Two New Spirostanol Saponins from *Allium tuberosum*

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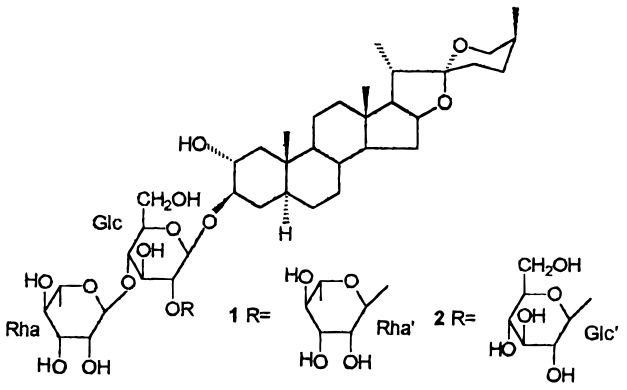
Received November 2, 1998

Two new spirostanol saponins, tuberosides D and E, have been isolated from the seeds of *Allium tuberosum*. On the basis of spectral data and chemical reactions, their structures were established as (25*S*)-5α-spirostane-2α,3*β*-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-*β*-D-glucopyranoside and (25*S*)-5α-spirostan-2α,3*β*-diol 3-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-*β*-D-glucopyranoside, respectively.

The plant *Allium tuberosum* Rottl. (Liliaceae) is distributed all over mainland China and 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] We have reported the isolation and structural elucidation of three new furostanol saponins, tuberosides A−C, from the seeds of this plant.[2] In a continued investigation of the seeds, two new spirostanol saponins, tuberosides D (**1**) and E (**2**) have been isolated. This paper deals with the isolation and structural elucidation of **1** and **2**.



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 **1** and **2**.

1, an amorphous solid, was assigned a molecular formula of $C_{45}H_{74}O_{17}$ determined by positive-ion FABMS ($[M + H]^+$ at *m*/*z* 887) 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 986, 922, 890, and 860 cm^{-1}, with the absorption at 922 cm^{-1} being of greater intensity than that at 890 cm^{-1}, implying the presence of a (25*S*)-spiroacetal moiety in the molecule.[3−5] The ^1H NMR spectrum of **1** showed signals for two angular methyl proton signals at *δ* 0.90 and 0.97 (each s), and four secondary methyl proton signals at *δ* 1.15 (d, *J* = 7.0 Hz), 1.70 (d, *J* = 6.2 Hz), 1.75 (d, *J* = 6.2 Hz), and 1.21 (d, *J* = 6.9 Hz), and three anomeric protons at *δ* 6.43 (s), 5.89 (s), and 5.08 (d, *J* = 7.2 Hz). The signals at *δ* 1.70 and 1.75 were due to the methyl group of a 6-deoxyhexopyranose sugar.[6] The ^{13}C NMR spectrum of **1** showed four signals at lower field than 100 ppm; the signals at *δ* 103.1, 102.3, and 101.0 were due to anomeric carbons, and the signal at *δ* 109.8 was assignable to the C-22 carbon of a spirostan skeleton.[7] The above data were consistent with **1** being a (25*S*)-spirostanol trisaccharide. Comparison of the signals from the aglycon moiety in the ^{13}C NMR spectra (Table 1) with those from neogitogenin[8] showed that the aglycon moiety of compound **1** was neogitogenin and sugars were bound to the C-3 position of neogitogenin. Acid hydrolysis of **1** gave neogitogenin, 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 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

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

	1		**2**	
position	δ_C	δ_H	δ_C	δ_H
glucose				
1	101.0 d	5.08 d (7.2)	100.6 d	5.07 d (7.5)
2	78.1 d[a]	4.28 m	82.0 d	4.27 m
3	78.0 d[a]	4.28 m	77.8 d	4.30 m
4	78.9 d	4.45 m	77.8 d	4.24 m
5	77.2 d	3.34 m	76.6 d	4.01 m
6	61.3 t	4.24 m	61.8 t	4.55 m
		4.34 m		4.58 m
glucose′				
1			105.3 d	5.20 d (7.8)
2			75.1 d	4.12 m
3			78.4 d	4.30 m
4			71.4 d	4.34 m
5			78.7 d	4.05 m
6			62.3 t	4.41 m
				4.55 m
rhamnose				
1	102.3 d	6.43 s	102.1 d	6.29 s
2	72.5 d	4.91 m	72.5 d	4.81 m
3	72.8 d	4.66 m	72.8 d	4.61 m
4	74.0 d	4.39 m	74.2 d	4.38 m
5	69.6 d	4.92 m	69.6 d	4.91 m
6	18.6 q	1.75 d (6.2)	18.7 q	1.77 d (6.2)
rhamnose′				
1	103.1 d	5.89 s		
2	72.6 d	4.72 m		
3	72.9 d	4.60 m		
4	74.2 d	4.39 m		
5	70.7 d	4.98 m		
6	18.6 q	1.70 d (6.2)		

[a] Signals may be interchanged in each column.

