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RESEARCH ARTICLE

Two new phenylpropane glycosides from *Allium tuberosum* Rottler

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Abstract A phytochemical investigation of *Allium tuberosum* Rottler afforded two new phenylpropane glycosides, named tuberonoid A (**1**) and B (**2**), along with four known flavonoids, kaempferol 3-*O*-β-sophoroside (**3**), 3-*O*-β-D-(2-*O*-feruloyl)-glucosyl-7,4′-di-*O*-β-D-glucosylkaempferol (**4**), 3-*O*-β-sophorosyl-7-*O*-β-D-(2-*O*-feruloyl)glucosyl kaempferol (**5**), kaempferol 3,4′-di-*O*-β-D-glucoside (**6**). The identification and structural elucidation of the new compounds were carried out based on spectral data analyses (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC) and HR-MS.

Keywords *Allium tuberosum* Rottler · Liliaceae · Phenylpropane glycosides

Introduction

The *Allium* species are fascinating plants, and *Allium* vegetables are consumed worldwide by a great number of people various and used in various American, European, Chinese, Japanese, and Korean cuisines (Nishimura et al. 2000). *Allium tuberosum* is a perennial herb which is distributed widely throughout the world, and its leaves are used mainly in dishes. *Allium tuberosum* has long been used in traditional medicine as a remedy for abdominal pain, diarrhea, hematemesis, snakebite, and asthma (Kuo et al. 2005). It was previously reported that the leaves contain sulfides (Kameoka and Miyake 1974), linalool (Mackenzie and Ferns 1977), and flavonoid glycosides (Kaneta et al. 1980; Yoshida et al. 1987).

Researchers have reported cytotoxic constituents and antitumor activities such as thiosulfinates (Park et al. 2007). As part of our continuing search for biologically active compounds from Korean medicinal plants, we investigated the constituents of the aerial parts of *A. tuberosum*. Column chromatography separation of the MeOH extract of *A. tuberosum* resulted in the isolation of two new phenylpropane glycosides, compounds **1** and **2**, together with four known flavonoid compounds (**3**–**6**). The new structures of compounds **1** and **2** were determined by spectroscopic methods including 1D, 2D NMR, and HR-MS data.

Materials and methods

General experimental procedure

Fast atom bombardment (FAB) and high resolution-fast atom bombardment (HR-FAB) mass spectra were obtained on a JEOL JMS700 mass spectrometer. Ultraviolet (UV) spectra were recorded with a Shimadzu UV-1601 UV–Visible spectrophotometer. Infrared (IR) spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian UNITY INOVA 700 NMR spectrometer. Preparative high performance liquid chromatography (HPLC) was performed using a Gilson 306 pump with a Shodex refractive index detector and an Apollo Silica 5 μ column

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(250 × 22 mm) or an Econosil® RP-18 10 μ column (250 × 22 mm). Thins-layer chromatography (TLC) was performed using Merck precoated Silica gel F_{254} plates and RP-18 F_{254s} plates. Silica gel 60 (Merck, 70–230 and 230–400 mesh) was used for column chromatography. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co., Uppsala, Sweden).

Plant materials

The aerial parts of *A. tuberosum* (5.0 kg) were collected in Taebaek in Gangwon-Do province, Korea in May 2010, and the plant was identified by one of the authors (K. R. Lee). A voucher specimen of the plant (SKKU-NPL-11-04) was deposited at the School of Pharmacy in Sungkyunkwan University.

