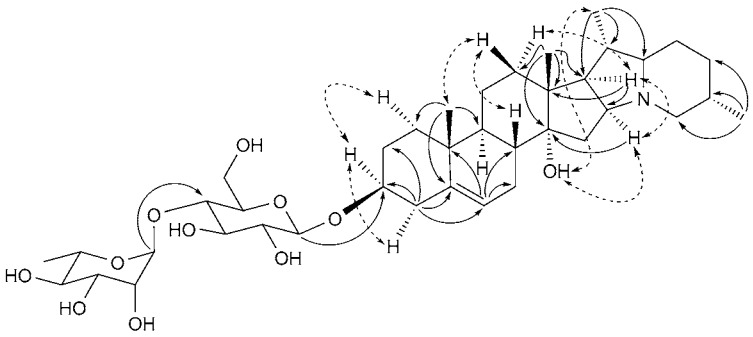

Fig. 2. *Key HMB* (H → C) *and ROESY* (H ← - - - → H) *correlations of compound* **1**

Table 2. *^{1}H- and ^{13}C-NMR* (500 and 125 MHz, resp.) *Data of the Sugar Moieties of* **1** *and* **2** *in (D_5)Pyridine.* δ in ppm, J in Hz. Assignments are based on HSQC, HMBC, ROESY, and TOCSY experiments.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
	Glc		Glc	
1′	4.93 (*d*, *J* = 7.7)	102.6 (*d*)	4.93 (*d*, *J* = 7.7)	100.7 (*d*)
2′	3.98 (*t*, *J* = 7.7)	75.7 (*d*)	4.20–4.23 (*m*)	78.2 (*d*)
3′	4.19 (*t*, *J* = 7.7)	76.9 (*d*)	4.20–4.23 (*m*)	78.3 (*d*)
4′	4.42 (*t*, *J* = 7.7)	78.7 (*d*)	4.33–4.35 (*m*)	79.1 (*d*)
5′	3.68–3.70 (*m*)	77.3 (*d*)	3.63 (*dt*, *J* = 8.9, 3.4)	77.2 (*d*)
6′	4.10 (*dd*, *J* = 11.5, 3.4), 4.25 (*d*, *J* = 11.5)	61.8 (*t*)	4.10 (*dd*, *J* = 12.1, 3.4), 4.19–4.21 (*m*)	61.7 (*t*)
	Rha		Rha	
1″	5.87 (br. *s*)	102.9 (*d*)	5.85 (br. *s*)	103.2 (*d*)
2″	4.58 (*dd*, *J* = 9.2, 3.2)	73.0 (*d*)	4.52 (*dd*, *J* = 9.2, 3.3)	73.0 (*d*)
3″	4.68–4.70 (*m*)	72.8 (*d*)	4.67–4.70 (*m*)	72.8 (*d*)
4″	4.34 (*t*, *J* = 9.2)	74.2 (*d*)	4.31–4.34 (*m*)	74.2 (*d*)
5″	4.99 (*dq*, *J* = 9.2, 6.2)	70.6 (*d*)	4.89–4.91 (*m*)	70.8 (*d*)
6″	1.70 (*d*, *J* = 6.2)	18.7 (*q*)	1.62 (*d*, *J* = 6.2)	18.2 (*q*)
			Rha	
1‴			6.38 (br. *s*)	102.4 (*d*)
2‴			4.61 (*dd*, *J* = 9.2, 3.4)	73.2 (*d*)
3‴			4.82–4.84 (*m*)	72.8 (*d*)
4‴			4.38–4.40 (*m*)	74.4 (*d*)
5‴			4.95–4.97 (*m*)	69.8 (*d*)
6‴			1.75 (*d*, *J* = 6.2)	18.9 (*q*)

