Four New Steroidal Saponins from the Seeds of *Allium tuberosum*

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Four new steroidal saponins, 26-*O*-*β*-D-glucopyranosyl-(25*S*,20*R*)-20-*O*-methyl-5α-furost-22(23)-en-2α,3*β*,20,26-tetraol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (**1**); 26-*O*-*β*-D-glucopyranosyl-(25*S*,20*R*)-5α-furost-22(23)-en-2α,3*β*,20,26-tetraol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L- rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (**2**); 26-*O*-*β*-D-glucopyranosyl-(25*S*,20*S*)-5α-furost-22(23)-en-2α,3*β*,20,26-tetraol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (**3**); and 26-*O*-*β*-D-glucopyranosyl-(25*S*,20*S*)-5α-furost-22(23)-en-3*β*,20,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (**4**), have been isolated from the seeds of *Allium tuberosum*. Their structures were established by spectroscopic studies such as MS, IR, NMR, and 2D-NMR and the results of acid hydrolysis and named tuberosides F, G, H, and I, respectively.

Keywords: *Steroidal saponins; tuberoside F, G, H, I; Allium tuberosum; Liliaceae*

INTRODUCTION

The scientific name of the Chinese chive is *Allium tuberosum* Rottl. (Liliaceae). It is known as "Jiucai" in China and Nira in Japan and is believed to have originated in China. It grows naturally in the central and northern parts of Asia and are cultured in China, Japan, Korea, India, Nepal, Thailand, and the Philippines (*1*). It is a perennial plant, and both the leaves and the inflorescences are eaten. Chinese chives have also been used as a herbal medicine for many diseases. According to the dictionary of Chinese drugs (*2*), the leaves have been used for treatment of abdominal pain, diarrhea, hematemesis, snakebite, and asthma, whereas the seeds are used as a tonic and aphrodisiac. In earlier studies, various volatile and nonvolatile sulfur-containing compounds (*3*−*5*), *N*-*p*-coumaroyltyramine and bis-(*p*-hydroxyphenyl) ether (*6*), the purine nucleoside, adenosine, and major free amino acids, alanine, glutamic acid, aspartic acid, and valine (*7*), 3-*O*-rhamnogalactosyl-7-*O*-rhamnosylkaempferol (*8*), and acylated flavonol glucosides (*9*) have been isolated from the leaves of *A. tuberosum*. However, there are no reports on phytochemicals in the seeds of this plant.

Plants of the genus *Allium* (Liliaceae) are well-known for their production of steroidal saponins (*10*). The steroidal saponins are naturally occurring glycosides that possess properties such as froth formation, hemolytic activity, toxicity to fish, and complex formation with cholestern (*11*). We have reported the isolation and structure elucidation of tubersides A−E (*12*, *13*). This paper deals with the isolation and structure elucidation of the other four novel steroidal saponins, named tuberosides F, G, H, and I, from the seeds of *A. tuberosum*.

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MATERIALS AND METHODS

General Procedures. Optical rotations were obtained on a JASCO DIP-181 polarimeter. IR spectra were recorded on a Perkin-Elmer model 599 infrared spectrometer. ^{1}H (400 Hz), ^{13}C (100 Hz), and all 2D NMR spectra were run on a Bruker AM-400 NMR spectrometer, with TMS as internal standard. FABMS were recorded on a MAT-95 mass spectrometer. GLC analysis was performed under the following conditions: Shimadzu GC-9A () glass column (300 × 0.32 cm) packed with OV 225; carrier gas, N_2; flow rate, 30 mL/min. Silica gel 60H and $HSGF_{254}$ (Qingdao Haiyang Chemical Group Co., Qingdao, People's Republic of China) were used for column chromatography and TLC, respectively.

