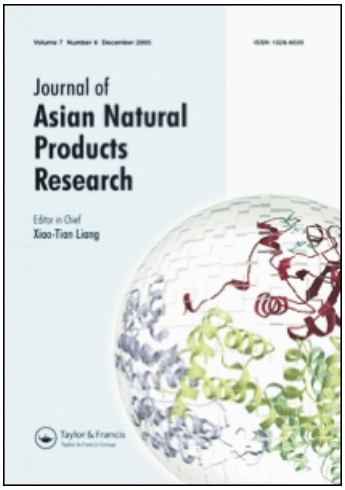

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Tuberoside M, a new cytotoxic spirostanol saponin from the seeds of Allium tuberosum

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TUBEROSIDE M, A NEW CYTOTOXIC SPIROSTANOL SAPONIN FROM THE SEEDS OF *ALLIUM TUBEROSUM*

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Tuberoside M (**1**), isolated from the seeds of *Allium tuberosum*, shows a significant inhibitory effect on the growth of the human promyelocytic leukemia cell line (HL-60) with IC_{50} value of 6.8 μg/ml. On the basis of spectral data and chemical reaction, its structure was established as (25*S*)-5β-spirostane-1β,3β-diol 3-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside.

Keywords: *Allium tuberosum*; Spirostanol saponin; Tuberoside M; HL-60

INTRODUCTION

The plant *Allium tuberosum* Rottl. (Liliaceae), commonly named garlic chives, Chinese chives or Chinese leeks, is a perennial herb cultivated widely, and its leaves and seeds have been used not only as food but also as medicine. The seeds of this plant are used in Chinese folk medicine as a tonic and aphrodisiac [1]. In the course of our search for new biologically active saponins from the seeds of *A. tuberosum*, we have previously reported nine new saponins, tuberosides A–I [2–4]. Further investigation of the seeds led to the isolation of a new spirostanol saponin, tuberoside M (**1**), which exerts a significant inhibitory effect on the growth of the human promyelocytic leukemia cell line (HL-60). In this paper, we wish to report its structural elucidation and cytotoxic activity against HL-60 cells.

RESULTS AND DISCUSSION

The *n*-butanol fraction from the ethanol extract of the seeds of *A. tuberosum* was chromatographed successively on Diaion HP-20, silica gel, and RP-18 silica gel to afford compound **1** (13 mg).

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Compound **1**, an amorphous solid, was assigned a molecular formula of $C_{39}H_{64}O_{13}$ determined by positive-ion FABMS ($[M+H]^+$ at *m/z* 741) as well as from its ^{13}C and DEPT NMR data. Its IR spectrum featured a strong absorption at 3400 cm^{-1} due to hydroxyl groups, and characteristic absorption at 987, 916, 900 and 850 cm^{-1}, with the intensity of absorption at 916 cm^{-1} being greater than that at 900 cm^{-1}. The ^{13}C NMR spectrum of **1** (Table I) showed a characteristic signal at δ 109.8 assigned to the C-22 of a spirostan skeleton [5]. These data indicated the presence of a (25*S*)-spiroacetal moiety in the molecule of **1** [6–8]. The ^1H NMR spectrum of **1** showed signals due to four steroidal methyl groups at δ 0.82 and 1.33 (each 3H, s), 1.16 (3H, d, $J = 6.7$ Hz) and 1.23 (3H, d, $J = 6.5$ Hz), one secondary methyl group of 6-deoxyhexapyranose sugar at 1.79 (1H, d, $J = 6.2$ Hz) [9], as well as two anomeric protons at δ 5.96 (1H, s) and 4.99 (1H, d, $J = 7.8$ Hz). The above data were consistent with **1** being a (25*S*)-spirostanol disaccharide. Comparison of the signals from the aglycon moiety in the ^{13}C NMR spectra (Table I) with those of phodeasapogenin [5] showed that the aglycon moiety of compound **1** was phodeasapogenin and sugars were bound to the C-3 position. Acid hydrolysis of **1** gave glucose, and rhamnose. The sugar chain and its sequence were determined by the analysis of DEPT, COSY, TOCSY, HMQC and HMBC spectra of **1**. Starting from the anomeric proton of each sugar unit, all the protons within each spin system were delineated by using COSY and TOCSY spectra. On the basis of the assigned protons, the ^{13}C NMR resonances of each sugar unit were assigned by HMQC and HMBC experiments. The α-anomeric configuration for the rhamnose was judged by the chemical shift of C-5 (δ 70.5). The β-anomeric configuration for the glucose unit was judged from the large $^3J_{H1,H2}$ coupling constant (7–8 Hz). From the HMBC spectrum, it was observed that C-3 (δ 74.8) with H-G_1 (δ

TABLE I ^{13}C (100 MHz) NMR spectral data of the aglycon part of compound **1** and phodeasapogenin and ^{13}C (100 MHz) and ^1H (400 MHz) NMR spectral data for the sugar moieties of compound **1** (C_5D_5N) (δ in ppm, *J* in Hz)

