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A steroidal saponin from the seeds of *Allium tuberosum*

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Abstract

A steroidal saponin, named tuberoside, together with seven known compounds, were isolated from the seeds of *Allium tuberosum* Rottl. ex Spreng. Its structure was established by spectroscopic data, hydrolysis, and comparison with spectral data of known compounds to be (2α, 3β, 5α, 25*S*)-2,3,27-trihydroxyspirostane 3-*O*-α-L-rhamnopyranoyl-(1→2)-*O*-[α-L-rhamnopyranoyl-(1→4)]-β-D-glucopyranoside. © 2001 Published by Elsevier Science Ltd.

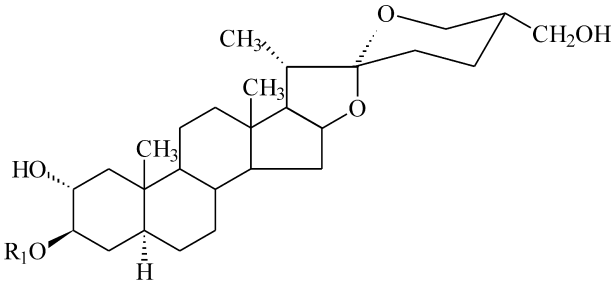
Keywords: *Allium tuberosum*; Liliaceae; Saponin; Spirostanol glycoside; Tuberoside

1. Introduction

Allium tuberosum Rottl. ex Spreng is widely cultivated and used as food in China, whose seeds have been reputedly used as a traditional Chinese medicine for treating both impotence and nocturnal emissions (Jiangsu New Medicinal College, 1979). Species of *Allium* genus are famous for their production of sulfur-containing biologically active natural products. With regard to the saponin constituents of the *Allium* genus, many steroidal saponins have been reported (Zou et al., 1999). However, there has been no exploration of the secondary metabolites of *A. tuberosum* seeds. Our detailed investigation of its seeds has led to the discovery of a new saponin (**1**), together with eight known compounds, gitogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside (Mimaki et al., 1996), S-allylcysteine (**3**), L-tyrosine (**4**), adenosine (**5**), inosine (**6**), allantoin (**7**) and mannitol (**8**). The known compounds were identified by direct comparison with authentic samples (co-TLC, IR, MS, NMR) or with reported spectral and physical data. This paper deals with the isolation and structural elucidation of the new saponin (**1**).

2. Results and discussion

Commercially available seeds of *Allium tuberosum* (9.5 kg) were defatted with petroleum ether followed by extraction with hot 70% EtOH. The concentrated extract was partitioned between $CHCl_3$ and H_2O, then between *n*-BuOH and H_2O. The *n*-BuOH soluble phase was subjected to silica gel column chromatography to give a new compound, named tuberoside (**1**).



1. R_1=α-L-Rha*p*-(1→2)-[α-L-Rha*p*-(1→4)]-β-D-Glc*p*

Tuberoside (**1**) was obtained as an amorphous powder, with the molecular formula $C_{45}H_{74}O_{18}$, which was deduced from the FAB–mass spectrum showing an $[M+H]^+$ ion at *m*/*z* 903 and the ^{13}C NMR spectrum (Table 1). The IR spectrum of **1** showed the characteristic absorption of hydroxyl groups at 3422 cm^{-1}. The FAB–mass spectrometry of compound **1** showed fragment ion

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peaks at m/z 757 (M + H-146), 611 (M + H-146-146), 449 (M + H-146-146-162), which suggested the sequential loss of two deoxy hexose and one hexose moiety. The NMR spectra (Tables 1 and 2) of **1** also showed the presence of three sugars from the three anomeric protons at δ 5.00 (*d*, $J = 7.3$ Hz), 6.38 (*s*) and 5.38 (*s*) in the ^{1}H NMR spectrum, and the three anomeric carbon signals at δ 102.9, 102.2, 100.9 in the ^{13}C NMR spectrum.

On acid hydrolysis, **1** afforded D-glucose, L-rhamnose, which were identified by co-TLC with authentic samples and aglycone (**1a**). The NMR spectral data (Table 1) indicated **1a** is a spirostanol derivative with three hydroxyl groups, and the base peak at m/z 155 in EI–MS spectrum of **1a** proved a hydroxyl group to be located at the F ring (Cong, 1987). Further analysis of

Table 1
^{13}C NMR spectroscopic data of compounds **1** and **1a** (125 MHz)[a]