Extraction and isolation

The half dried aerial parts of *A. tuberosum* (5.0 kg) were extracted at room temperature with 80 % MeOH and evaporated under reduced pressure to give a residue (200 g), that was dissolved in water (800 mL three times) and subsequently partitioned into *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH fractions. After the solvents were evaporated, the residues found in each were as follows; *n*-hexane (18 g), $CHCl_3$ (1 g), EtOAc (2 g), and *n*-BuOH (25 g). The *n*-BuOH soluble fraction (25 g) was subjected to silica gel column chromatography with a solvent system of $CHCl_3$:MeOH (4:1–1:1) to give eight fractions (B1–B8). Fraction B6 was separated over a RP-18 column with a solvent system of 35 % MeOH–100 % MeOH to give five subfractions (B61–B65). Subfraction B63 (120 mg) was purified with by RP-C_{18} prep-HPLC (45 % MeCN) to yield compound **3** (4 mg, $R_t = 15$ min). Subfraction B64 (65 mg) was purified by RP-C_{18} prep-HPLC (45 % MeOH) to yield compound **4** (5 mg, $R_t = 18$ min). Subfraction B65 (103 mg) was purified by RP-C_{18} prep-HPLC (50 % MeOH) to yield compounds **5** (4 mg, $R_t = 19$ min) and **6** (8 mg, $R_t = 21$ min). Fraction B5 was separated over a RP-18 column with a solvent system of 50 % MeO-H = and 100 % MeOH to give four subfractions (B51–B54). Subfraction B52 (69 mg) was purified by RP-C_{18} prep-HPLC (20 % MeCN) to yield compounds **1** (6 mg, $R_t = 13$ min), and **2** (4 mg, $R_t = 17$ min).

Tuberonoid A (1)

Colourless gum, UV (MeOH) nm (log ε): 297 (3.85), 325 (3.87); HR-FAB-MS *m/z*: 517.1552 $[M-H]^-$ (calcd for $C_{22}H_{29}O_{14}$, 517.1557); ^{1}H-NMR (700 MHz, CD_3OD) and ^{13}C-NMR (175 MHz, CD_3OD) see Table 1.

Table 1 ^{1}H- (700 MHz) and ^{13}C-NMR (175 MHz) spectral data of **1** and **2** in CD_3OD (δ in ppm)

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		127.6		127.3
2	7.26 (d, 1.9)	112.0	7.94 (d, 1.8)	113.3
3		149.7		148.3
4		151.3		150.6
5	6.84 (d, 8.2)	116.7	6.75 (d, 8.2)	116.1
6	7.14 (dd, 1.9, 8.2)	124.6	7.18 (dd, 1.8, 8.2)	124.6
7	6.44 (d, 15.9)	115.0	5.82 (d, 12.9)	112.7
8	7.73 (d, 15.9)	148.3	6.95 (d, 12.9)	145.7
9		167.6		166.3
1′	5.74 (d, 7.7)	94.5	5.70 (d, 7.8)	93.0
2′	3.73 (m)	83.0	3.63 (m)	83.1
3′	3.87 (m)	78.1	3.78 (m)	78.8
4′	3.71 (m)	71.2	3.70 (m)	71.5
5′	3.20 (m)	77.8	3.20 (m)	77.9
6′	3.74 (m)	62.4	3.73 (m)	62.3
1″	4.62 (d, 7.7)	105.7	4.50 (d, 7.8)	105.1
2″	3.21 (m)	76.0	3.22 (m)	76.2
3″	3.36 (m)	78.0	3.35 (m)	78.0
4″	3.46 (m)	70.9	3.45 (m)	70.7
5″	3.70 (m)	77.8	3.71 (m)	76.7
6″	3.84 (m)	62.4	3.83 (m)	61.7
OCH_3	3.95 (s)	56.6	3.90 (s)	55.3

Assignments were based on 2D NMR including COSY, HMQC and HMBC. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses

Tuberonoid B (2)

Colourless gum, UV (MeOH) nm (log ε): 270 (3.83), 293 (3.97); HR-FAB-MS *m/z*: 517.1552 $[M-H]^-$ (calcd for $C_{22}H_{29}O_{14}$, 517.1557); ^{1}H-NMR (700 MHz, CD_3OD) and ^{13}C-NMR (175 MHz, CD_3OD) see Table 1.

Acid hydrolysis of 1 and 2

Compound **1** (1 mg) and **2** (1 mg) were treated with 1 N HCl (2 mL) at 80 °C for 1.5 h. After cooling, each hydrolysate was extracted with $CHCl_3$ and the extract was evaporated in vacuo to yield compounds **1a** and **2a**, both as a colorless gum. The sugar in water layer was identified as D-glucose by co-TLC (EtOAc–MeOH–H_2O = 9:3:1, *Rf* value: 0.2) with D-glucose standard (Aldrich Co., U.S.A.).