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10.1021/np980486l CCC: $18.00
Published on Web 06/12/1999

by HMQC and further confirmed by HMBC experiments. The α-anomeric configurations for the two rhamnoses were judged by their chemical shifts at C-5 (δ 69.6 and 70.7). A β-anomeric configuration for the glucose unit was judged from its large $^3J_{H1,H2}$ coupling constant (7−8 Hz). From the HMBC spectrum, it was observed that C-3 (δ 85.2) with H−G1 (δ 5.08), C−G2 (δ 78.1) with H−R′1 (δ 5.89), and C−G4 (δ 78.9) with H−R1(δ 6.43) had cross-peaks. Thus, **1** was determined as (25*S*)-5α-spirostane-2α,3β-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-β-D-glucopyranoside.

2, an amorphous solid, was assigned a molecular formula of $C_{45}H_{74}O_{18}$ determined by FABMS ([M + H]$^+$ at *m/z* 903) as well as its ^{13}C and DEPT NMR data. Of the 45 carbons observed, 27 were assigned to the aglycon part and 18 to the oligosaccharide moiety (see Table 1). The spectral data of **2** showed that the aglycon and the number of sugar units were the same as those of **1**, but compound **2** has two glucoses and one rhamnose while compound **1** has one glucose and two rhamnoses. Acid hydrolysis of **2** gave neogitogenin, glucose, and rhamnose. The trisaccharide nature of compound **2** was also manifested by its ^{1}H [δ 6.29, s; δ 5.20, d, *J* = 7.8 Hz and δ 5.07, d, *J* = 7.5 Hz] and ^{13}C [δ 105.3, 102.1, 100.6] NMR data, respectively (Table 1). The identity of the single sugar chain and the sequence of the oligosaccharide chain were determined from the DEPT, COSY, TOCSY, HMQC, and HMBC NMR spectra, as described for compound **1** above. An α-anomeric configuration for the rhamnose unit was concluded from its C-5 chemical shift (δ 69.6). 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 cross-peaks were observed between C-3 (δ 85.0) and H-Gl (δ 5.07), C−G2 (δ 82.0) and H−G′1 (δ 5.20), and C−G4 (δ 77.8) and H−R1 (δ 6.29). Thus, tuberoside E (**2**) was determined as (25*S*)-5α-spirostan-2α, 3β-diol 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-β-D-glucopyranoside.

Experimental Section

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. ^{1}H (400 Hz), ^{13}C (100 Hz) and all 2D spectra NMR were run on Bruker AM-400 NMR spectrometer, with TMS as internal standard. FABMS were recorded on a MAT-95 mass spectrometer. GLC analysis was performed with a Shimadzu GC-9A instrument, glass column (300 × 0.32 cm) packed with OV 225, carrier gas, N_2, flow rate, 30 mL/min. Silica gel 60H (Qingdao Haiyang Chemical Group Co., Qingdao, People's Republic of China) was used for column chromatography. TLC was performed on silica gel $HSGF_{254}$.

Plant Material. The seeds of *A. tuberosum* were purchased in Shanghai 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, Shanghai Institute of Materia Medica, 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 a EtOH−H_2O gradient system (0−100%). The fraction (60 g) eluted by 70% EtOH was objected to silica gel column chromatgraphy 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 (5:1:0.2) was subjected to RP-18 silica gel column chromatgraphy with 80% MeOH to give compounds **1** (45 mg) and **2** (26 mg).

Compound 1: amorphous solid, $[\alpha]^{24}_D$ −31.2° (*c* 0.33, MeOH); IR (KBr) ν_{max} 3417, 1641, 1452, 1381, 1000−1100, 986, 922, 890, 860 cm^{-1}; ^{1}H NMR (C_5D_5N) of the aglycon part of **1**, δ 0.90 (H-18, s), 0.97 (H-19, s), 1.15 (H-27, d, *J* = 7.0 Hz), 1.21 (H-21, d, *J* = 6.9 Hz), 3.42 (H-26a, m), 3.92 (H-3, m), 4.11 (H-26b, m), 4.12 (H-2, m), 4.60 (H-16, m); ^{13}C NMR (C_5D_5N) of the aglycon part of **1**, δ 45.9 (t, C-1), 70.6 (d, C-2), 85.2 (d, C-3), 33.6 (t, C-4), 44.7 (d, C-5), 28.3 (t, C-6), 32.3 (t, C-7), 34.8 (d, C-8), 54.6 (d, C-9), 37.0 (s, C-10), 21.6 (t, C-11), 40.2 (t, C-12), 40.9 (s, C-13), 56.5 (d, C-14), 32.4 (t, C-15), 81.3 (d, C-16), 63.0 (d, C-17), 16.7 (q, C-18), 13.7 (q, C-19), 42.6 (d, C-20), 15.0 (q, C-21), 109.8 (s, C-22), 26.5 (t, C-23), 26.3 (t, C-24), 27.7 (d, C-25), 65.2 (t, C-26), 16.4 (q, C-27); ^{1}H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moiety of **1**, see Table 1; FABMS *m/z* 887 [M + H]$^+$, 741 [M + H − 146]$^+$, 595 [M + H − 146 × 2]$^+$, 433 [M + H − 146 × 2 − 162]$^+$.

