New Spirostanol Saponins from Chinese Chives (*Allium tuberosum*)

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Three new spirostanol saponins have been isolated from the seeds of *Allium tuberosum*. On the basis of acid hydrolysis and comprehensive spectroscopic analysis, their structures were established as tuberoside **J**, (25*R*)-5α-spirostan-2α,3*β*,27-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside; tuberoside **K**, (25*R*)-5α-spirostan-2α,3*β*,27-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside; and tuberoside **L**, 27-*O*-*β*-D-glucopyranosyl-(25*R*)-5α-spirostan-2α,3*β*,27-triol 3-*O*-α-D-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside.

Keywords: *Allium tuberosum; Liliaceae; spirostanol saponins, tuberosides **J**, **K**, and **L***

INTRODUCTION

The steroidal saponins of the spirostane and furostane series are naturally occurring glycosides that possess properties such as froth formation, hemolytic activity, toxicity to fish, and complex formation with cholesterin (*1*). During recent years, steroidal glycosides have attracted a growing interest owing to the wide range of their biological action on living organisms, including antidiabetic (*2*), antitumor (*3*), antitussive (*4*), and platelet aggregation inhibitor activities (*5*). It is well-known that the *Allium* genus with ∼500 species has a wide distribution in the northern hemisphere and is a rich source of steroidal saponins as well as sulfur-containing compounds (*6*). These publications have prompted us to carry out systematic studies on steroidal saponins of one of the important *Allium* genus plants, Chinese chives. The scientific name of Chinese chives is *Allium tuberosum* Rottl. (Liliaceae). It is known as "jiucai" in China and "nira" in Japan. It is believed to have originated in China. It grows naturally in central and northern parts of Asia and is cultured in China, Japan, Korea, India, Nepal, Thailand, and the Philippines (*7*). It is a perennial plant, and both the leaves and the inflorescences are eaten. It has also been used as an herbal medicine for many diseases. According to the dictionary of Chinese drugs (*8*), the leaves have been used for treatment of abdominal pain, diarrhea, hematemesis, snakebite, and asthma; the seeds are used as a tonic and aphrodisiac. Nine new steroidal saponins, tuberosides **A**−**I**, from the seeds of this plant have been reported previously (*9*−*11*). Further investigation of this seed led to the isolation of three novel spirostanol saponins, named tuberosides **J**, **K**, and **L** (Figure 1). This paper deals with their isolation and structural elucidation.

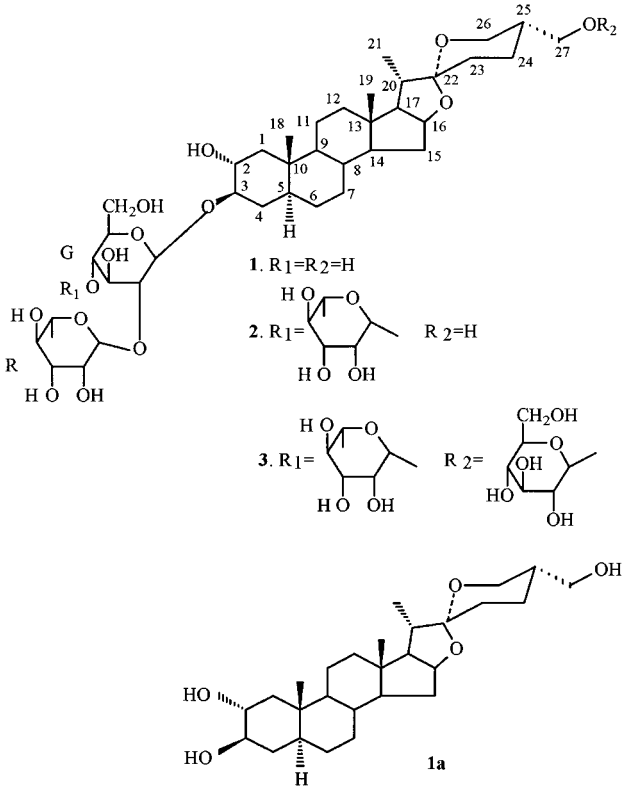

Figure 1. Structures of compounds **1**−**3** (tuberosides **J**, **K**, and **L**) and **1a**.