Compound **2** was isolated as a white amorphous powder. Its positive-ion-mode HR-ESI-MS displayed a quasimolecular-ion peak at *m*/*z* 868.5062 ($[M+H]^+$; calc. 868.5053), in accordance with the molecular formula $C_{45}H_{73}NO_{15}$. The IR spectrum indicated the presence of OH groups (3426 cm^{-1}) and of an olefinic bond (1642 cm^{-1}). Upon acid hydrolysis, compound **2** afforded the sugar moieties L-rhamnose and D-glucose in a ratio of 2 : 1 based on the GC analysis of their chiral derivatives. The ^{1}H-

and ^{13}C-NMR spectra of the aglycone of **2** (*Table 1*) were compared with those of solanidine [6], showing considerable structural similarity, except for the absence of a CH_2 C-atom resonance at δ(C) 32.1 (C(7)). Instead, the appearance of an O-bearing C-atom signal at δ(C) 73.2 (C(7)), and the observed downfield shifted C-atom resonances at δ(C) 128.9 (C(6) (+7.6 ppm)) and δ(C) 41.1 (C(8) (+8.6 ppm)), suggested a hydroxylation at C(7), which was confirmed by the observed HMBC features from δ(H) 4.03 (H–C(7)) to δ(C) 142.0 (C(5)) and 128.9 (C(6)), from δ(H) 1.78 (H–C(8)) and 1.08 (H–C(9)) to δ(C) 73.2 (C(7)), respectively. In the ROESY spectrum of **2**, the correlations from H–C(7) (δ(H) 4.03) to H_α–C(9) (δ(H) 1.08) and H_α–C(14) (δ(H) 1.38) established α-configuration for H–C(7) and thus β-configuration HO–C(7).

Three anomeric H-atom signals at δ(H) 4.93 (*d*, $J = 7.7$), 5.85 (br. *s*), and 6.38 (br. *s*), and three anomeric C-atom signals at δ(C) 100.7 (C(1′)), 103.2 (C(1″)), and 102.4 (C(1‴)) were observed in the ^{1}H- and ^{13}C-NMR spectra, respectively (*Table 2*). The HMBC features between δ(H) 5.85 (H–C(1″)) and δ(C) 79.1 (C(4′)), between δ(H) 6.38 (H–C(1‴)) and δ(C) 78.2 (C(2′)), and between δ(H) 4.93 (H–C(1′)) and δ(C) 78.3 (C(3)) revealed that the trisaccharide chain was a α-L-Rha-(1 → 2)-[α-L-Rha-(1 → 4)]-β-D-Glc moiety. Therefore, **2** was elucidated as (3β,7β)-7-hydroxysolanid-5-en-3-yl 6-deoxy-α-L-mannopyranosyl-(1 → 2)-[6-deoxy-α-L-mannopyranosyl-(1 → 4)]-β-D-glucopyranoside.

Compound **3** was obtained as a white amorphous powder. Its positive-ion-mode HR-ESI-MS displayed a quasimolecular-ion peak at *m*/*z* 866.4906 ($[M + H]^+$; calc. 866.4896), indicating the molecular formula $C_{45}H_{71}NO_{15}$. The IR spectrum showed the presence of OH groups (3427 cm^{-1}) and of an α,β-unsaturated ketone unit (1713 and 1644 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of the aglycone of **3** (*Table 3*) resembled those of **2**, except for the absence of the signal of an O-bearing CH group at δ(C) 73.2 (C(7)). Instead, the appearance of a C=O functionality at this position, forming a conjugated enone group was indicated by the H-atom resonances at δ(H) 5.76 (H–C(6)) and C-atom resonances at δ(C) 201.8 (C(7)), 167.8 (C(5)) and 124.9 (C(6)) (*Table 3*), and confirmed by the observed HMBCs (*Fig. 3*) from δ(H) 2.53 (H–C(8)) to δ(C) 124.9 (C(6)) and 201.8 (C(7)), from δ(H) 2.75 (H–C(4)) to δ(C) 167.8 (C(5)) and 124.9 (C(6)), and from δ(H) 1.30 (H–C(19)) to δ(C) 167.8 (C(5)). The NMR spectroscopic data (*Table 4*) for the sugar part of **3** resembled a closely those of **2**, revealing that **3** had the same sugar substitution pattern as **2**. The HMBC feature between δ(H) 4.56 (H–C(1′)) and δ(C) 76.5 (C(3)) showed that the linkage of the sugar chain was at O–C(3) of the aglycone. The structure of **3** was thus assigned as (3β)-7-oxosolanid-5-en-3-yl 6-deoxy-α-L-mannopyranosyl-(1 → 2)-[6-deoxy-α-L-mannopyranosyl-(1 → 4)]-β-D-glucopyranoside.