Compound 2: amorphous solid, $[\alpha]^{22}_D$ −29.5° (*c* 0.30, MeOH); IR (KBr) ν_{max} 3400, 1635, 1452, 1383, 1000−1100, 987, 922, 890, 860 cm^{-1}; ^{1}H NMR (C_5D_5N) of the aglycon part of **2**, δ 0.88 (H-18, s), 0.97 (H-19, s), 1.15 (H-27, d, *J* = 7.0 Hz), 1.21 (H-21, d, *J* = 7.0 Hz), 2.51 (H-17, d, *J* = 9.9 Hz), 3.44 (H-26a, brd, *J* = 10.8 Hz), 3.94 (H-3, m), 4.12 (H-26b, m), 4.16 (H-2, m), 4.58 (H-16, m); ^{13}C NMR (C_5D_5N) of the aglycon part of **1**, δ 46.0 (t, C-1), 70.7 (d, C-2), 85.0 (d, C-3), 33.5 (t, C-4), 44.7 (d, C-5), 28.3 (t, C-6), 32.3 (t, C-7), 34.7 (d, C-8), 54.5 (d, C-9), 37.0 (s, C-10), 21.6 (t, C-11), 40.2 (t, C-12), 40.9 (s, C-13), 56.5 (d, C-14), 32.4 (t, C-15), 81.3 (d, C-16), 63.0 (d, C-17), 16.7 (q, C-18), 13.7 (q, C-19), 42.6 (d, C-20), 15.0 (q, C-21), 109.8 (s, C-22), 26.5 (t, C-23), 26.3 (t, C-24), 27.7 (d, C-25), 65.2 (t, C-26), 16.4 (q, C-27); ^{1}H NMR (C_5D_5N) and ^{13}C NMR (C_5D_5N) of the sugar moiety of **2**, see Table 1; FABMS *m/z* 903 [M + H]$^+$, 741 [M + H − 162]$^+$, 579 [M + H − 162 × 2]$^+$, 433 [M + H − 162 × 2−146]$^+$.

Acid Hydrolysis of 1 and 2. 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 extracts were washed with H_2O and dried with Na_2SO_4. Evaporation of the solvent gave neogitogenin (co-TLC with an authentic sample).[2,9] After repeated evaporation of the solvent of the aqueous layer, the sugars were analyzed by silica gel TLC in comparison with standard sugars (CH_3Cl−MeOH−H_2O, 7:3:0.5). This procedure was repeated for compound **2**.

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 was 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 adding AcOH, the mixture was evaporated to dryness by repeated co-distillation with MeOH. 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 2:1. By the same method, compound **2** afforded the alditol acetates of rhamnose and glucose in a molar ratio of 1:2.

References and Notes

(1) Jiangsu New Medical College. *Zhong-yao-da-ci-dian*; Shanghai Science and Technology Publisher: Shanghai, 1986.
(2) Sang, S. M.; Lao, A. N.; Wang, H. C., Chen, Z. L. *Phytochemistry*, in press.
(3) Wall, M. E.; Eddy, C. R.; McClennan, M. L.; Klumpp, M. E. *Anal. Chem.* **1952**, *24*, 1337−1340.
(4) Eddy, C. R.; Wall, M. E.; Scott, M. K. *Anal. Chem.* **1953**, *25*, 266−268.
(5) Jones, R. N.; Katzenellenbogen, K.; Dobriner, K. *J. Am. Chem. Soc.* **1953**, *75*, 158−161.
(6) Mimaki, Y.; Sashida, Y.; Nikaido, T.; Ohmoto, T. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 458−492.
(7) Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S. *Phytochemistry* **1985**, *24*, 2479−2496.
(8) Achenbach, H.; Hubner, H.; Brand, W.; Reiter, M. *Phytochemistry* **1994**, *35*, 1527−1543.
(9) Tori, K.; Nishikawa, J.; Seo, S. *Steroids* **1982**, 39, 73−80.

NP980486L