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Food Chemistry 83 (2003) 499–506

Food Chemistry

www.elsevier.com/locate/foodchem

New steroid saponins from the seeds of *Allium tuberosum* L.

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Received 5 February 2003; accepted 25 February 2003

Abstract

Eight new steroidal saponins, including four spirostanol, three furostanol and one cholesterol saponin, have been isolated from the seeds of *Allium tuberosum*. On the basis of acid hydrolysis and comprehensive spectroscopic analyses, their structures were established as tuberoside **N**, (25*S*)-5β-spirostan-2β, 3β-diol 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl (1→4)]-β-D-glucopyranoside; tuberoside **O**, (25*S*)-5β-spirostan-2β, 3β, 5-triol 3-*O*-β-D-glucopyranoside; tuberoside **P**, (25*S*)-5β-spirostan-2β, 3β, 5-triol 3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside; tuberoside **Q**, (24*S*, 25*S*)-5β-spirostan-2β, 3β, 5, 24-tetraol 3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside; tuberoside **R**, 26-*O*-β-D-glucopyranosyl-(25*S*)-5β-furost-20(22)-ene-2β, 3β, 5, 26-tetraol 3-*O*-β-D-glucopyranoside; tuberoside **S**, 26-*O*-β-D-glucopyranosyl-(25*S*)-5β-furost-20(22)-ene-3β, 26-diol 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl (1→4)]-β-D-glucopyranoside; tuberoside **T**, 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furost-20(22)-ene-3β, 26-diol 3-*O*-α-L-rhamnopyranosyl (1→2)-[α-L-rhamnopyranosyl (1→4)]-β-D-glucopyranoside and tuberoside **U**, 16-*O*-β-D-glucopyranosyl-(22*S*, 25*S*)-cholest-5-ene-3β, 16β, 22, 26-tetraol 3-*O*-α-L-rhamnopyranosyl (1→2)-[α-L-rhamnopyranosyl (1→4)]-β-D-glucopyranoside, respectively.

Keywords: *Allium tuberosum*; Liliaceae; Steroid saponins; tuberosides **N–U**

1. Introduction

Since ancient time, many *Allium* species, such as onion, garlic, leek, and chives, have been used as foods, spices, and herbal remedies in widespread areas of the world, especially in the northern hemisphere. It is well known that the *Allium* genus, with about 500 species, is a rich source of steroidal saponins, as well as sulfur-containing compounds (Hostettmann & Marston, 1995). The steroidal saponins are naturally occurring glycosides that possess properties such as froth formation, hemolytic activity, toxicity to fish and complex formation with cholesterin (Agrawal, Jain, Guptq, & Thakur, 1985). During recent years, steroidal glycosides have attracted a growing interest owing to the wide range of their biological actions on living organisms, including antidiabetic (Nakashima, Kimura, Kimura, & Matsuura, 1993), antitumor (Wu, Chiang, & Fu, 1990), antitussive (Miyata, 1992) actions, and as platelet aggregation inhibitors (Niwa, Takeda, & Ishimaru, 1988). These reports 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). According to the dictionary of Chinese drugs (Jiangsu New Medical College, 1986), the leaves have been used for treatment of abdominal pain, diarrhea, hematemesis, snakebite and asthma, while the seeds are used as a tonic and aphrodisiac. Thirteen new steroidal saponins, tuberosides **A**–**M**, from the seeds of this plant have been reported previously (Sang, Lao, Wang, & Chen, 1999a,b; Sang, Mao, Lao, Chen, & Ho, 2001; Sang et al., 2001; Sang, Zou, Zhang, Lao, & Chen, 2002). Further investigation of this seed led to the isolation of eight novel steroid saponins, named tuberosides **N**–**U**. This paper deals with their isolation and structural elucidation.

2. Materials and methods

2.1. General procedures

Optical rotations were obtained on a JASCO DIP-181 polarimeter (Jasco Inc., Norwalk). IR spectra were

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doi:10.1016/S0308-8146(03)00131-6

recorded on a Perkin-Elmer model 599 Infrared spectrometer (PerkinElmer Co., Shelton, CT). ^{1}H (400 Hz), ^{13}C (100 Hz) and all 2D NMR spectra were run on a Brüker AM-400 NMR spectrometer (Brüker Co., Fallanden), with TMS as internal standard. FABMS were recorded on a MAT-95 mass spectrometer (Finnegan Co, Bremen). ESIMS were run on a Quattro mass spectrometer. 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.

2.2. Plant material

The seeds of *Allium 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.

2.3. Extraction and isolation procedures

The powdered seeds of *A. tuberosum* (50 kg) were extracted successively with petroleum ether (×2) and 95% EtOH (×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 a 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 (5:1:0.15) was subjected to RP-18 silica gel CC with 75 and 80% MeOH to get tuberoside **O** (**2**) (40 mg), tuberoside **P** (**3**) (150 mg), tuberoside **Q** (**4**) (18 mg), and tuberoside **R** (**5**) (80 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 **N** (**1**) (35 mg), the fraction eluted by CH_2Cl_2–MeOH–H_2O (2:1:0.2) was subjected to RP-18 silica gel CC with 65% MeOH to get tuberoside **S** (**6**) (50 mg), tuberoside **T** (**7**) (570 mg), tuberoside **U** (**8**) (25 mg).

Tuberoside **N** (**1**): an amorphous solid, $[\alpha]_D^{24}$ −34.5° (MeOH, *c* 0.43). IR_{max}^{KBr} cm^{-1}: 3396, 1452, 1070, 986, 918, 900, 848. FABMS: *m*/*z* 903 $[M+H]^+$, 757 $[M+H-146]^+$, 741 $[M+H-162]^+$, 595 $[M+H-146-162]^+$, 433 $[M+H-146-162\times2]$. ^{1}H NMR (C_5D_5N) of the sterol part of **1**: δ 0.88 (H-18, *s*), 1.05 (H-19, *s*), 1.15 (H-27, *d*, $J=7.0$ Hz), 1.21 (H-21, *d*, $J=6.9$ Hz), 1.90 (H-17, m), 3.47 (H-26a, *d*, $J=10.9$ Hz), 3.91 (H-2, *ddd*, $J=10.2$, 6.7, 3.5 Hz), 4.15 (H-26b, *m*), 4.40 (H-16, *m*) and 4.66 (H-3, *m*). ^{13}C NMR (C_5D_5N) of the sterol part of **1**: (Table 1); ^{1}H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **1** (Table 2).