Compound **4** was obtained as a white amorphous powder. Its positive-ion-mode HR-ESI-MS displayed a quasimolecular-ion peak at *m*/*z* 886.5166 ($[M + H]^+$; calc. 886.5159), in accordance with the molecular formula $C_{45}H_{75}NO_{16}$. The ^{1}H-NMR spectra of the aglycone of **4** (*Table 3*) displayed signals of two tertiary Me groups at δ(H) 0.88 (*s*, 3 H) and 1.17 (*s*, 3 H), two secondary Me groups at δ(H) 0.91 (*d*, $J = 5.8$, 3 H) and 0.97 (*d*, $J = 5.6$, 3 H). The ^{1}H- and ^{13}C-NMR spectra of the aglycone of **4** (*Table 3*) were in good agreement with those of (22*R*,25*S*)-solanid-5-enine-3β,5α,6β-triol [7]. The NMR spectroscopic data for the sugar part of **4** was very similar to those of **3**, revealing that **4** had the same sugar substitution pattern as **3**. Based on HSQC and

Table 3. *^{1}H- and ^{13}C-NMR* (500 and 125 MHz, resp.) *Data for the Aglycone Moieties of* **3**–**5** *in CD_3OD*. δ in ppm, J in Hz. Assignments are based on HSQC, HMBC, ROESY, and TOCSY experiments.