*Compound **1a***

Colourless gum; m.p. 168–169 °C; UVλmax (MeOH) nm (log ε): 289 (3.83), 318 (3.86); IRυmax (KBr)

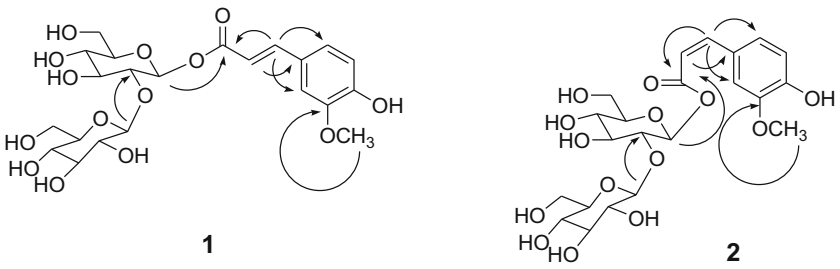

Fig. 1 Key HMBC correlations (H → C) of **1** and **2**

cm^{-1}: 3437, 1691, 1665, 1517; ^{1}H-NMR (700 MHz, CD_3OD) δ 7.18 (1H, d, $J = 1.9$ Hz, H-2), 7.07 (1H, dd, $J = 8.2$, 1.9 Hz, H-6), 6.82 (1H, d, $J = 8.2$ Hz, H-5), 6.31 (1H, d, $J = 16$ Hz, H-8), 7.59 (1H, d, $J = 16$ Hz, H-7), 3.89 (3H, *s*, OCH_3); ^{13}C-NMR (175 MHz, CD_3OD) δ 171.19 (C-9), 151.50 (C-3), 149.90 (C-4), 127.76 (C-1), 123.97 (C-6), 116.46 (C-5), 115.89 (C-8), 111.64 (C-2), 146.95 (C-7), 56.45 (OCH_3); FAB-MS *m/z* (% relative intensity): 194 (M^+, 100), 179 (16), 161(5), 148 (6), 133 (17), 105 (5), 77 (6).

Compound ***2a***

Colourless gum; ^{1}H-NMR (700 MHz, DMSO-d_6): δ 7.66 (1H, d, $J = 1.8$ Hz, H-2), 7.13 (1H, dd, $J = 8.2$, 1.8 Hz, H-6), 6.75 (1H, d, $J = 8.1$ Hz, H-5), 6.64 (1H, d, $J = 13$ Hz, H-8), 5.75 (1H, d, $J = 13$ Hz, H-7), 3.74 (3H, s, 3-OCH_3), ESIMS *m/z* 193.0 $[M-H]^-$.

Results and discussion

The known compounds were identified as kaempferol 3-*O*-β-sophoroside (**3**) (Wolfram et al. 2010), 3-*O*-β-D-(2-*O*-feruloyl)-glucosyl-7,4′-di-*O*-β-D-glucosyl kaempferol (**4**), 3-*O*-β-sophorosyl-7-*O*-β-D-(2-*O*-feruloyl)glucosyl kaempferol (**5**) (Yoshida et al. 1987), and kaempferol 3,4′-di-*O*-β-D-glucoside (**6**) (Lee et al. 2001) by comparison of their spectroscopic and physical data with previously reported values.