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. Silica gel 60H and $HSGF_{254}$ (Qingdao Haiyang Chemical Group Co., Qingdao, China) were used for column chromatography and TLC, respectively.

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Table 1. ^{13}C (100 MHz) NMR Spectral Data for the Sterol Parts of Compounds 1 (Tuberoside J), 2 (Tuberoside K), and 3 (Tuberoside L) (C_5D_5N) and Gitogenin ($CDCl_3$) (δ in ppm)

carbon	1	2	3	gitogenin
1	45.6t	45.5t	46.0t	45.1t
2	70.5d	70.3d	70.8d	73.0d
3	85.2d	84.8d	85.1d	76.4d
4	33.5t	33.2t	33.6t	35.6t
5	44.4d	44.4d	44.8d	44.9d
6	27.9t	27.9t	28.3t	27.9t
7	32.1t	32.0t	32.5t	32.1t
8	34.4d	34.4d	34.8d	34.5d
9	54.2d	54.2d	54.6d	54.3d
10	36.7s	36.3s	37.1s	37.6s
11	21.3t	21.2t	21.6t	21.2t
12	39.9t	39.8t	40.2t	40.0t
13	40.6s	40.5s	40.9s	40.6s
14	56.2d	56.1d	56.5d	56.3d
15	32.0t	31.9t	32.3t	31.8t
16	81.0d	80.9d	81.4d	80.7d
17	62.8d	62.8d	63.0d	62.2d
18	16.4q	16.3q	16.7q	16.8q
19	13.3q	13.2q	13.7q	14.4q
20	41.9d	41.8d	42.6d	42.6d
21	14.8q	14.8q	15.0q	14.4q
22	109.5s	109.4s	109.9s	109.0s
23	31.4t	31.3t	30.2t	31.4t
24	23.9t	23.8t	27.3t	28.6t
25	39.0d	38.9d	33.6d	30.2d
26	64.2t	64.2t	63.1t	67.1t
27	63.9t	63.8t	69.7t	17.1q

Plant Material. 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. Powdered seeds of *A. tuberosum* (50 kg) were extracted successively with petroleum ether (50 L × 2) and 95% EtOH (50 L × 3). 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 (CC) with a CH_2Cl_2/MeOH/H_2O solvent system (5:1:0.15−1:1:0.3). Finally, the fraction eluted by CH_2Cl_2/MeOH/H_2O (4:1:0.2) was subjected to RP-18 silica gel CC with 70% and 75% MeOH to get tuberoside **J** (compound **1**) (40 mg) and tuberoside **K** (compound **2)** (200 mg); the fraction eluted by CH_2Cl_2/MeOH/H_2O (3:1:0.2) was subjected to RP-18 silica gel CC with 70% MeOH to get tuberoside **L** (compound **3**) (38 mg).

*Compound **1**:* amorphous solid, $[\alpha]_D^{24}$ −35.1° (MeOH, *c* 0.28); IR_{max}^{KBr} cm^{-1} 3400, 1452, 1381, 1045, 989, 912, 816; FABMS, *m/z* 757 $[M + H]^+$, 611 $[M + H - 146]^+$, 595 $[M + H - 162]^+$, 449 $[M + H - 162 - 146]^+$; ^1H NMR (C_5D_5N) of the sterol part of **1** δ 0.87 (H-18, s), 0.97 (H-19, s), 1.25 (H-21, d, $J = 7.1$ Hz) 1.90 (H-17, m), 3.73 (H-26a, dd, $J = 7.3$, 0.6 Hz), 3.81 (H-26b, dd, $J = 5.2$, 10.6 Hz), 3.95 (H-27a, m), 3.97 (H-3, m), 4.20 (H-2, m), 4.21 (H-27b, m), 4.62(H-16, m); ^{13}C NMR (C_5D_5N) of the sterol part of **1** (see Table 1); ^1H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **1** (see Table 2).

*Acid Hydrolysis of **1**.* A solution of **1** (20 mg) in 2 N HCl (1,4 dioxane/H_2O 1:1, 2 mL) was heated at 100 °C for 2 h. The reaction mixture was neutralized with 10% KOH and subjected to a Sephadex LH-20 column using MeOH as eluant to give compound **1a** (7 mg) and the sugar fractions. The sugar fractions were compared with standard sugars on an HR-TLC silica gel plate developed with *n*-BuOH/Me_2CO/H_2O (4:5:1) and $CHCl_3$/MeOH/H_2O (7:3:0.5), detected by spraying with aniline−phthalic acid reagent [aniline/phthalic acid/*n*-BuOH (2:3:200)] and then heated to 110 °C.