Position	**3**		**4**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.26–1.28 (*m*), 2.02–2.04 (*m*)	36.0 (*t*)	0.96–0.99 (*m*), 1.78–1.80 (*m*)	33.6 (*t*)	1.25–1.27 (*m*), 2.01–2.04 (*m*)	36.0 (*t*)
2	1.76 (*dd*, *J* = 13.8, 3.2), 1.98–2.01 (*m*)	29.0 (*t*)	1.59–1.61 (*m*), 1.83–1.87 (*m*)	30.1 (*t*)	1.74 (*dd*, *J* = 14.0, 3.6), 2.04–2.07 (*m*)	29.0 (*t*)
3	3.77–3.79 (*m*)	76.5 (*d*)	4.12–4.15 (*m*)	76.4 (*d*)	3.78–3.80 (*m*)	76.2 (*d*)
4	2.50–2.52 (*m*), 2.75–2.78 (*m*)	38.1 (*t*)	1.67 (*dd*, *J* = 13.5, 4.6), 2.07 (*d*, *J* = 13.5)	38.3 (*t*)	2.48–2.51 (*m*), 2.73–2.76 (*m*)	38.0 (*t*)
5		167.8 (*s*)		76.8 (*s*)		168.0 (*s*)
6	5.76 (*s*)	124.9 (*d*)	3.26–3.28 (*m*)	76.7 (*d*)	5.73 (*d*, *J* = 1.5)	124.9 (*d*)
7		201.8 (*s*)	1.55 (*dt*, *J* = 13.4, 2.8), 1.70–1.73 (*m*)	35.4 (*t*)		201.9 (*s*)
8	2.53–2.55 (*m*)	43.9 (*d*)	1.90–1.93 (*m*)	31.3 (*d*)	2.52–2.55 (*m*)	44.0 (*d*)
9	1.71–1.73 (*m*)	49.6 (*d*)	1.38–1.41 (*m*)	46.8 (*d*)	1.69–1.72 (*m*)	49.6 (*d*)
10		38.5 (*s*)		39.7 (*s*)		38.5 (*s*)
11	1.62–1.64 (*m*), 1.71–1.73 (*m*)	20.6 (*t*)	1.32–1.34 (*m*), 1.39–1.42 (*m*)	22.2 (*t*)	1.61–1.64 (*m*), 1.69–1.72 (*m*)	20.6 (*t*)
12	1.30–1.33 (*m*), 1.89 (*dt*, *J* = 12.6, 3.2)	38.4 (*t*)	1.19–1.22 (*m*), 1.75–1.78 (*m*)	41.6 (*t*)	1.28–1.30 (*m*), 1.88 (*dt*, *J* = 12.6, 3.2)	38.4 (*t*)
13		40.8 (*s*)		41.9 (*s*)		40.8 (*s*)
14	1.57–1.60 (*m*)	50.2 (*d*)	1.27–1.30 (*m*)	58.3 (*d*)	1.57–1.59 (*m*)	50.2 (*d*)
15	1.56–1.59 (*m*), 3.03 (br. *t*, *J* = 7.0)	29.4 (*t*)	1.32–1.34 (*m*), 1.58–1.61 (*m*)	33.7 (*t*)	1.56–1.59 (*m*), 3.00 (br. *s*)	29.3 (*t*)
16	3.72–3.75 (*m*)	70.2 (*d*)	3.02 (br. *s*)	71.2 (*d*)	3.72–3.75 (*m*)	70.2 (*d*)
17	2.04–2.07 (*m*)	59.0 (*d*)	1.70–1.73 (*m*)	63.6 (*d*)	2.02–2.04 (*m*)	58.9 (*d*)
18	0.96 (*s*)	14.9 (*q*)	0.88 (*s*)	17.2 (*q*)	0.94 (*s*)	14.9 (*q*)
19	1.30 (*s*)	16.2 (*q*)	1.17 (*s*)	17.4 (*q*)	1.27 (*s*)	16.2 (*q*)
20	1.96–1.99 (*m*)	36.5 (*d*)	1.74–1.77 (*m*)	38.2 (*d*)	1.95–1.98 (*m*)	36.5 (*d*)
21	1.12 (*d*, *J* = 6.0)	15.0 (*q*)	0.97 (*d*, *J* = 5.6)	17.6 (*q*)	1.10 (*d*, *J* = 6.1)	15.1 (*q*)
22	2.91 (br. *s*)	75.2 (*d*)	2.05–2.07 (*m*)	76.4 (*d*)	2.89 (br. *s*)	75.2 (*d*)
23	1.56–1.59 (*m*), 2.09–2.12 (*m*)	25.7 (*t*)	1.28–1.31 (*m*), 1.82–1.85(*m*)	29.0 (*t*)	1.53–1.57 (*m*), 2.07–2.09 (*m*)	25.7 (*t*)
24	1.21–1.24 (*m*), 1.96–1.98 (*m*)	30.5 (*t*)	1.27–1.30 (*m*), 1.89–1.91 (*m*)	30.9 (*t*)	1.19–1.21 (*m*), 1.93–1.96 (*m*)	30.5 (*t*)
25	2.06–2.09 (*m*)	29.0 (*d*)	1.73–1.75 (*m*)	31.5 (*d*)	2.06–2.09 (*m*)	29.0 (*d*)
26	2.50–2.53 (*m*), 3.52–3.55 (*m*)	58.0 (*t*)	1.75–1.78 (*m*), 3.10 (br. *s*)	61.2 (*t*)	2.47–2.50 (*m*), 3.49–3.53 (*m*)	58.0 (*t*)
27	1.03 (*d*, *J* = 6.5)	17.1 (*q*)	0.91 (*d*, *J* = 5.8)	19.5 (*q*)	1.01 (*d*, *J* = 6.4)	17.2 (*q*)