Tuberoside **O** (**2**): an amorphous solid, $[\alpha]_D^{24}$ −37.8° (MeOH, *c* 0.28). IR_{max}^{KBr} cm^{-1}: 3400, 1450, 1068, 1041, 987, 921, 900, 852. FABMS: *m*/*z* 611 $[M+H]^+$, 449 $[M+H-162]^+$. ^{1}H NMR (C_5D_5N) of the sterol part of **2**: δ ppm 0.79 (H-18, *s*), 1.05 (H-27, *d*, $J=7.0$ Hz), 1.12 (H-21, *d*, $J=6.3$ Hz), 1.13 (H-19, *s*), 1.79 (H-17, *m*), 3.36 (H-26a, *d*, $J=11.8$ Hz), 3.99 (H-2, *m*), 4.02 (H-16, *m*), 4.04 (H-26b, *m*) and 4.69 (H-3, *m*). ^{13}C NMR (C_5D_5N) of the sterol part of **2**: (Table 1); ^{1}H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **2** (Table 2).

Acid hydrolysis of **2**. A soln of **2** (20 mg) in 2N 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 was subjected to a Sephadex LH-20 column using MeOH as eluant to give compound **2a** (13 mg) and the sugar fraction. The sugar fraction was compared with standard sugar on 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.

2a: an amorphous solid, EIMS: *m*/*z* 448 $[M]^+$, 389, 376, 316, 287, 269, 175, 139. ^{1}H NMR ($CDCl_3$): δ ppm 0.75 (H-18, *s*), 0.98 (H-19, *s*), 1.01 (H-27, *d*, $J=7.2$ Hz), 1.08 (H-21, *d*, $J=7.1$ Hz), 3.30 (H-26a, *d*, $J=11.0$ Hz), 3.67 (H-2, *ddd*, $J=11.9$, 4.8, 3.3 Hz), 3.94 (H-26b, *dd*, $J=12.0$, 2.7 Hz), 4.02 (H-16, *d*, $J=2.7$ Hz) and 4.39 (H-3, *ddd*, $J=7.4$, 3.3, 1.0 Hz). ^{13}C NMR ($CDCl_3$) (Table 1).

Tuberoside **P**(**3**): an amorphous solid, $[\alpha]_D^{24}$, *c* 0.25). cm^{-1}: 3400, 1452, 1041, 986, 922, 900, 850. FABMS: *m*/*z* 757 $[M+H]^+$, 611 $[M+H-146]^+$, 449 $[M+H-146-162]^+$. ^{1}H NMR (C_5D_5N) of the sterol part of **3**: δ ppm 0.91 (H-18, *s*), 1.16 (H-27, *d*, $J=7.0$ Hz), 1.21 (H-19, *s*), 1.22 (H-21, *d*, $J=6.4$ Hz), 1.90 (H-17, *m*), 3.45 (H-26a, *d*, $J=11.8$ Hz), 4.10 (H-2, *m*), 4.15 (H-26b, *m*), 4.65 (H-16, *m*) and 4.70 (H-3, *m*). ^{13}C NMR (C_5D_5N) of the sterol part of **3**: (Table 1); ^{1}H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **3** (Table 2).

Tuberoside **Q** (**4**): an amorphous solid, $[\alpha]_D^{24}$ −53.2° (MeOH, *c* 0.18). IR_{max}^{KBr} cm^{-1}: 3400, 1452, 1379, 1041, 993, 895. FABMS: *m*/*z* 773 $[M+H]^+$, 627 $[M+H-146]^+$, 465 $[M+H-146-162]^+$. ^{1}H NMR (C_5D_5N) of the sterol part of **4**: δ ppm 0.89 (H-18, *s*), 1.21 (H-19, *s*), 1.26 (H-21, *d*, $J=7.0$ Hz), 1.39 (H-27, *d*, $J=7.0$ Hz), 3.64 (H-26a, *d*, $J=10.3$ Hz), 4.10 (H-2, *m*), 4.13 (H-26b, *m*), 4.65 (H-16, *m*), 4.68 (H-24, *m*) and 4.73 (H-3, *m*). ^{13}C NMR (C_5D_5N) of the sterol part of **4** (Table 1); ^{1}H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **4** (Table 2).

Tuberoside **R** (**5**): an amorphous solid, $[\alpha]_D^{24}$ −18.2° (MeOH, *c* 0.18). IR_{max}^{KBr} cm^{-1}: 3400, 1448, 1381, 1078, 1039, 914, 856. FABMS: *m*/*z* 773 $[M+H]^+$, 611 $[M+H-162]^+$, 449 $[M+H-162\times2]^+$. ^{1}H NMR

Table 1
^{13}C (100 MHz) NMR spectral data for the aglycone part of compounds **1–8** and **2a** (C_5D_5N) (δ in ppm)