Table 2. ^{13}C (100 MHz) and ^1H (400 MHz) NMR Spectral Data for the Sugar Moieties of Compounds 1 (Tuberoside J), 2 (Tuberoside K), and 3 (Tuberoside L) (C_5D_5N) (δ in ppm; *J* in Hz)

	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
glucose						
1	101.1d	5.14, d, 7.5	101.0d	5.06, d, 7.1	101.0d	5.07, d, 7.1
2	77.9d	4.29, dd, 7.7, 9.0	78.4d	4.23, m	78.3d	4.24, m
3	79.3d	4.34, m	78.2d	4.23, m	78.0d	4.24, m
4	71.3d	4.16, m	78.9d	4.40, m	78.9d	4.39, m
5	78.1d	4.08, m	78.0d	4.02, m	77.3d	3.83, m
6	62.3t	4.46, m	60.9t	4.01, m	61.1t	3.85, m
		4.61, m		4.40, m		4.10, m
glucose′						
1					105.1d	4.97, d, 7.7
2					75.4d	4.11, m
3					78.7d	4.31, m
4					71.9d	4.31, m
5					78.6d	4.03, m
6					61.4t	4.14, m
						4.32, m
rhamnose						
1	102.0d	6.41, s	102.3d	6.39, s	102.3d	6.39, s
2	72.3d	4.88, m	72.5d	4.88, m	72.6d	4.88, m
3	72.6d	4.64, m	72.8d	4.64, m	72.9d	4.64, m
4	73.9d	4.38, m	74.0d	4.39, m	74.0d	4.39, m
5	69.3d	4.96, m	69.7d	4.91, m	69.7d	4.90, m
6	18.4q	1.82, d, 6.1	18.7q	1.74, d, 6.1	18.7q	1.74, d, 6.2
rhamnose′						
1			103.0d	5.86, s	103.1d	5.86, s
2			72.6d	4.72, m	72.7d	4.72, m
3			72.9d	4.58, m	72.9d	4.59, m
4			74.2	4.39, m	74.2	4.39, m
5			70.6d	4.95, m	70.6d	4.95, m
6			18.7q	1.68, d, 6.1	18.7q	1.68, d, 6.2

*Compound **1a**:* amorphous solid; EIMS, *m/z* 448 $[M]^+$, 363, 289, 155; ^1H NMR ($CDCl_3$) δ 4.52 (H-16, m), 4.10 (H-27b, m), 4.00 (H-2, m), 3.81 (H-27a, m), 3.79 (H-26b, m), 3.50 (H-26a, m), 3.19 (H-3, m), 1.60 (H-17, m), 1.10 (H-21, d, $J = 6.7$ Hz), 0.81 (H-19, s), 0.79 (H-18, s).

*Compound **2**:* amorphous solid; $[\alpha]_D^{23}$ −39.8° (MeOH, *c* 0.30); IR_{max}^{KBr} cm^{-1} 3400, 1452, 1381, 1043, 980, 912, 814; FABMS, *m/z* 903 $[M + H]^+$, 757 $[M + H - 146]^+$, 741 $[M + H - 162]^+$, 595 $[M + H - 146 - 162]^+$, 449 $[M + H - 162 - 146 \times 2]^+$; ^1H NMR (C_5D_5N) of the sterol part of **2** δ 0.87 (H-18, s), 0.96 (H-19, s), 1.22 (H-21, d, $J = 6.9$ Hz), 1.91 (H-17, m), 3.72 (H-26a, m), 3.86 (H-26b, m), 3.95 (H-27a, m), 4.14 (H-3, m), 4.20 (H-2, m), 4.21 (H27b, m), 4.62 (H-16, m); ^{13}C NMR (C_5D_5N) of the sterol part of **2** (see Table 1); ^1H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **2** (see Table 2).