Plant Material. The seeds of *A. tuberosum* were purchased from Shanghai Traditional Chinese Medicine Inc. in September 1997 and were identified by Professor Xuesheng Bao (Shanghai Institute of Drug Control). A voucher specimen (No. 334) has been deposited at the Herbarium of the Department of Phytochemistry, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Extraction and Isolation Procedures. The powdered seeds of *A. tuberosum* (50 kg) were extracted successively with petroleum ether (two times) and 95% EtOH (three times). After evaporation of ethanol in vacuo, the residue was suspended in water and then extracted successively with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH fraction (270 g) was subjected to passage over Diaion HP-20 using an $EtOH/H_2O$ gradient system (0−100%). The fraction (60 g) eluted by 70% EtOH was subjected to silica gel column chromatography with a $CH_2Cl_2/MeOH/H_2O$ solvent system (5:1:0.15−1:1:0.3). Finally, a fraction eluted by $CH_2Cl_2/MeOH/H_2O$ (2.5:1:0.2) was subjected to RP-18 silica gel column chromatography with 70% MeOH to give compounds **1** (50 mg), **2** (20 mg), **3** (35 mg), and **4** (20 mg).

Acid Hydrolysis of 1. A solution of **1** (3 mg) in 2 N aqueous CF_3COOH (5 mL) was refluxed on a water bath for 3 h. After this period, the reaction mixture was diluted with H_2O (15 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2

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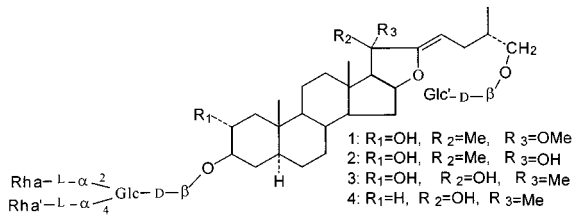

Figure 1. Structures of compounds **1—4**.

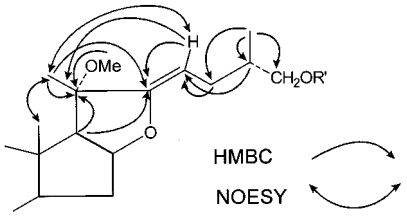

Figure 2. Significant HMBC (H→C) and NOESY correlations of **1**.

Table 1. ^{13}C (100 MHz) NMR Spectral Data for the Aglycon Part of Compounds 1−4 (C_5D_5N) (δ in Parts per Million)

	1	2	3	4		1	2	3	4
1	45.9 t	45.9 t	46.0 t	37.4 t	15	33.5 t	33.5 t	33.6 t	33.6 t
2	70.7 d	70.7 d	70.3 d	30.1 t	16	84.0 d	84.0 d	84.4 d	84.4 d
3	85.2 d	85.3 d	85.2 d	77.1 d	17	66.9 d	66.9 d	68.1 d	68.1 d
4	33.6 t	33.5 t	33.6 t	34.6 t	18	14.0 q	13.9 q	13.9 q	13.9 q
5	44.8 d	44.8 d	44.8 d	44.8 d	19	13.6 q	13.6 q	13.7 q	12.5 q
6	28.2 t	28.2 t	28.3 t	29.1 t	20	82.5 s	82.4 s	77.0 s	76.9 s
7	32.2 t	32.2 t	32.3 t	32.4 t	21	15.4 q	15.4 q	22.0 q	22.0 q
8	34.0 d	34.0 d	34.2 d	34.8 d	22	157.4 s	157.4 s	163.8 s	163.7 s
9	54.3 d	54.3 d	54.4 d	54.4 d	23	96.2 d	96.1 d	91.6 d	91.5 d
10	37.0 s	37.0 s	37.0 s	36.0 s	24	29.8 t	29.7 t	29.8 t	29.8 y
11	21.0 t	21.0 t	21.1 t	21.0 t	25	35.0 d	35.0 d	35.0 d	34.8 d
12	39.5 t	39.5 t	39.6 t	39.7 t	26	75.5 t	75.5 t	75.3 t	75.5 t
13	40.7 s	40.7 s	40.9 s	40.8 s	27	17.7 q	17.7 q	17.7 q	17.6 q
14	56.6 d	56.6 d	56.9 d	57.0 d					

extracts were washed with H_2O and dried with Na_2SO_4. Evaporation of the solvent gave neogitogenin (co-TLC with an authentic sample) (*11*, *12*). The sugars were analyzed by silica gel TLC in comparison with standard sugars (CH_3Cl/MeOH/H_2O, 7:3:0.5).