	1	2	2a	3	4	5	6	7	8
1	40.6 *t*	35.4 *t*	34.9 *t*	35.4 *t*	35.7 *t*	35.8 *t*	30.9 *t*	37.5 *t*	37.7 *t*
2	67.2 *d*	66.0 *d*	67.1 *d*	65.9 *d*	66.3 *d*	66.0 *d*	27.0 *t*	30.1 *t*	30.4 *t*
3	81.5 *d*	78.9 *d*	70.9 *d*	78.9 *d*	79.3 *d*	79.4 *d*	75.6 *d*	77.1 *d*	78.3 *d*
4	31.4 *t*	35.7 *t*	36.3 *t*	35.6 *t*	36.0 *t*	35.4 *t*	31.0 *t*	34.6 *t*	39.1 *t*
5	36.4 *d*	72.9 *s*	74.6 *s*	72.9 *s*	73.1 *s*	73.3 *s*	36.9 *d*	44.8 *d*	140.9 *s*
6	26.6 *t*	35.0 *t*	34.0 *t*	34.9 *t*	35.2 *t*	30.6 *t*	27.1 *t*	29.1 *t*	122.2 *d*
7	26.9 *t*	28.9 *t*	28.7 *t*	28.9 *t*	29.2 *t*	29.5 *t*	26.9 *t*	32.8 *t*	32.3 *t*
8	35.7 *d*	34.5 *d*	34.4 *d*	34.5 *d*	34.8 *d*	34.5 *d*	35.4 *d*	35.2 *d*	32.0 *d*
9	41.5 *d*	44.4 *d*	44.6 *d*	44.4 *d*	44.7 *d*	44.8 *d*	40.4 *d*	54.6 *d*	50.6 *d*
10	37.2 *s*	42.9 *s*	42.7 *s*	42.9 *s*	43.2 *s*	43.3 *s*	35.4 *s*	36.1 *s*	37.2 *s*
11	21.5 *t*	21.6 *t*	21.5 *t*	21.6 *t*	21.9 *t*	21.7 *t*	21.5 *t*	21.6 *t*	21.3 *t*
12	40.4 *t*	39.9 *t*	39.9 *t*	39.9 *t*	40.1 *t*	40.0 *t*	40.3 *t*	40.1 *t*	40.1 *t*
13	41.0 *s*	40.4 *s*	40.4 *s*	40.4 *s*	40.7 *s*	43.8 *s*	44.0 *s*	43.9 *s*	42.6 *s*
14	56.5 *d*	56.2 *d*	56.3 *d*	56.2 *d*	56.5 *d*	54.8 *d*	54.9 *d*	55.0 *d*	55.3 *d*
15	32.3 *t*	32.0 *t*	31.7 *t*	32.0 *t*	32.2 *t*	34.6 *t*	34.6 *t*	34.6 *t*	37.1 *t*
16	81.2 *d*	81.0 *d*	80.8 *d*	81.0 *d*	81.7 *d*	84.7 *d*	84.8 *d*	84.7 *d*	82.9 *d*
17	63.1 *d*	62.6 *d*	61.9 *d*	62.6 *d*	62.6 *d*	64.7 *d*	64.9 *d*	64.8 *d*	58.1 *d*
18	16.7 *q*	16.1 *q*	16.4 *q*	16.1 *q*	16.6 *q*	14.5 *q*	14.6 *q*	14.5 *q*	13.7 *q*
19	24.0 *q*	17.4 *q*	16.9 *q*	17.4 *q*	17.7 *q*	17.8 *q*	24.2 *q*	12.6 *q*	19.6 *q*
20	42.6 *d*	42.3 *d*	42.2 *d*	42.3 *d*	42.7 *d*	103.8 *s*	103.8 *s*	103.8 *s*	36.1 *d*
21	15.0 *q*	14.7 *q*	14.3 *q*	14.7 *q*	14.9 *q*	12.0 *q*	12.0 *q*	12.0 *q*	12.7 *q*
22	109.7 *s*	109.5 *s*	109.8 *s*	109.5 *s*	111.6 *s*	152.6 *s*	152.6 *s*	152.6 *s*	73.4 *d*
23	26.4 *t*	26.2 *t*	25.9 *t*	26.2 *t*	36.2 *t*	23.8 *t*	23.8 *t*	23.8 *t*	33.4 *t*
24	26.3 *t*	26.0 *t*	25.8 *t*	26.0 *t*	66.6 *t*	31.6 *t*	31.6 *t*	31.6 *t*	31.4 *t*
25	27.7 *d*	27.3 *d*	27.1 *d*	27.3 *d*	36.0 *d*	33.9 *d*	33.9 *d*	33.9 *d*	37.3 *d*
26	65.2 *t*	64.9 *t*	65.2 *t*	64.9 *t*	64.7 *t*	75.4 *t*	75.4 *t*	75.4 *t*	67.8 *t*
27	16.4 *q*	16.3 *q*	16.0 *q*	16.3 *q*	9.9 *q*	17.4 *q*	17.4 *q*	17.4 *q*	17.8 *q*

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 ppm, J in Hz)

	1		2		3		4	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
G								
1	102.5 *d*	4.99 *d*, 7.1	101.9 *d*	5.08 *d*, 7.8	101.7 *d*	5.07 *d*, 7.8	102.0 *d*	5.10 *d*, 7.9
2	82.5 *d*	4.33 *m*	74.6 *d*	4.05 *m*	74.7 *d*	3.98 *t*, 8.4	75.0 *d*	3.98 *dd*, 8.3, 8.7
3	77.3 *d*	4.33 *m*	78.4 *d*	4.23 *m*	76.5 *d*	4.25 *t*, 9.0	76.9 *d*	4.27 *t*, 9.0
4	77.3 *d*	4.50 *m*	71.4 *d*	4.23 *m*	78.1 *d*	4.45 *t*, 9.3	78.4 *d*	4.51 *t*, 9.2
5	76.5 *d*	3.74 *m*	78.7 *d*	3.99 *m*	77.3 *d*	3.81 *brd*, 8.5	77.7 *d*	3.82 *brd*, 8.3
6	61.2 *t*	4.31 *m*	62.3 *t*	4.51 *m*	60.9 *t*	4.30 *m*	61.3 *t*	4.33 *d*, 11.2
		4.16 *m*		4.37 *dd*, 11.8, 5.4		4.15 *m*		4.20 *m*
G′								
1	105.7 *d*	5.46 *d*, 7.7						
2	77.1 *d*	4.15 *m*						
3	78.7 *d*	4.36 *m*						
4	72.1 *d*	4.35 *m*						
5	78.1 *d*	4.04 *m*						
6	63.2 *t*	4.54 *m*						
		4.67 *m*						
R								
1	102.5 *d*	5.90 *brs*			102.5 *d*	5.90 *brs*	102.9 *d*	5.96 *brs*
2	72.6 *d*	4.70 *s*			72.3 *d*	4.72 *m*	72.7 *d*	4.77 *m*
3	72.9 *d*	4.59 *m*			72.5 *d*	4.60 *m*	72.9 *d*	4.62 *m*
4	74.1 *d*	4.39 *m*			73.7 *d*	4.40 *m*	74.1 *d*	4.41 *t*, 9.4
5	70.4 *d*	5.02 *m*			70.2 *d*	5.00 *m*	70.5 *d*	5.03 *m*
6	18.6 *q*	1.70 *d*, 6.1			18.3 *q*	1.88 *d*, 6.2	18.7 *q*	1.78 *d*, 6.1