*Compound **3**:* amorphous solid, $[\alpha]_D^{25}$ −46.4° (MeOH, *c* 0.44); IR_{max}^{KBr} cm^{-1} 3417, 1452, 1381, 1043, 980, 912, 815; ESIMS, *m/z* 1088 $[M + Na]^+$; ^1H NMR (C_5D_5N) of the sterol part of **3** δ 0.84 (H-18, s), 1.00 (H-19, s), 1.14 (H-21, d, $J = 68$ Hz), 1.80 (H-17, m), 3.94 (H-3, m), 3.98 (H-27a, m), 4.14 (H-2, m), 4.41 (H-27b, m), 4.44 (H-26a, m), 4.52 (H-16, m), 4.61 (H-26b, m); ^{13}C NMR (C_5D_5N) of the sterol part of **3** (see Table 1); ^1H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **3** (see Table 2).

*Acid Hydrolysis of **2** and **3**.* An MeOH solution of compounds **2** and **3** together with standard sugar samples was applied at points ∼1 cm from the bottom of an HR-TLC silica gel plate and hydrolyzed with HCl vapor for 2 h at 50 °C; the plate was then heated at 60 °C for 4 h to remove residual HCl. Identification of sugars was performed as described under compound **1** above.

RESULTS AND DISCUSSION

The *n*-butanol fraction from the ethanol extract of the seeds of *A. tuberosum* was chromatographed on Diaion

HP-20, silica gel, and RP-18 silica gel to afford tuberosides **J** (**1**), **K** (**2**), and **L** (**3**).

Tuberoside **J** (compound **1**), an amorphous solid, had a molecular formula of $C_{39}H_{64}O_{14}$ determined by positive ion FABMS (at *m*/*z* 757 $[M + H]^+$) as well as ^{13}C DEPT NMR data. Its IR spectrum showed characteristic absorption for hydroxyl (3400 cm^{-1}) and a glycosidic linkage (1000−1100 cm^{-1}). Its spectral features and physicochemical properties suggested **1** to be a sterol saponin. Of the 39 carbons, 27 were assigned to the aglycon part and 12 to the oligosaccharide moiety (Tables 1 and 2). The ^1H NMR spectrum of **1** showed signals due to three steroidal methyl groups at δ 0.87, 0.97 (each 3H, s) and 1.25 (3H, d, $J = 7.1$ Hz) and one secondary methyl group of 6-deoxyhexapyranose at 1.82 (3H, d, $J = 6.1$ Hz) as well as two anomeric protons at δ 6.41 (1H, brs) and 5.14 (1H, d, $J = 7.5$ Hz). Comparing the ^1H and ^{13}CNMR spectra of the aglycon part of 1 with those of gitogenin (*1*) (Table 1), the appearance of CH_2 bearing hydroxy signals [δ_H 3.95, 4.21 (each 1H, m) and δ_C 63.9 t], the disappearance of a CH_3 signal at C-27 (δ 17.1 q), the downfield shifts of C-25 and C-27 (8.8 and 46.8), and the upfield shifts of C-24 and C-26 (4.7 and 2.9) indicated that the aglycon of **1** should be 27-hydroxy-1-gitogenin. Acid hydrolysis of **1** gave **1a**, D-glucose, and L-rhamnose. The ^1H NMR, IR, and MS spectral data of **1a** and the ^1H and ^{13}C NMR spectral data of the aglycon part of **1** allowed the identification of **1a** as (25*R*)-5α-spirostan-2α,3*β*,27-triol (*1*). Thus, on the basis of the above spectral data and chemical evidence, the structure of the sterol part of **1** was (25*R*)-5α-spirostan-2α,3*β*,27-triol glycosylated in the C-3 positions. The disaccharide nature of compound **1** was manifested by its ^1H [δ 6.41 (1H, br s), 5.14 (1H, d, $J =$ 7.5 Hz)] and ^{13}C (δ 102.0, 101.1) NMR data, respectively (Table 2). The identity of the single sugar chain and the sequence of the oligosaccharide chain were determined by a combination of DEPT, COSY, TOCSY, HMQC, and HMBC spectra. Starting from the anomeric proton of each sugar unit, all of the hydrogens within each spin system were delineated using COSY with the aid of TOCSY spectra. On the basis of the assigned protons, the ^{13}C resonances of each sugar unit were identified by HMQC and further confirmed by HMBC experiments. The α anomeric configuration for the rhamnose was judged by its C_5 data (δ 69.3). The *β* anomeric configuration for the glucose was judged from its large $^3J_{H1,H2}$ coupling constants (7.5 Hz). From the HMBC spectrum, the cross-peaks of C_3 (δ 85.2) with H_{Gl} (δ 5.14) and C_{G2} (δ 77.9) with H_{R1} (δ 6.41) were observed. Thus, tuberoside **J** (compound **1**) was determined to be (25*R*)-5α-spirostan-2α,3*β*,27-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside.