A 2 mg quantity of saponin **1** was refluxed in 2 N aqueous CF_3COOH (2 mL) in a sealed serum vial at 120 °C for 2 h and the solution evaporated to dryness with MeOH. The residue was dissolved in 5 mL of H_2O, and the mixture was reduced with $NaBH_4$ at room temperature for 3 h. After neutralization by the addition of AcOH, the mixture was evaporated to dryness. The resulting alditol mixture was refluxed with Ac_2O for 1 h, and the solution was evaporated to dryness. A sample was subjected to GLC to give the alditol acetates of rhamnose and glucose in a molar ratio of 1:1.

RESULTS AND DISCUSSION

Compounds **1**−**4** were shown to be furostanol saponins by Ehrlich's test (*14*).

Compound **1**, an amorphous solid [[α]$^{25}_D$ −27.8 (*c* 0.22, MeOH)], had a molecular formula of $C_{52}H_{86}O_{23}$ determined by ESIMS (*m*/*z* 1102 [M + Na]$^+$) as well as its ^{13}C and DEPT NMR data [IR (KBr cm^{-1}) 3415 (OH), 1641 (C═C), 1043 (glycoside linkage)]. The ^{1}H NMR spectrum of the aglycon part of **1** showed three angular methyl signals at δ 0.95, 0.96, and 1.43 (each 3H, s), one secondary methyl signal at δ 1.15 (3H, d, J = 6.4 Hz), one methoxy signal at δ 3.26 (3H, s), and one olefinic proton at δ 4.60 (1H, m). Comparison of the signals from the aglycon part of **1** in the ^{13}C NMR spectra (Table 1) with those from neogitogenin (*15*) showed that they have the same structure of A, B, C, and D rings. Detailed analyses of the ^{1}H−^{1}H COSY and TOCSY spectra of **1** gave the structure of the side chain (Figure 2). The structures of the E ring and the side chain were also confirmed by the HMBC correlations (Figure 2). The large difference between the chemical shifts of two protons of C-26 at δ 3.60 (1H, dd, J = 7.0, 9.2 Hz) and 4.21 (1H, m) indicated that C-25 of **1** had an *S* configuration (*16*). The NOESY cross-peaks between H-21/H-18 and H-21/H-23 (Figure 2) showed that C-20 of **1** should be the rare *R* configuration in spirostanols and furostanols. On the basis of the above spectra data, the aglycon of compound **1** was identified as (25*S*,20*R*)-20-*O*-methyl-5α-furost-22(23)-en-2α,3*β*,20,-26-tetraol. The tetrasaccharide nature of compound **1** was manifested by its ^{1}H and ^{13}C NMR data, respectively (Table 2). Acid hydrolysis of **1** gave glucose and rhamnose. The identity of the single sugar chain and the sequence of the oligosaccharide chain were determined by the analysis of a combination of its DEPT, COSY, TOCSY, HMQC, and HMBC NMR spectra. Starting from the anomeric proton of each sugar unit, all of the protons within each spin system were delineated using COSY NMR, with the aid of the TOCSY spectrum. On the basis of the assigned protons, the ^{13}C NMR resonances of each sugar unit were identified by HMQC and further confirmed by HMBC experiments. The α-anomeric configurations for the two rhamnoses were concluded from their chemical shifts at C-5 (δ 69.6 and 70.6). The *β*-anomeric configuration for the glucose units was judged from their large $^3J_{H1,H2}$ coupling constants (7−8 Hz) (*17*). From the HMBC spectrum, it was observed that H-G1/C-3, H-R′1/C-G2, H-R1/C-G4, and H-G′1/C-26 had cross-peaks. Thus, compound **1** was determined as 26-*O*-*β*-D-glucopyranosyl-(25*S*,20*R*)-20-*O*-methyl-5α-furost-22(23)-en-2α,3*β*,20,26-tetraol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside, named tuberoside F.