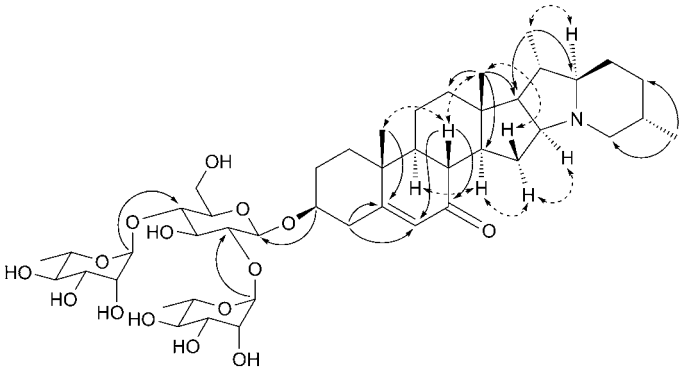

Fig. 3. *Key HMB* (H → C) *and ROESY* (H ← - - - → H) *correlations of compound* **3**

Table 4. *^{1}H- and ^{13}C-NMR* (500 and 125 MHz resp.) *Data for the Sugar Moieties of* **3**–**5** *in CD_3OD.* δ in ppm, J in Hz. Assignments are based on HSQC, HMBC, ROESY, and TOCSY experiments.

Position	**3**		**4**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
	Glc		Glc		Gal	
1′	4.56 (*d*, *J* = 7.8)	99.3 (*d*)	4.45 (*d*, *J* = 7.8)	101.0 (*d*)	4.52 (*d*, *J* = 7.5)	99.7 (*d*)
2′	3.43–3.45 (*m*)	77.6 (*d*)	3.37–3.39 (*m*)	79.5 (*d*)	3.80–3.82 (*m*)	74.0 (*d*)
3′	3.62–3.64 (*m*)	76.6 (*d*)	3.56 (*t*, *J* = 8.7)	78.3 (*d*)	3.75–3.77 (*m*)	84.2 (*d*)
4′	3.51–3.53 (*m*)	78.7 (*d*)	3.51 (*t*, *J* = 8.7)	80.3 (*d*)	4.09–4.11 (*m*)	68.8 (*d*)
5′	3.36–3.38 (*m*)	75.2 (*d*)	3.44–3.46 (*m*)	76.7 (*d*)	3.53–3.55 (*m*)	74.7 (*d*)
6′	3.66–3.68 (*m*), 3.81–3.83 (*m*)	60.6 (*t*)	3.64 (*dd*, *J* = 12.1, 4.3), 3.79 (*dd*, *J* = 12.1, 1.8)	62.1 (*t*)	3.68–3.70 (*m*), 3.71–3.73 (*m*)	61.1 (*t*)
	Rha		Rha		Glc	
1″	4.85 (*d*, *J* = 1.5)	101.6 (*d*)	4.83 (*d*, *J* = 1.5)	103.2 (*d*)	4.47 (*d*, *J* = 7.7)	104.4 (*d*)
2″	3.84–3.86 (*m*)	71.0 (*d*)	3.82 (*dd*, *J* = 3.2, 1.5)	72.6 (*d*)	3.26–3.28 (*m*)	73.6 (*d*)
3″	3.63–3.65 (*m*)	70.8 (*d*)	3.60 (*dd*, *J* = 9.4, 3.2)	72.3 (*d*)	3.33–3.35 (*m*)	76.8 (*d*)
4″	3.44–3.46 (*m*)	72.3 (*d*)	3.41 (*dd*, *J* = 9.4, 6.2)	73.9 (*d*)	3.32–3.34 (*m*)	69.8 (*d*)
5″	3.93–3.95 (*m*)	69.3 (*d*)	3.90–3.93 (*m*)	70.9 (*d*)	3.26–3.29 (*m*)	76.5 (*d*)
6″	1.30 (*d*, *J* = 5.8)	16.5 (*q*)	1.26 (*d*, *J* = 6.2)	18.0 (*q*)	3.65–3.68 (*m*), 3.83–3.85 (*m*)	61.0 (*t*)
	Rha		Rha		Rha	
1‴	5.25 (*d*, *J* = 1.5)	100.8 (*d*)	5.20 (*d*, *J* = 1.5)	102.4 (*d*)	5.22 (*d*, *J* = 1.5)	100.7 (*d*)
2‴	3.67–3.69 (*m*)	71.0 (*d*)	3.91–3.93 (*m*)	72.4 (*d*)	3.64 (*dd*, *J* = 3.3, 1.5)	71.0 (*d*)
3‴	3.93–3.95 (*m*)	70.7 (*d*)	3.67 (*dd*, *J* = 9.5, 3.3)	72.3 (*d*)	3.94 (*dd*, *J* = 9.4, 3.3)	70.6 (*d*)
4‴	3.42–3.44 (*m*)	72.5 (*d*)	3.38 (*dd*, *J* = 9.5, 6.2)	74.4 (*d*)	3.39–3.41 (*m*)	72.6 (*d*)
5‴	4.12 (*dq*, *J* = 12.3, 6.2)	68.3 (*d*)	4.12–4.15 (*m*)	69.7 (*d*)	4.12 (*dq*, *J* = 12.3, 6.2)	68.3 (*d*)
6‴	1.26 (*d*, *J* = 6.2)	16.4 (*q*)	1.24 (*d*, *J* = 6.2)	18.1 (*q*)	1.24 (*d*, *J* = 6.2)	16.6 (*q*)