Tuberoside **K** (compound **2**) was isolated as an amorphous solid. The molecular formula was determined to be $C_{45}H_{74}O_{18}$ by positive ion FABMS (at *m*/*z* 903 $[M + H]^+$) as well as ^{13}C DEPT NMR data. Of the 45 carbons, 27 were assigned to the aglycon part and 18 to the oligosaccharide moiety (Tables 1 and 2). The spectral data of **2** showed that it possessed the same aglycon as **1** but differed from the saccharide structure of **1**. The molecular weight of **2** was 146 mass units greater than that of **1**, indicating that **2** had three sugar units. The trisaccharide nature of compound **2** was also manifested by its ^1H [δ 6.39, 5.86 (each 1H, s), and 5.06 (1H, d, $J =$ 7.1 Hz)] and ^{13}C (δ 103.0, 102.3, 101.0) NMR data, respectively (Table 2). Acid hydrolysis of **2** on an HR-TLC silica gel plate gave D-glucose and L-rhamnose. The identity of the monosaccharide and the sequence of the oligosaccharide chain were determined as described under compound **1** above. The α anomeric configuration for the rhamnoses was judged by their C_5 data (δ 69.7 and 70.6). The *β* anomeric configuration for the glucose was judged from its large $^3J_{H1,H2}$ coupling constants (7.1 Hz). From the HMBC spectrum, the cross-peaks of C_3 (δ 84.8) with H_{Gl} (δ 5.06), C_{G2} (δ 78.4) with H_{R1} (δ 6.39), and C_{G4}(δ 78.9) with $H_{R'1}$ (5.86) were observed. Thus, tuberoside **K** (compound **2**) was determined to be (25*R*)-5α-spirostan-2α,3*β*,27-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside.

Tuberoside **L** (compound **3**), an amorphous solid, had a molecular formula of $C_{51}H_{84}O_{23}$ determined by ESIMS (at *m*/*z* 1088 $[M + Na]^+$) as well as ^{13}C DEPT NMR data. Of the 51 carbons, 27 were assigned to the aglycon part and 24 to the oligosaccharide moiety (Tables 1 and 2). The spectral data of **3** showed that it possessed the same aglycon as **2** but differed from the saccharide structure of **2**. The molecular weight of **3** was 162 mass units greater than that of **2**, indicating that **3** had four sugar units. Acid hydrolysis of **3** on an HR-TLC silica gel plate gave D-glucose and L-rhamnose. The four-saccharide nature of compound **3** was also manifested by its ^1H [δ 6.39, 5.86 (each 1H, s), 5.07 (1H, d, $J = 7.1$ Hz), and 4.97 (1H, d, $J = 7.7$ Hz)] and ^{13}C (δ 105.1, 103.1, 102.3, 101.0) NMR data, respectively (Table 2). The identity of the monosaccharide and the sequence of the oligosaccharide chain were determined by a combination of DEPT, COSY, TOCSY, HMQC, and HMBC spectra as described under compound **1** above. The α anomeric configuration for the rhamnoses was judged by their C_5 data (δ 70.6 and 69.7). The *β* anomeric configurations for the two glucoses were judged from their large $^3J_{H1,H2}$ coupling constants (7−8 Hz). From the HMBC spectrum, there were cross-peaks between C_3 (δ 85.1) and H_{Gl} (δ 5.07), C_{G2} (δ 78.3) and H_{R1} (δ 6.39), C_{G4}(δ 78.9) and $H_{R'1}$ (5.86), and C_{27} (δ 69.7) and $H_{G'1}$ (δ 4.97). Thus, tuberoside **L** (compound **3**) was determined to be 27-*O*-*β*-D-glucopyranosyl-(25*R*)-5α-spirostan-2α,3*β*,27-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside.

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