(C_5D_5N) of the sterol part of **5**: δ ppm 0.76 (H-18, *s*), 1.18 (H-27, *d*, $J=76.6$ Hz), 1.22 (H-19, *s*), 1.69 (H-21, *s*), 2.53 (H-17, *d*, $J=10.1$ Hz), 3.57 (H-26a, *dd*, $J=7.0$, 9.0 Hz), 4.10 (H-2, *m*), 4.20 (H-26b, *m*), 4.78 (H-3, *m*) and 4.90 (H-16, *m*). ^{13}C NMR (C_5D_5N) of the sterol part of **5**: (Table 1); ^{1}H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **5** (Table 3).

Tuberoside **S** (**6**): an amorphous solid, $[\alpha]_D^{24}$ −25.2° (MeOH, *c* 0.32). IR_{max}^{KBr} cm^{-1}: 3400, 1448, 1379, 1074, 910. positive ESIMS: *m*/*z* 1072 $[M+H+Na]^+$. ^{1}H NMR (C_5D_5N) of the sterol part of **6**: δ ppm 0.78 (H-18, *s*), 1.05 (H-19, *s*), 1.12 (H-27, *d*, $J=6.6$ Hz), 1.70 (H-21, *s*), 2.56 (H-17, *d*, $J=10.1$ Hz), 3.57 (H-26a, *dd*, $J=7.0$, 9.1 Hz), 4.15 (H-26b, *m*), 4.35 (H-3, *m*) and 4.91 (H-16, *m*). ^{13}C NMR (C_5D_5N) of the sterol part of **6**: (Table 1); ^{1}H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **6** (Table 3).

Tuberoside **T** (**7**): an amorphous solid, $[\alpha]_D^{24}$ −29.4° (MeOH, *c* 0.33). IR_{max}^{KBr} cm^{-1}: 3415, 1450, 1381, 1041, 910, 812. FABMS: *m*/*z* 1033 $[M+H]^+$. ^{1}H NMR (C_5D_5N) of the sterol part of **7**: δ ppm 0.77 (H-18, *s*), 0.90 (H-19, *s*), 1.11 (H-27, *d*, $J=6.5$ Hz), 1.70 (H-21, *s*), 2.57 (H-17, *d*, $J=10.0$ Hz), 3.55 (H-26a, *dd*, $J=7.0$, 9.0 Hz), 4.00 (H-3, *m*), 4.11 (H-26b, *m*) and 4.88 (H-16, *m*). ^{13}C NMR (C_5D_5N) of the sterol part of **7** (Table 1); ^{1}H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **7** (Table 3).

Tuberoside **U** (**8**): an amorphous solid, $[\alpha]_D^{24}$ −57.0° (MeOH, *c* 0.30). IR_{max}^{KBr} cm^{-1}: 3400, 1641, 1460, 1383, 1041. ESIMS: *m*/*z* 1074 $[M+Na]^+$. ^{1}H NMR (C_5D_5N) of the sterol part of **8**: δ ppm 1.08 (H-18, *s*), 1.11 (H-19, *s*), 1.20 (H-27, *d*, $J=6.3$ Hz), 1.30 (H-21, *d*, $J=6.9$), 2.07 (H-17, *m*), 3.79 (H-26a, *m*), 3.91 (H-3, *m*), 3.93 (H-26b, *m*), 4.61 (H-16, *m*) and 5.34 (H-6, *m*). ^{13}C NMR (C_5D_5N) of the sterol part of **8** (Table 1); ^{1}H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **8** (Table 3).

Acid hydrolysis of **1**, **3–8**. MeOH soln of each compound together with standard sugar samples, were applied at points ca. 1 cm from the bottom of a HR-TLC sillica 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 were performed as described under tuberoside **O** (**2**).

3. 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 **N–U** (**1–8**).