HMBC evidence, the structure of **4** was determined to be (3β,5α,6β)-5,6-dihydroxy-solanidan-3-yl 6-deoxy-α-L-mannopyranosyl-(1 → 2)-[6-deoxy-α-L-mannopyranosyl-(1 → 4)]-β-D-glucopyranoside.

Compound **5** was isolated as a white amorphous powder. Its positive-ion-mode HR-ESI-MS displayed a quasimolecular-ion peak at *m/z* 882.4852 ($[M+H]^+$; calc. 882.4846), indicating the molecular formula $C_{45}H_{71}NO_{16}$. The IR spectrum revealed the presence of OH groups (3441 cm^{-1}) and of an α,β-unsaturated ketone unit (1710 and 1641 cm^{-1}). Upon acid hydrolysis of **5**, three sugar monomers were identified as D-glucose, L-rhamnose, and D-galactose by GC/MS analysis of their silyl derivatives. The anomeric H-atom signals at δ(H) 4.47 (d, $J=7.7$), 4.52 (d, $J=7.5$), and 5.22 (d, $J=1.5$) correlated with the C-atom resonances at δ(C) 104.4 (C(1″)), 99.7 (C(1′)), and 100.7 (C(1‴)), respectively, in the HSQC spectrum. The connectivity of the three sugars was determined by the following HMBC features: from δ(H) 4.52 (H–C(1′)) to δ(C) 76.2 (C(3)) from δ(H) 4.47 (H–C(1″)) to δ(C) 84.2 (C(3′)), and from δ(H) 5.22 (H–C(1‴)) to δ(C) 74.0 (C(2′)). The ^{1}H- and ^{13}C-NMR signals (*Table 3*) for the aglycone of **5** were in good agreement with those of **3**. Therefore, the structure of **5** was elucidated as (3β)-7-oxosolanid-5-en-3-yl 6-deoxy-α-L-mannopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-galactopyranoside.

The known steroidal alkaloid glycoside α-solanine (**6**) was identified by comparison of its NMR and MS data with those reported in the literature [8].

Biological Study. Cytotoxic activities of compounds **1**–**4** and **6** were evaluated *in vitro* against SMMC-7721 (human hepatoma), NCI-H460 (non-small cell lung cancer), and A-549 (human lung adenocarcinoma) cell lines, by the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method. Compound **6** showed cytotoxicity against to SMMC-7721, NCI-H460, and A-549 cell lines, with IC_{50} values of 14.4, 39.0, and 35.7 μM, respectively. The other compounds showed no or little cytotoxic activity (IC_{50} values > 100 μM) against the tested tumor cells.