Compound **1** was isolated as a colorless gum. The molecular formula of $C_{22}H_{30}O_{14}$ was determined by the FAB-MS *m/z*: 517.1552 $[M-H]^-$ (calcd for $C_{22}H_{29}O_{14}$, 517.1557). The ^{1}H-NMR spectrum of **1** showed three aromatic protons at δ_H 7.26 (1H, d, $J = 1.7$ Hz, H-2), 7.14 (1H, dd, $J = 8.2$, 1.7 Hz, H-6), and 6.84 (1H, d, $J = 8.2$ Hz, H-5), a *trans*-double bond at δ_H 7.73 (1H, d, $J = 15.8$ Hz, H-7) and 6.44 (1H, d, $J = 15.8$ Hz, H-8) and one methyl proton at δ_H 3.92 (3H, s) and two sugar anomeric protons at δ_H 5.74 (1H, d, $J = 7.7$ Hz, H-1′) and 4.62 (1H, d, $J = 7.7$ Hz, H-1″). In the ^{13}C-NMR spectrum, 15 carbon signals of an aglycone were discerned at δ_C 167.6 (C-9), 151.3 (C-4), 149.7 (C-3), 148.3 (C-8), 127.6 (C-1), 124.6 (C-6), 116.7 (C-5), 115.0 (C-7) and 112.0 (C-2), which were attributable to ferulic acid (Supaluk et al. 2009), as well as other sugar signals at δ 105.7 (C-1″), 94.5 (C-1′), 83.0 (C-2′), 78.1 (C-3′), 78.0 (C-3″), 77.8 (C-5′), 77.8 (C-5″), 76.0(C-2″), 71.2 (C-4′), 70.9 (C-4″), 62.4 (C-6′), and 62.4 (C-6″), which indicated the presence of two glucose moieties (Table 1). The NMR data of **1** were very similar to those of 1-*O*-β-feruloyl glucopyranose (Zhu and Ralph 2011). The major difference was the existence of additional sugar unit at δ_H 4.62 (1H, d, $J = 7.7$ Hz, H-1″), 3.84 (1H, m, H-6″), 3.70 (1H, m, H-5″), 3.46 (1H, m, H-4″), 3.36 (1H, m, H-3″) and 3.21 (1H, m, H-2″) in the ^{1}H-NMR spectrum, and at δ_C 105.7, 78.0, 77.8, 76.0, 70.9 and 62.4 in the ^{13}C-NMR spectrum. There were also differences in the downfield shift of C-2 for 1-*O*-β-feruloyl glucopyranose (Zhu and Ralph 2011). The positions of the glucoses were confirmed by the HMBC correlations of H-1′/C-9 and H-1″/C-2′ (Fig. 1). The anomeric configurations of the two glucoses were found to be a *β*-form from the coupling constant of 7.7 Hz of anomeric protons. (Yoshida et al. 1987) Acid hydrolysis of **1** with 1 N HCl yielded *trans*-ferulic acid (**1a**), whose ^{1}H-NMR and MS data were in good agreement with previously reported values (Supaluk et al. 2009), and D-glucose ($[\alpha]_D^{25}$ +49.4° $c = 0.04$ in H_2O), which was identified by co-TLC (EtOAc–MeOH–H_2O = 9:3:1, *Rf* value: 0.2) (Lee et al. 2012) with the glucose standard (Aldrich Co., U.S.A.), Thus, the structure of **1** was determined as shown in Fig. 2, and was named tuberonoid A.

Compound **2** was isolated as a colorless gum. The molecular formula of $C_{22}H_{30}O_{14}$ was determined by the HR-FAB-MS *m/z*: 517.1552 $[M-H]^-$ (calcd for $C_{22}H_{29}O_{14}$, 517.1557). The ^{1}H-NMR spectrum of **1** showed three aromatic protons at δ_H 7.94 (1H, d, $J = 1.8$ Hz, H-2), 7.18 (1H, dd, $J = 8.2$, 1.8 Hz, H-6), and 6.75 (1H, d, $J = 8.2$ Hz, H-5), a *cis*-double bond at δ_H 6.95 (1H, d, $J = 12.9$ Hz, H-7) and 5.82 (1H, d, $J = 12.9$ Hz, H-8), one methoxy proton at δ_H 3.90 (3H, s) and two sugar anomeric protons at δ_H 5.70 (1H, d, $J = 7.8$ Hz, H-1′) and 4.50 (1H, d, $J = 7.8$ Hz, H-1″). In the ^{13}C-NMR spectrum, 15 carbon signals of an aglycone were identified at δ_C 166.3 (C-9), 150.6 (C-4), 148.3 (C-3), 145.7 (C-8), 127.3 (C-1), 124.6