Tuberoside **N** (**1**), an amorphous solid, had a molecular formula of $C_{45}H_{74}O_{18}$ determined by positive ion FABMS (at *m*/*z* 903 $[M+H]^+$) as well as ^{13}C, DEPT NMR data. Its IR spectrum featured a strong absorptions at 3396 cm^{-1} due to hydroxyl groups, and characteristic absorption at 986, 918, 900 and 848 cm^{-1}, with the absorption at 918 cm^{-1} being of greater intensity than that at 900 cm^{-1}, implying the presence of a (25*S*)-spiroacetal moiety in the molecule (Eddy, Wall, & Scott, 1953; Jones, Katzenellenbogen, & Dobriner, 1953; Wall, Eddy, McClennan, & Klumpp, 1952). The ^{1}H NMR spectrum of **1** showed signals for two angular methyl groups at δ 0.88 and 1.05 (each *s*), and three secondary methyl proton signals at δ 1.15 (*d*, $J=7.0$ Hz), 1.21 (*d*, $J=6.9$ Hz) and 1.70 (*d*, $J=6.1$ Hz), and three anomeric protons at δ 5.90 (s), 5.46 (*d*, $J=7.7$ Hz) and 4.99 (*d*, $J=7.1$ Hz). The doublet at δ 1.70 ($J=6.1$ Hz) was due to the methyl group of a 6-deoxyhexopyranose sugar. The ^{13}C NMR spectrum of **1** showed four signals at lower field than 100 ppm; the signals at δ 105.7, 102.5 and 102.5 were due to anomeric carbons, and the signal at δ 109.7 was assignable to the C-22 carbon of a spirostan skeleton (Agrawal et al., 1985). The above data were consistent with **1** being a (25*S*)-spirostanol trisaccharide. Comparison of the signals from the aglycone moiety in the ^{13}C NMR spectra (Table 1) with those from markogenin [(25*S*)-5β-spirostane-2β, 3β-diol] (Kaneda, Nakanishi, & Staba, 1987) showed that the aglycone moiety of **1** was markogenin and sugars were attached to the C-3 position of markogenin. The coupling constants of H-2 (*ddd*, $J=10.2$, 6.7 and 3.5 Hz) observed from the 2D J-resolved spectroscopy of **1** further confirmed the β configuration of the hydroxyl groups at positions 2 and 3 of the aglycone. The 5β configuration was further confirmed by the chemical shift of C-19 (δ 24.0) (Agrawal et al., 1985). Acid hydrolysis of **1** gave glucose and rhamnose. The identity of the sugars 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 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 configuration for the rhamnose was judged by its chemical shifts at C-5 (δ 70.4). The β-anomeric configurations for the two glucose units were judged from their large $^3J_{H1,H2}$ coupling constants (7–8 Hz). The HMBC spectrum showed cross peaks between C-3 (δ 81.5) and H-G_1 (δ 4.99), C-G_2 (δ 82.5) and H-G'_1 (δ 5.46), and C-G_4 (δ 77.3) and H-R_1 (δ 5.90). Thus, tuberoside **N** (**1**) was determined as (25*S*)-5β-spirostan-2β, 3β-diol 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl (1→4)]-β-D-glucopyranoside (Fig. 1).

Tuberoside **O** (**2**) was assigned a molecular formula of $C_{33}H_{54}O_{10}$, determined by positive ion FABMS (at *m*/*z* 611 $[M+H]^+$), as well as ^{13}C, DEPT NMR data. Of the

Table 3
^{13}C (100 MHz) and ^{1}H (400 MHz) NMR spectral data for the sugar moieties of compounds **5–8** (C_5D_5N) (δ in ppm, J in Hz)

	5		**6**		**7**		**8**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
G								
1	102.3 *d*	5.13 *d*, 7.8	102.0 *d*	4.94 *d*, 7.1	100.0 *d*	5.04 *d*, 7.4	100.4 *d*	5.02 *d*, 7.0
2	74.9 *d*	4.03 *m*	83.0 *d*	4.30 *m*	78.3 *d*	4.30 *m*	78.1 *d*	4.30 *m*
3	78.7 *d*	4.30 *m*	77.2 *d*	4.33 *m*	78.1 *d*	4.30 *m*	78.0 *d*	4.29 *m*
4	71.7 *d*	4.25 *m*	77.5 *d*	4.51 *m*	78.9 *d*	4.42 *m*	78.9 *d*	4.43 *m*
5	78.6 *d*	4.02 *m*	76.6 *d*	3.70 *m*	77.4 *d*	3.80 *m*	77.1 *d*	3.72 *m*
6	62.6 *t*	4.59 *m*	61.5 *t*	4.29 *m*	61.6 *t*	4.34 *m*	61.5 *t*	4.30 *m*
		4.44 *m*		4.12 *m*		4.19 *m*		4.18 *m*
G′								
1	105.3 *d*	4.88 *d*, 7.7	105.3 *d*	4.91 *d*, 7.7	105.3 *d*	4.90 *d*, 7.6	107.2 *d*	4.82 *d*, 7.6
2	75.4 *d*	4.03 *m*	75.4 *d*	4.11 *m*	75.4 *d*	4.09 *t*, 8.0	75.8 *d*	4.07 *t*, 8.2
3	78.9 *d*	4.30 *m*	78.7 *d*	4.31 *m*	78.7 *d*	4.28 *m*	78.8 *d*	4.23 *m*
4	71.8 *d*	4.25 *m*	71.9 *d*	4.31 *m*	71.9 *d*	4.28 *m*	71.9 *d*	4.31 *m*
5	78.7 *d*	4.02 *m*	78.7 *d*	4.05 *m*	78.6 *d*	4.01 *m*	78.3 *d*	3.91 *m*
6	63.0 *t*	4.59 *m*	63.0 *t*	4.62 *m*	63.0 *t*	4.60 *m*	63.1 *t*	4.58 *m*
		4.44 *m*		4.46 *m*		4.44 *m*		4.45 *m*
G″								
1			105.8 *d*	5.51 *d*, 7.7				
2			77.2 *d*	4.16 *m*				
3			78.1 *d*	4.29 *m*				
4			77.0 *d*	4.30 *m*				
5			78.7 *d*	4.05 *m*				
6			63.1 *t*	4.65 *m*				
				4.55 *m*				
R								
1					102.3 *d*	6.42 *s*	102.2 *d*	6.45 *s*
2					72.6 *d*	4.76 *s*	72.7 *d*	4.90 *m*
3					72.9 *d*	4.59 *m*	72.9 *d*	4.68 *dd*, 3.3, 9.2
4					74.0	4.39 *m*	74.1 *d*	4.42 *m*
5					69.7 *d*	4.96 *m*	69.7 *d*	5.02 *m*
6					18.8 *q*	1.69 *d*, 6.3	18.8 *q*	1.84 *d*, 6.2
R′								
1			102.6 *d*	5.96 *s*	103.1 *d*	5.90 *s*	103.1 *d*	5.91 *s*
2			72.9 *d*	4.74 *s*	72.7 *d*	4.76 *s*	72.7 *d*	4.76 *m*
3			72.7 *d*	4.58 *m*	73.0 *d*	4.59 *m*	73.0 *d*	4.62 *m*
4			74.1 *d*	4.37 *m*	74.2 *d*	4.39 *m*	74.3 *d*	4.40 *m*
5			70.4 *d*	5.03 *m*	70.6 *d*	4.96 *m*	70.6 *d*	4.97 *m*
6			18.6 *q*	1.73 *d*, 6.2	18.7 *q*	1.69 *d*, 6.3	18.7 *q*	1.70 *d*, 6.1