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Experimental Part

General. All the reagents and solvents were of the anal. grade (*Jiangsu Hanbang Sci. & Tech. Co., Ltd.*, Huaian, China). Column chromatography (CC): silica gel *H* (SiO_2; 100–200 and 200–300 mesh; *Qingdao Marine Chemical Factory*, Qingdao, China), *RP-18* (40–63 μm; *Fuji Silysia Chemical Ltd.*), *D101* macroporous resin (*The Chemical Plant of Nankai University*, Tianjin, China), *Sephadex LH-20* (*Pharmacia*, *Amersham Biosciences*, S-Uppsala, Sweden). TLC: SiO_2 GF_{254} (*Qingdao Marine Chemical Co., Ltd.*). Optical rotations: *JASCO P-1020* polarimeter. IR Spectra: *Bruker Tensor-27* spectrometer; KBr pellets; in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AV-500* spectrometer; at 500 (^{1}H) and 125 MHz (^{13}C); δ in ppm rel. to TMS as an internal standard, *J* in Hz. GC/MS: *Agilent 6890* gas chromatograph and *Agilent 5975* mass spectrometer. ESI-MS: *Agilent 1100 Series LC/MSD Trap* mass spectrometer; in *m/z*. HR-ESI-MS: *Micro Q-TOF MS* instrument; in *m/z*.

Plant Material. Aerial parts of *S. tuberosum* were collected from Nanjing City, Jiangsu Province, China, in May 2009. The identity of the plant was confirmed by Prof. *Min-Jian Qin*, Department of Medicinal Plants, China Pharmaceutical University. A voucher specimen (No. 20090525) has been deposited with the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. Dried aerial parts of *S. tuberosum* (10.6 kg) were extracted three times with 90% EtOH (3 × 40 l) under reflux for 2 h each time. The extract was evaporated under reduced pressure.

Then, the residue (640.8 g) was suspended in H_2O and, by standing, partitioned with supernatant and precipitation successively. The supernatant was passed through a *D101* macroporous adsorption resin column and eluted with EtOH/H_2O 0:100, 30:70, 70:30, and 100:0 to yield four fractions, *Frs. 1–4*, resp. *Fr. 3* (24.2 g) was separated by CC (SiO_2; ($CHCl_3$/MeOH/H_2O 7:3:0.2 → 6:4:0.5) to give further ten subfractions, *Subfrs. 3.1–3.10*. *Subfr. 3.9* (3.0 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ 7:3:0.3; and *ODS*; MeOH/H_2O 30:70, 50:50, 70:30) to give **1** (5 mg) and **2** (7 mg), resp. *Subfr. 3.10* (2.2 g) was submitted to CC(ODS; MeOH/H_2O 45:55; and SiO_2; ($CHCl_3$/MeOH/$NH_3 \cdot H_2O$ 6.5:3.5:0.4) to afford **3** (3 mg), **4** (4 mg), and **5** (1.5 mg).

*(3β)-14-Hydroxysolanid-5-en-3-yl 4-O-(6-Deoxy-α-*L*-mannopyranosyl)-β-*D*-glucopyranoside* (**1**). White amorphous powder. $[\alpha]_D^{20} = -16.4$ ($c = 0.11$, MeOH). IR (KBr): 3423, 2925, 1641, 1400, 1066, 618. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 722 ($[M+H]^+$), 576 ($[M-146+H]^+$), 414 ($[M-146-162+H]^+$). HR-ESI-MS: 722.4481 ($[M+H]^+$, $C_{39}H_{64}NO_{11}^+$; calc. 722.4474).

*(3β,7β)-7-Hydroxysolanid-5-en-3-yl 6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)-[6-deoxy-α-*L*-mannopyranosyl-(1 → 4)]-β-*D*-glucopyranoside* (**2**). White amorphous powder. $[\alpha]_D^{20} = -34.4$ ($c = 0.10$, MeOH). IR (KBr): 3426, 2938, 1642, 1402, 1044, 620. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 868 ($[M+H]^+$), 722 ($[M-146+H]^+$), 576 ($[M-146-146+H]^+$), 414 ($[M-146-146-162+H]^+$). HR-ESI-MS: 868.5062 ($[M+H]^+$, $C_{45}H_{74}NO_{15}^+$; calc. 868.5053).