C	1	1a
1	45.9	45.0
2	70.6	73.1
3	85.1	76.4
4	33.5	37.4
5	44.6	44.9
6	28.1	27.8
7	32.3	32.1
8	34.6	35.6
9	54.4	54.3
10	36.9	37.6
11	21.5	21.2
12	40.1	39.9
13	40.6	41.7
14	56.4	56.2
15	32.2	32.8
16	81.2	80.9
17	63.1	63.0
18	16.6	16.2
19	15.6	13.6
20	42.1	41.7
21	15.1	14.4
22	109.7	109.2
23	31.6	31.9
24	24.1	24.5
25	39.2	38.2
26	64.1	63.1
27	64.4	63.4
Glc- 1	100.9	
2	78.0	
3	78.0	
4	78.7	
5	77.2	
6	61.1	
Rha- 1	102.2	
2	72.6a[b]	
3	72.7a	
4	73.9b	
5	70.5	
6	18.6	
Rha- 1	102.9	
2	72.5a	
3	72.7a	
4	74.1b	
5	69.6	
6	18.6	

[a] Spectra were measured in pyridine-d_5 (**1**) and chloroform-*d* (**1a**).
[b] a and b signals may be interchangeable.

Table 2
^{1}H NMR spectroscopic data of compound **1** (500 MHz, pyridine-d_5)[a]

Position	δ_H	J (Hz)
H-1α	2.22 *dd*	12.7, 4.7
H-1β	1.16 *m*	
H-2β	4.07 *ddd*	12.1, 11, 4.7
H-3α	3.88 *dd*	11.4, 11.0
H-4α	2.0 overlap	
H-4β	1.39 overlap	
H-5α	1.01 overlap	
H-6	1.20 *m*	
	1.08 *m*	
H-7	0.83 *m*	
	1.48 *m*	
H-8	1.39 overlap	
H-9	0.62 *ddd*	11, 11, 2.5
H-11	1.48 *m*	
	1.23 *m*	
H-12	1.63 overlap	
	1.03 *dd*	11.8, 11.8
H-14	1.00 *m*	
H-15	2.00 overlap	
	1.39 overlap	
H-16	4.54 *m*	
H-17	1.39 overlap	
H-18	0.80 *s*	
H-19	0.94 *s*	
H-20	1.95 *m*	
H-21	1.12 *d*	6.9
H-23	1.74 *m*	
H-23	1.74 *m*	
H-24	1.80 overlap	
	0.96 *m*	
H-25	2.06 *m*	
H-26eq	4.13 *dd*	11.4, 5.2
H-26ax	3.89 *dd*	11.4, 11.1
H-27a	3.71 *dd*	10.7, 5.1
H-27b	3.63 *dd*	10.7, 7.2
Glc H-1	5.00 *d*	7.3
H-2	4.19 *t*	8.9, 7.3
H-3	4.21 *t*	8.9
H-4	4.38 *t*	8.9
H-5	3.75 *d*	8.9
H-6	4.07 *dd*	12.3, 3.6
	4.24 *dd*	12.3, 5.1
Rha H-1	6.38 *s*	
H-2	4.82 *br.s*	
H-3	4.59 *dd*	9.3, 3.3
H-4	4.34 *dd*	9.4, 9.3
H-5	4.92 *dd*	9.4, 6.1
H-6	1.63 *d*	6.1
Rha H-1	5.85 *s*	
H-2	4.66 *brs*	
H-3	4.47 *dd*	9.3, 3.4
H-4	4.32 *dd*	9.4, 9.3
H-5	4.85 *dd*	9.4, 6.3
H-6	1.66 *d*	6.3

[a] All assignments were confirmed by ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY and DEPT spectra.

the ^1H and ^{13}C NMR spectra of **1a** allowed its identification as (2α, 3β, 5α, 25*S*)-spirostan-2,3,27-triol (crestagenin), isolated previously from the leaves of *Digitalis canariensis* (Dolgado et al., 1969; Gonzalez, et al. 1983).

The ^1H and ^{13}C NMR spectral data assignments of compound **1** were performed via analyses of the ^1H–^1H COSY, ^{13}C–^1H COSY and DEPT spectra. The presence of two terminal α-L-rhmnopyranosyl moieties in the molecule of **1** was demonstrated by the two sets of characteristic six carbon signals at δ 102.2, 72.6, 72.7, 73.9, 70.5, 18.6; 102.9, 72.5, 72.7, 74.1, 69.6, 18.6 in the ^{13}C NMR spectrum. The C-5 signals of two rhamnose units at δ 70.5 and 69.6, respectively, indicated their α-configurations (Seo et al., 1978). Comparison of ^{13}CNMR data of the sugar moieties with the corresponding methyl β-D-glucopyranoside (Agrawal et al., 1985) indicated that the C-2 and C-4 of the glucosyl moiety were shifted downfield to δ 78.0 and 78.7 from 73.7 and 70.3, respectively. The significant glycosylation shifts clearly showed the two terminal rhamnopyranoses were linked to the C-2 and C-4 positions of the inner glucopyranose. The β-configuration of the glucopyranose was further confirmed by the large $J_{1,2}$ couplings of its anomeric proton.