Compound **2** ($C_{51}H_{84}O_{23}$) is an amorphous solid [[α]$^{17}_D$ −46.0 (*c* 0.30, MeOH)]. On comparison of the NMR spectra of **2** with those of **1** (Table 1), all signals appeared at almost the same positions except the loss of a methoxyl group. **2** can be converted into **1** in methanol at 90 °C for 48 h. Thus, **2** was identified as 26-*O*-*β*-D-glucopyranosyl-(25*S*,20*R*)-5α-furost-22(23)-en-2α,3*β*,20,26-tetraol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside, named tuberoside G.

Compound **3** ($C_{51}H_{84}O_{23}$) is an amorphous solid [[α]$^{25}_D$ −41.8 (*c* 0.34, MeOH); IR (KBr, cm^{-1}) 3421 (OH), 1637 (C═C), 1041 (glycoside linkage)]. The spectral data of **3** showed that the structure of the oligosaccharide unit was the same as that of **2** (Table 2). The ^{1}H NMR spectrum of the aglycon part of **3** showed signals for three angular methyl groups at δ 0.86, 0.90, and 1.69 (each 3H, s), one secondary methyl group at δ 1.05 (3H, d, J = 6.0 Hz), an olefinic proton at δ 4.55 (1H, m), and two protons of C-26 at δ 3.51 (1H, dd, J = 7.0, 9.2 Hz) and δ 4.12 (1H, m). The ^{1}H−^{1}H COSY, TOCSY, HMQC, and HMBC spectra and the molecular formula of **3** indicated that the plane structure of **3** was the same as that of **2**. Comparison of the ^{13}C NMR of **3** with that of **2** showed that all signals of **3** and **2** were the same except those of C-17, -20, -21, -22, and -23 (Table 1). Thus, the differences between **3** and **2** should be in the configuration of C-20. The C-20 of **2** had an *R* configuration, so the configuration of **3** should be *S*. This was

Table 2. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR Spectral Data for the Sugar Moieties of Compounds 1−4 (C_5D_5N) (δ in Parts per Million, J in Hertz)