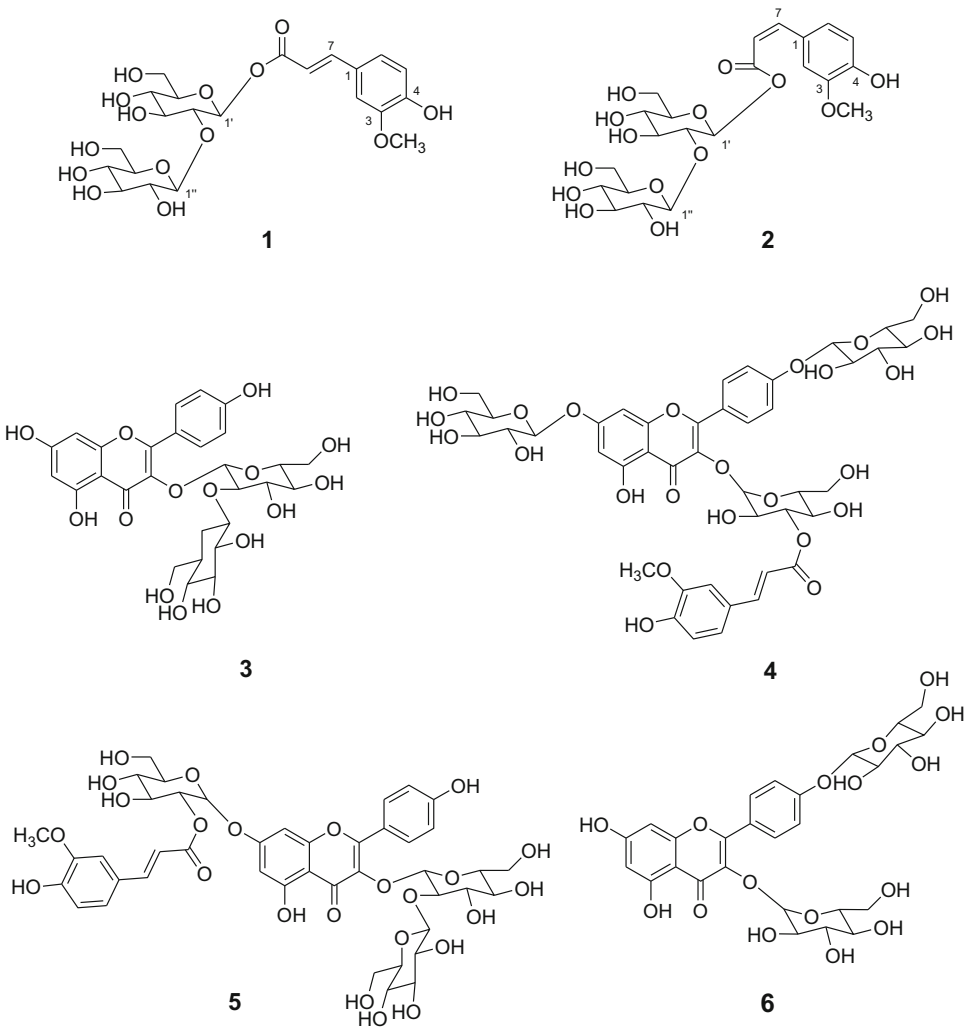

Fig. 2 Structures of compounds **1–6**

(C-6), 116.1 (C-5), 112.7 (C-7) and 113.3 (C-2), which implied the presence of *cis*- ferulic acid (Wei et al. 2014), as well as two glucose units at δ 105.1 (C-1″), 93.0 (C-1′), 83.1 (C-2′), 78.8 (C-3′), 78.0 (C-3″), 77.9 (C-5′), 76.7 (C-5″), 76.2(C-2″), 71.5 (C-4′), 70.7 (C-4″), 62.3 (C-6′), and 61.7 (C-6″) (Table 1). The NMR spectrum data of **2** was very similar to those of **1**. The major difference of the signals, which are of the two olefinic protons, appeared at δ_H 6.95 (1H, d, $J = 12.9$ Hz, H-7), and 5.82 (1H, d, $J = 12.9$ Hz, H-8). The positions of the glucoses were confirmed by the HMBC correlations of H-1′/C-9 and H-1″/C-2′ (Fig. 1). Acid hydrolysis of **1** with 1 N HCl yielded *cis*-ferulic acid (**2a**), whose ^{1}H-NMR and MS data were in good agreement with previously reported values (Wei et al. 2014), and D-glucose ($[\alpha]_D^{25}+$ 49.4° $c = 0.04$ in H_2O), which was identified by co-TLC (EtOAc–MeOH–H_2O = 9:3:1, R_f value: 0.2) (Lee et al. 2012) with the glucose standard (Aldrich Co., U.S.A.), Thus, the structure of **2** was determined as shown in Fig. 2 and named tuberonoid B.

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