	Aglycon of **1**, δ_C	*Phodeasapogenin*, δ_C	*Sugar moieties of compound* ***1***		
				δ_C	δ_H
1	72.5 d	73.4 d	G		
2	29.2 t	32.9 t	1	101.2 d	4.99 d (7.8)
3	74.8 d	68.2 d	2	74.8 d	3.97 m
4	32.3 t	34.4 t	3	76.9 d	4.26 t (9.0)
5	31.1 d	31.2 d	4	78.4 d	4.50 t (9.3)
6	26.6 t	26.7 t	5	77.4 d	3.82 m
7	26.6 t	26.7 t	6	61.6 t	4.35 m
8	35.8 d	35.9 d			4.18 m
9	42.6 d	42.2 d	R		
10	40.5 s	40.4 s	1	102.8 d	5.96 s
11	21.7 t	21.1 t	2	72.7 d	4.77 m
12	40.4 t	40.4 t	3	72.8 d	4.62 m
13	40.7 s	40.7 s	4	74.1 d	4.42 t (9.3)
14	56.5 d	56.4 d	5	70.5 d	5.03 m
15	32.0 t	32.2 t	6	18.7 q	1.79 d (6.2)
16	81.3 d	81.2 d			
17	63.1 d	63.2 d			
18	16.7 q	16.6 q			
19	19.2 q	19.4 q			
20	42.3 d	42.2 d			
21	15.0 q	14.3 q			
22	109.8 s	109.7 s			
23	26.5 t	26.0 t			
24	26.3 t	25.8 t			
25	27.6 d	27.1 d			
26	65.2 t	65.2 t			
27	16.4 q	16.1 q			

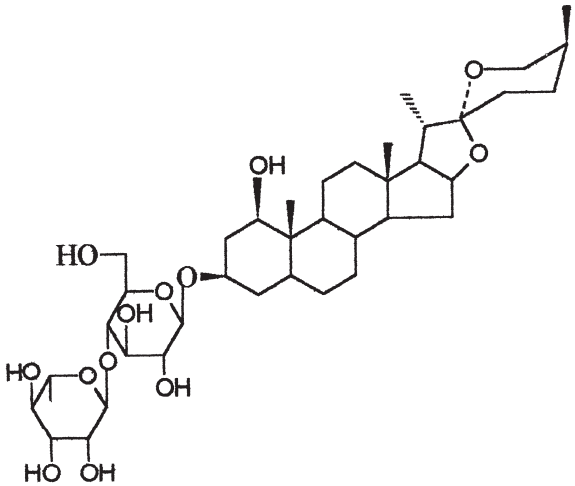

FIGURE 1 Structure of Tuberoside M (**1**).

4.99), and C-G_4 (δ 78.4) with H-R_1 (δ 5.96) had cross-peaks. Thus, compound **1** was determined as (25*S*)-5β-spirostane-1β,3β-diol 3-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside, named tuberoside M (Fig. 1).

Tuberoside M exhibited strong activity against the human promyelocytic leukemia cell line (HL-60) with observed IC_{50} value of 6.8 μg/ml as determined by using an MTT assay.

Nohara *et al.* [10] have previously reported that the spirostanol oligoglycosides possessing a chacotrisyl moiety {α-L-rhamnopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyl} were effective [11]. However, tuberoside M was α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl spirostanol. So, it is worth to study the structure–bioactivity relationship of spirostanol steroidal saponins.

EXPERIMENTAL

General Experimental Procedures

Optical rotations were obtained on a JASCO DIP-181 polarimeter. IR spectra were recorded on a Perkin–Elmer model 599 Infrared spectrometer. ^1H (400 MHz), ^{13}C (100 MHz) 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.

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

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 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, the fraction eluted by CH_2Cl_2–MeOH–H_2O (4:1:0.2) was subjected to RP-18 silica gel column chromatography with 80% MeOH to get compound **1** (13 mg).

Tuberoside M (**1**): an amorphous solid, $[\alpha]_D^{24}$ − 47.9 (MeOH, c 0.20); IR_{max}^{KBr} (cm^{-1}): 3406, 1450, 1051, 987, 916, 900, 850; ^1H NMR (C_5D_5N) of the sterol part of **1**: δ 0.82 (H-18, s), 1.16 (H-27, d, $J = 6.7$ Hz), 1.23 (H-21, d, $J = 6.5$ Hz) 1.33 (H-19, s), 1.87 (H-17, m), 3.45 (H-26a, d, $J = 10.9$ Hz), 3.94 (H-1, m), 4.14 (H-26b, m), 4.60 (H-3, m), 4.62 (H-16, m); ^1H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moieties of **1**, see Table I; FABMS: *m/z* 741[M+H]$^+$, 595[M+H − 146]$^+$, 433[M+H − 162 − 146]$^+$.

Acid hydrolisis of **1**, compound **1** (3 mg) was dissolved in 2N HCl (1,4-dioxane–H_2O 1:1, 2 ml) and heated at 100°C for 2 h. The reaction mixture was neutralized with 10% KOH and extracted with $CHCl_3$. The aqueous layer was concentrated and then compared with standard sugars 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.

HL-60 cell culture assay, HL-60 leukemia cells were maintained in RPMI 1640 medium containing 15% fetal bovine serum. The leukemia cells were washed and suspended in the above medium to 3×10^4 cell/ml, and 90 μl of this cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated in 5% CO_2/air for 24 h at 37°C. After incubation, 10 μl of DMSO solution containing the sample was added to give the final concentrations of 1, 10, 100 μg/ml, respectively, and 10 μl of DMSO was added into control wells. The cells were incubated for six days. And then cell growth was evaluated with an MTT assay procedure [12]. A dose–response curve was plotted for compound **1**, which showed more than 50% of cell growth inhibition at the sample concentration of 10 μl/ml. The concentration giving 50% inhibition (IC_{50}) was calculated.

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