	1		**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
G								
1	101.0 d	5.10 d, 7.6	101.0 d	4.92 d,7.6	101.1 d	5.01d, 6.8	100.0 d	5.04 d, 7.2
2	78.0 d	4.30 m	78.0 d	4.23 m	78.0 d	4.23 m	78.1 d	4.28 m
3	78.1 d	4.30 m	78.1 d	4.23 m	78.3 d	4.23 m	78.3 d	4.28 m
4	78.9 d	4.45 m	78.9 d	4.37 m	79.0 d	4.39 m	78.9 d	4.45 m
5	77.2 d	3.84 m	77.3 d	3.75 m	77.3 d	3.75 m	77.1 d	3.79 m
6	61.3 t	4.14 m	61.3 t	4.08 m	61.5 t	4.07 m	61.6 t	4.13 m
		4.32 m		4.25 m		4.27 m		4.36 m
G′								
1	105.3 d	4.93 d, 7.2	105.3 d	4.85 d, 7.3	105.6 d	4.84 d, 7.2	105.3 d	4.91 d, 7.9
2	75.4 d	4.12 m	75.4 d	4.02 m	75.4 d	4.02 m	75.4 d	4.10 m
3	78.3 d	4.31 m	78.6 d	4.24 m	78.4 d	4.22 m	78.8 d	4.28 m
4	71.8 d	4.31 m	71.8 d	4.24 m	71.8 d	4.22 m	71.8 d	4.33 m
5	78.6 d	4.02 m	78.8 d	3.96 m	78.6 d	3.92 m	78.6 d	4.00 m
6	62.9 t	4.48 m	62.9 t	4.57 m	62.9 t	4.46 m	62.9 t	4.46 m
		4.60 m		4.41 m		4.61 m		4.60 m
R								
1	102.2 d	6.45 s	102.2 d	6.38 s	102.3 d	6.37 s	102.3 d	6.42 s
2	77.5 d	4.90 s	72.6 d	4.84 s	75.6 d	4.84 s	72.7 d	4.91 s
3	72.8 d	4.66 m	72.8 d	4.55 m	72.8 d	4.58 m	73.0 d	4.67 m
4	74.0 d	4.40 m	74.0 d	4.35 m	74.1 d	4.35 m	74.2 d	4.41 m
5	69.6 d	4.96 m	69.7 d	4.91 m	69.7 d	4.91 m	70.6 d	4.98 m
6	18.6 q	1.75 d, 6.2	18.7 q	1.72 d, 6.0	18.7 q	1.67d, 6.0	18.8 q	1.82 d, 6.1
R′								
1	103.0 d	5.90 s	103.1 d	5.85 s	103.1 d	5.83 s	103.1 d	5.91 s
2	72.6 d	4.75 s	72.7 d	4.69 s	72.7 d	4.67 s	72.6 d	4.75 s
3	72.9 d	4.60 m	72.9 d	4.61 m	72.9 d	4.54 m	72.9 d	4.61 m
4	74.2 d	4.40 m	74.2 d	4.35 m	74.2 d	4.35 m	74.0 d	4.41 m
5	70.6 d	5.00 m	70.6 d	5.01 m	70.7 d	4.96 m	69.6 d	5.01 m
6	18.7 q	18.67d,6.2	18.7 q	1.69 d, 5.9	18.7 q	1.62 d, 6.0	18.7 q	1.68 d, 6.2

confirmed by the NOESY spectrum of **3**. Thus, compound **3** was elucidated as 26-*O*-β-D-glucopyranosyl-(25*S*,20*S*)-5α-furost-22(23)-en-2α,3β,20,26-tetraol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside, named tuberoside H.

Compound **4** ($C_{51}H_{84}O_{22}$) is an amorphous solid [$[\alpha]^{25}_D$ −41.8 (*c* 0.28, MeOH); IR (KBr, cm^{-1}) 3406 (OH), 1641 (C═C), 1041 (glycoside linkage)]. The ^1H NMR spectrum of the aglycon part of **4** revealed the following signals: δ 0.95, 0.96, and 1.79 (each 3H, s), 1.15 (3H, d, J = 6.4 Hz), 3.60 (1H, dd, J = 7.0 Hz, H-26a), 4.00 (1H, m, H-26b), 4.60 (1H, m, H-23), an olefinic proton at δ 4.55 (1H, m), and two protons of C-26 at δ 3.51 (1H, dd, J = 7.0, 9.2 Hz) and δ 4.12 (1H, m). The $^1H-^1H$ COSY, TOCSY, HMQC, and HMBC spectra of **4** indicated that the E ring and the side chain of **4** were the same as those of **3**. Comparison of the ^{13}C NMR of **4** with that of **3** showed that the chemical shifts of **4** were very close to those of **3** except the upfield shifts of C-1, -2, and -3 and the downfield shift of **4** (Table 1). The above spectral data together with the molecular formula of **4** suggested that C-2 of **4** was the absence of a hydroxy group. Acid hydrolysis of **4** gave glucose and rhamnose. The COSY, TOCSY, HMQC, and HMBC spectrum of **4** indicated that the single sugar chain and the sequence of the oligosaccharide chain were the same as those of **3** (Table 2), so the structure of **4** was formulated as 26-*O*-β-D-glucopyranosyl-(25*S*,20*S*)-5α-furost-22(23)-en-3β,20,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside, named tuberoside I.

Compounds **1**−**4** are all unusual 22(23)-ene-furostanol saponins in furostanols. In addition, it is novel in spirostanols and furostanols that the C-20 of compounds **1** and **2** possess all *R* configurations.

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