The linkage of the sugar chain was concluded to be at the C-3 hydroxyl position of the aglycone because, in the ^{13}C NMR spectrum of **1**, the signal due to C-3 shifted to a lower field by 8.7 ppm, whereas the signals due to C-2 and C-4 moved to upper fields by 2.5 and 3.9 ppm, as compared with those of **1a**. Accordingly, the structure of **1** was elucidated as (2α, 3β, 5α, 25*S*)-2,3,27-trihydroxyspirostane 3-*O*-α-L-rhamnopyranoyl-(1→2)-*O*-[α-L-rhamnopyranoyl-(1→4)]-β-D-glucopyranoside (**1**).

3. Experimental

3.1. General

Mps were obtained on a Boetius miromelting apparatus and are uncorrected. Optical rotations were measured with Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 683 instrument. EIMS was obtained on a VG Zab-2f and FABMS on a VG Zabspec mass spectrometer. The ^1H NMR spectra (500 MHz) and ^{13}C NMR (125 MHz) were recorded on a Brüker AM-500 spectrometer and the chemical shifts are reported in ppm using the solvent as reference.

3.2. Plant materials.

The seeds of *Allium tuberosum* Rottl. ex Spreng were purchased from Beijing Tong-Ren-Tang Group, China, and were identified by Mr. Wei-Ze Liu, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

3.3. Extration and isolation.

Air-dried and powdered seeds of *A. tuberosum* Rottl. Ex Spreng (9.5 kg) were defatted by percolation with petroleum ether followed by extraction with hot 75% EtO. The combined EtOH extracts were filtered, evap. under red. press. and the residue suspended in H_2O and partitioned with $CHCl_3$ and *n*-BuOH, respectively.

The *η*-BuOH extract (62 g) was fractionated on a silica gel column with a gradient mixture of $CHCl_3$–MeOH and finally with MeOH to give five Frs. (A_1–A_5). Fr. A_1 was chromatographied on silica gel, eluting with $CHCl_3$–MeOH (9:1) to give compounds **2** (10 mg) and **5** (176 mg), while Fr. A_2 was repeatedly chromatographied on silica gel to give compounds **6** (15 mg) and **7** (7 mg). Fr.A_3 was subjected to silica gel with $CHCl_3$-MeOH (20:3) to give compounds **1** (31mg) and **8** (38 mg). Fr.A_4 was repeatedly chromatographied on silica gel CC to give compound **3** (162mg). Fr. A_5 was subjected to silica gel CC with $CHCl_3$–MeOH (5:1) to give compound **4** (16 mg).

3.4. Tuberoside A (*1*)

$C_{45}H_{74}O_{18}$, amorphous powder, mp 292–293°, $[\alpha]_D^{25}$ −33°; *c* 0.02, MeOH. IR (KBr) cm^{-1} 3422, 2932, 1452, 1381, 1043, 987, 912. FABMS *m/z* 903 $[M+H]^+$, 757 $[M+H\text{-}146]^+$, 611 $[M+H\text{-}2\times146]^+$, 449 [aglycone + H]$^+$, 431, 413. ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.5. Acid hydrolysis of *1*

Compound **1** (20 mg) was heated with 3% H_2SO_4 (EtOH, 1:1) at 100°C for 3 h. After cooling, the reaction mixture was neutralized with 1 N NaOH and partitioned between AcOEt and H_2O. The AcOEt soluble phase was concentrated and subjected to silica gel CC with $CHCl_3$ to give the known crestaganin **1a**.

Compound **1a**: white powder, EI–MS *m/z* 448 (M^+), 155 (100). ^1H NMR ($CDCl_3$) δ 0.97 (3H, *d*, *J* = 7.8 Hz), 0.86 (3H, *s*), 0.76 (3H, *s*), 3.71 (*dd*, *J* = 10.7, 5.1 Hz, CH_2-27), 3.63 (*dd*, *J* = 10.7, 7.1 Hz, CH_2-27), 3.89 (1H, *dd*, *J* = 11.4, 11.1 Hz, CH_2-26), 4.13 (*dd*, *J* = 11.4, 5.2 Hz, CH_2-26). ^{13}C NMR data, see Table 1.

The H_2O soluble phase was concentrated and examined by comparison with authentic samples through TLC ($CHCl_3$–MeOH–H_2O, 7:3:0.1) to detect D-glucose and L-rhamnose.

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