33 carbons, 27 were assigned to the aglycone part, six to the oligosaccharide moiety (Tables 1 and 2). Acid hydrolysis of **2** gave **2a** and glucose. The molecular formula of **2a** was determined to be $C_{27}H_{44}O_5$ by EIMS (at m/z 448 $[M]^+$) as well as ^{13}C, DEPT NMR data. This indicated that the aglycone of **2** had one more hydroxyl group than that of **1**. Comparing the ^{1}H and ^{13}C NMR spectra of the aglycone part of **2** with that of **1**, the downfield shifts of C-4, C-5, C-6 and C-10 (4.3, 36.5, 8.4 and 5.7), the upfield shifts of C-1, C-3 and C-19 (5.2, 2.6 and 6.6), the appearance of a quaternary carbon signal bearing hydroxyl group (δ_C 72.9 *s*) and the disappearance of a methine signal at C-5 (δ_C 36.4 *d*), indicated that the aglycone of **2** was 5β-hydroxyl markogenin [(25*S*)-5β-spirostane-2β, 3β, 5-triol]. The β configurations of the hydroxyl groups at positions 2 and 3 of this aglycone were further confirmed by the coupling constants of H-2 (δ 3.67, *ddd*, $J = 11.9$, 9.8, 3.3 Hz) and H-3 (δ 4.40, *ddd*, $J = 7.4$, 3.3, 1.0 Hz) observed from the 2D *J*-resolved spectroscopy of **2a**. While the chemical shift of C-19 (δ 17.4) (Agrawal et al., 1985) further confirmed the β configuration of the hydroxyl groups at positions 5. Thus, based on the above spectral data and chemical evidence, the structure of the sterol part of **2** was (25*S*)-5β-spirostane-2β, 3β, 5-triol glycosylated in the C-3 position. The monosaccharide nature of **2** was manifested by its ^{1}H [δ 5.08 (d, $J = 7.8$ Hz)] and ^{13}C [δ 101.9] NMR data, respectively (Tables 1 and 2). The β

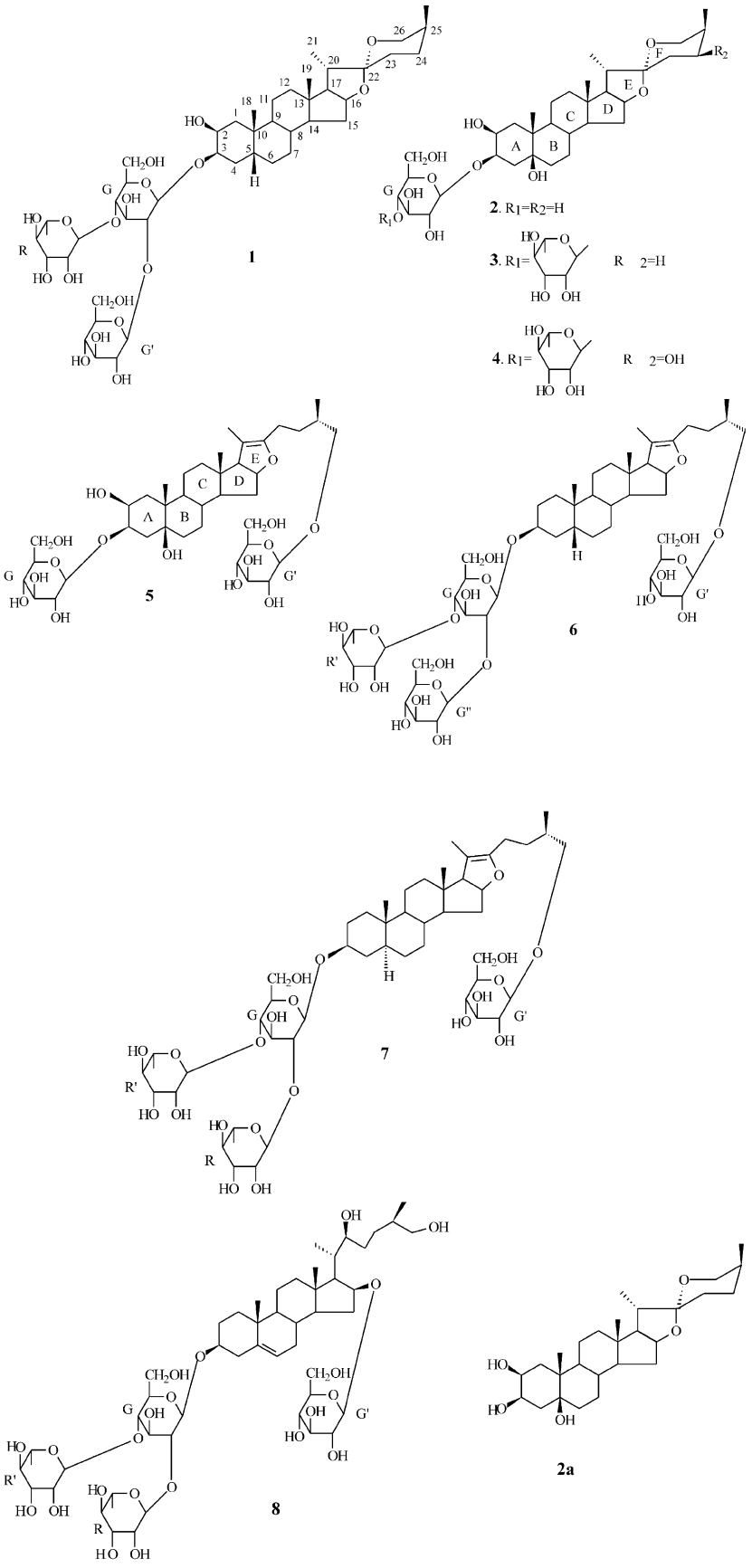

Fig. 1. Structures of compounds **1–8** and **2a**.

anomeric configuration for the glucose was judged from its large $^3J_{H1,H2}$ coupling constants (7.8 Hz). Thus, tuberoside **O** (**2**) was determined to be (25*S*)-5β-spirostan-2β, 3β, 5-triol 3-*O*-β-D-glucopyranoside (Fig. 1).