*(3β)-7-Oxosolanid-5-en-3-yl 6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)-[6-deoxy-α-*L*-mannopyranosyl-(1 → 4)]-β-*D*-glucopyranoside* (**3**). White amorphous powder. $[\alpha]_D^{20} = -50.7$ ($c = 0.09$, MeOH). IR (KBr): 3427, 1713, 1644, 1403, 670. ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. ESI-MS: 866 ($[M+H]^+$), 720 ($[M-146+H]^+$), 574 ($[M-146-146+H]^+$), 412 ($[M-146-146-162+H]^+$). HR-ESI-MS: 866.4906 ($[M+H]^+$, $C_{45}H_{72}NO_{15}^+$; calc. 866.4896).

*(3β,5α,6β)-5,6-Dihydroxysolanidan-3-yl 6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)-[6-deoxy-α-*L*-mannopyranosyl-(1 → 4)]-β-*D*-glucopyranoside* (**4**). White amorphous powder. $[\alpha]_D^{20} = -34.4$ ($c = 0.09$, MeOH). IR (KBr): 3425, 2925, 1400, 1046. ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. ESI-MS: 886 ($[M+H]^+$), 740 ($[M-146+H]^+$), 594 ($[M-146-146+H]^+$), 432 ($[M-146-146-162+H]^+$). HR-ESI-MS: 886.5166 ($[M+H]^+$, $C_{45}H_{76}NO_{16}^+$; calc. 886.5159).

*(3β)-7-Oxosolanid-5-en-3-yl 6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)-[β-*D*-glucopyranosyl-(1 → 3)]-β-*D*-galactopyranoside* (**5**). White amorphous powder. $[\alpha]_D^{20} = -37.3$ ($c = 0.09$, MeOH). IR (KBr): 3441, 1710, 1641, 1400, 668. ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. ESI-MS: 882 ($[M+H]^+$), 736 ($[M-146+H]^+$), 574 ($[M-146-162+H]^+$), 412 ($[M-146-162-162+H]^+$). HR-ESI-MS: 882.4852 ($[M+H]^+$, $C_{45}H_{72}NO_{16}^+$; calc. 882.4846).

Absolute Configuration. Each compound (1–2 mg) was dissolved in MeOH (4 ml) and treated with 3 ml of 5% H_2SO_4 at 90° for 2 h. After addition of H_2O (3 ml), each mixture was concentrated to 3 ml under reduced pressure and then neutralized with *Amberlite MB-3* resin (D-Darmstadt). Each residue, evaporated to dryness *in vacuo*, was mixed with L-cysteine methyl ester hydrochloride (2 mg) and dissolved in pyridine (2 ml), with the solns. being kept at 60° for 1 h, followed by addition of Me_3SiCl (0.5 ml) and then keeping for 30 min. Each soln. was diluted with H_2O and extracted with hexane (1 ml × 3). Each extract was analyzed by GC/MS [9][10]. The monosaccharides were confirmed as L-rhamnose, D-glucose, and D-galactose by comparison of the retention times of their derivatives with those of standard samples (L-rhamnose (14.19 min), D-glucose (15.49 min), and D-galactose (15.77 min), resp).

Cytotoxicity Assay. SMMC-7721 (human hepatoma carcinoma), NCI-H460 (human lung cancer), and A-549 (human lung adenocarcinoma) cell lines were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and grown in the indicated media supplemented with 10% *FBS* and 50 *IU* penicillin/streptomycin in a humidified atmosphere of 5% CO_2 at 37°. The cytotoxicity assay was performed according to the MTT method in 96-well microplates [11]. Briefly, 200 µl of adherent cells were seeded into 96-well cell-culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with initial density of 1×10^5 cells/ml. Each tumor cell line was exposed to the test compound at concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 µM in triplicates for 48 h, with 5-fluorouracil (5-FU, *Sigma*, USA) as a positive control. After compound treatment, the optical density was measured at 570 nm using a *Spectra Shell Microplate Reader* and a cell growth curve was plotted.

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