Tuberoside **P** (**3**) was isolated as an amorphous solid. The molecular formula was determined to be $C_{39}H_{64}O_{14}$ by positive ion FABMS (at *m*/*z* 757 $[M+H]^+$) as well as ^{13}C, DEPT NMR data. Of the 39 carbons, 27 were assigned to the aglycone part, and 12 to the oligosaccharide moiety (Tables 1 and 2). The spectral data of **3** showed that it possessed the same aglycone as that of **2**, but differed from the saccharide structure. The molecular weight of **3** was 146 mass units greater than that of **2,** indicating that **3** had two sugar units. The disaccharide nature of **3** was also manifested by its ^1H [δ 5.90 (1H, *s*) and δ 5.07 [1H, *d*, *J* = 7.8 Hz] and ^{13}C [δ 102.5 and 101.7] NMR data, respectively (Table 2). Acid hydrolysis of **3** gave glucose and rhamnose. The identity of the monosaccharide and the sequence of the oligosaccharide chain were determined as described under **1**. The α anomeric configuration for the rhamnose was judged by its C-5 data (δ 70.2). The β anomeric configuration for the glucose was judged from its large $^3J_{H1,H2}$ coupling constants (7.8 Hz). From the HMBC spectrum, the cross peaks of C-3 (δ 78.9) with H-G_1 (δ 5.07) and C-G_4 (δ 78.1) with H-R_1 (δ 5.90) were observed. Thus, tuberoside **P** (**3**) was determined to be (25*S*)-5β-spirostan-2β, 3β, 5-triol 3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside.

Tuberoside **Q** (**4**) was isolated as an amorphous solid. The molecular formula was determined to be $C_{39}H_{64}O_{15}$ by positive ion FABMS (at *m*/*z* 773 $[M+H]^+$) as well as ^{13}C, DEPT NMR data. The spectral data of **4** showed that it possessed the same saccharide structure as that of **3**, but differed from the aglycone portion. The molecular weight of **4** was 16 mass units greater than that of **3**, indicating that **4** had one more hydroxyl group. Comparing the ^1H and ^{13}C NMR spectra of the aglycone part of **4** with those of **3**, the downfielded shifts of C-23, C-24 and C-25 (10.0, 40.6 and 8.7) and the upfielded shifts of C-26 and C-27 (0.2 and 6.2) indicated that the additional hydroxyl group was located at position 24. The configuration of position 24 was identified as *S* by comparing the NMR data with those of (24*S*, 25*S*)-5α-spirostan-2α, 3β, 5α, 6β, 24-pentol (Mimaki, Nikaido, Matsumoto, Sashida, & Ohmoto, 1994); they had the same structures for the C, D, E and F rings. Therefore, the aglycone of **4** was (24*S*, 25*S*)-5β-spirostane-2β, 3β, 5, 24-tetraol. Tuberoside **Q** (**4**) was identified as (24*S*, 25*S*)-5β-spirostan-2β, 3β, 5, 24-tetraol 3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside (Fig. 1).

Tuberoside **R** (**5**) had a molecular formula of $C_{39}H_{64}O_{15}$, determined by positive ion FAB-MS (at *m*/*z* 773 $[M+H]^+$) as well as from its ^{13}C and DEPT NMR data. **5** was shown to be a 20(22)-ene-furostanol saponin from ^1H and ^{13}C NMR spectra [δ_H 1.70 (H-21, s), δ_C 152.6 (C-22), and 103.8 (C-20)] (Mimaki, Takaashi, Kuroda, & Sashida, 1997). Comparing the ^{13}C NMR spectra of the aglycone part of **5** with those of **2** indicated that both compounds had the same structures of the A, B, C and D rings, but differed in the E and F rings. Comparison of the signals from the sterol part of **5** in the ^{13}C NMR spectra with those from the sterol part of 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furost-20(22)-ene-1β,3α,26-triol 3-*O*-β-D-glucopyranoside (Mimaki et al., 1997) showed that they had the same sterol part except A ring. Thus, based on the above spectral data, the structure of the sterol part of **5** was determined as (25*S*)-5β-furost-20(22)-ene-2β, 3β, 5, 26-tetraol glycosylated at the C-3 and C-26 positions. Acid hydrolysis of **5** gave glucose. The disaccharide nature of **5** was manifested by its ^1H [δ 5.13 (1H, *d*, *J* = 7.8 Hz) and 4.88 (1H, *d*, *J* = 7.7 Hz)] and ^{13}C [δ 105.3, 102.3] NMR data, respectively (Table 3). The β anomeric configurations for the glucose were judged from their large $^3J_{H1,H2}$ coupling constants (7.8 and 7.7 Hz). Finally in the HMBC spectrum, the cross peaks of C-3 (δ 79.4) with H-G_1 (δ 5.13) and C-26 (δ 75.4) with H-G'_1 (δ 4.88) identified the linkage sites. Thus, tuberoside **R** (**5**) was determined to be 26-*O*-β-D-glucopyranosyl-(25*S*)-5β-furost-20(22)-ene-2β, 3β, 5, 26-tetraol 3-*O*-β-D-glucopyranoside.

Tuberoside **S** (**6**) was isolated as an amorphous solid. The molecular formula was determined to be $C_{51}H_{84}O_{22}$ by positive ion ESIMS (at *m*/*z* 1072 $[M+H+Na]^+$), as well as ^{13}C, DEPT NMR data. **6** was also suggested to be a 20(22)-ene-furostanol saponin from ^1H and ^{13}C NMR spectra [δ_H 1.70 (H-21, *s*), δ_C 152.6 (C-22), and 103.8 (C-20)]. Comparison of the signals from the aglycone moiety in the ^{13}C NMR spectra (Table 1) with those from anemarsaponin B (Dong & Han, 1991) showed that the aglycone moiety of **6** was the same as that of anemarsaponin B and sugars were bound to the C-3 and C-26 positions. Acid hydrolysis of **6** gave glucose, and rhamnose. The tetrasaccharide nature of **6** was manifested by its ^1H [δ 5.96 (1H, *s*), 5.51 (1H, *d*, *J* = 7.7 Hz), 4.94 (1H, *d*, *J* = 7.1 Hz) and 4.91 (1H, *d*, *J* = 7.7 Hz)] and ^{13}C [δ 105.8, 105.3, 102.6 and 102.0] NMR data, respectively (Table 3). In the HMBC spectrum, the following diagnostic cross peaks of C-3 (δ 75.6) with H-G_1 (δ 4.94), C-26 (δ 75.4) with H-G'_1 (δ 4.91), C-G_2 (δ 83.0) with H-G''_1 (δ 5.51) and C-G_4 (δ 77.5) with H-R'_1 (δ 5.96) were observed. Thus, tuberoside **S** (**6**) was determined to be 26-*O*-β-D-glucopyranosyl-(25*S*)-5β-furost-20(22)-ene-3β, 26-diol 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside.

Tuberoside **T** (**7**) had a molecular formula of $C_{51}H_{84}O_{21}$ determined by FABMS (at *m*/*z* 1033 $[M+H]^+$) as well as ^{13}C, DEPT NMR data. Comparing the ^{13}C NMR spectra of the aglycone part of **7** with those of **6** indicated the sterol part of **7** to be the same as that of **6** except for the A ring and B ring. Comparison of the signals from the sterol part of **7** in the ^{13}C NMR

spectra with those of neotigogenin (Agrawal et al., 1985) showed that they had the same structures for rings A–D. Based on the earlier spectral data, the structure of the sterol part of **7** was (25*S*)-5α-furost-20(22)-ene-3β, 26-diol, glycosylated in the C-3 and C-26 positions. Acid hydrolysis of **7** gave glucose and rhamnose. The tetrasaccharide nature of **7** was also manifested by its ^{1}H [δ 6.42, 5.90 (each 1H, *s*), 5.04 (1H, *d*, $J=7.4$ Hz) and 4.90 (1H, *d*, $J=7.6$ Hz)] and ^{13}C [δ 105.3, 103.1, 102.3, 100.0] NMR data, respectively (Table 3). From the HMBC spectrum, there were cross peaks between C-3 (δ 77.1) with H-G_1 (δ 5.04), C-26 (δ 75.4) with H-G'_1 (δ 4.90), C-G_2 (δ 78.3) with H-R_1 (δ 6.42) and C-G_4 (δ 78.9) with H-R'_1 (δ 5.90). Therefore, tuberoside **T** (**7**) was determined to be 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furost-20(22)-ene-3β, 26-diol 3-*O*-α-L-rhamnopyranosyl(1→2)-[α-L-rhamnopyranosyl (1→4)]-β-D-glucopyranoside.

Tuberoside **U** (**8**) had a molecular formula of $C_{51}H_{86}O_{22}$ determined by ESIMS (at *m*/*z* 1074 $[M+Na]^+$) as well as ^{13}C, DEPT NMR data. Comparing the ^{13}C NMR spectra of the aglycone part of **8** with those of (25*S*)-cholest-5-ene-3β, 16β, 26-triol (Uomori, Seo, Sato, Yoshimura, & Takeda, 1987), the downfielded shifts of C-3 and C-16 (5.9 and 10.1 ppm), and the upfielded shifts of C-2, C-4, C-15 and C-17 (0.8, 3.8, 4.1 and 4.6), indicated glycosylation at the C-3 and C-26 positions; downfield shifts of C-20, C-22 and C-23 (3.5, 38.6 and 8.5 ppm), and the upfield shifts of C-21 and C-24 (6.1 and 1.5), suggested one hydroxyl group located at position 22. The configuration at position 22 was assumed as *S* by comparison of the ^{13}C NMR signals of the part structures for ring C, ring D and side chain from the sterol part of **8** with the corresponding signals of (22S)-cholest-5-ene-1β, 3β, 16β, 22-tetraol 1-*O*-α-L-rhamnopyranoside 16-*O*-β-D-glucopyranoside (Mimaki, Kawashima, Kanmoto, & Sashida, 1993) which showed that they had the same structures for rings C and D and side chain except for the downfielded shifts of C-26 and C-25 (44.7 and 8.4 ppm) and the upfielded shifts of C-24 and C-27 (5.4 and 5.3 ppm). Based on the earlier spectral data, the structure of the sterol part of **8** was (22*S*, 25*S*)-cholest-5-ene-3β, 16β, 22, 26-tetraol, glycosylated in the C-3 and C-16 positions. Acid hydrolysis of **8** gave glucose and rhamnose. The tetrasaccharide nature of **8** was also manifested by its ^{1}H [δ 6.45, 5.91 (each 1H, *s*), 5.02 (1H, *d*, $J=7.0$ Hz) and 4.82 (1H, *d*, $J=7.6$ Hz)] and ^{13}C [δ 107.2, 103.1, 102.2, 100.4] NMR data, respectively (Table 3). From the HMBC spectrum, the following cross peaks between C-3 (δ 78.3) with H-G_1 (δ 5.02), C-16 (δ 82.9) with H-G'_1 (δ 4.82), C-G_2 (δ 78.1) with H-R_1 (δ 6.45) and C-G_4 (δ 78.9) with H-R'_1 (δ 5.91) were observed. Therefore, tuberoside **U** (**8**) was determined to be 16-*O*-β-D-glucopyranosyl-(22*S*, 25*S*)-cholest-5-ene-3β, 16β, 22, 26-tetraol 3-*O*-α-L-rhamnopyranosyl (1→2)-[α-L-rhamnopyranosyl (1→4)]-β-D-glucopyranoside.

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