http://dx.doi.org/10.5012/bkcs.2014.35.8.2529

Notes

p-Coumaroyl Anthocyanins from the Tuber Epidermis of a Colored Potato *Solanum tuberosum* L. cv Jayoung

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Received February 26, 2014, Accepted April 2, 2014

Key Words : *Solanum tuberosum* L. cv Jayoung, Solanaceae, Colored potato, Anthocyanin

Potatoes (*Solanum tuberosum* L.) rank as the world's fourth most important food crop, after maize, wheat, and rice[1] and colored potato cultivars are becoming popular, because of their color appeal, outstanding taste and mashability and their potential use in salads and novelty crisps.[2-4] The pigments in colored potatoes have been identified as the *p*-coumaroyl or feruloyl 5-glucoside-3-rhamnosylglucoside derivatives of pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin.[5] A new colored potato cultivar *Solanum tuberosum* L. cv Jayoung is widely cultivated in Korea, which was originally bred in the Republic of Korea during a joint program between the Highland Agriculture Research Center (HARC), the Korean National Institute of Crop Science, and the Rural Development Administration (RDA).[6] The colored potato 'Jayoung' has dark purple-flesh, and contains substantial amounts of polyphenols, such as, anthocyanin and phenolic acid.[6,7] Recent studies on 'Jayoung' showed that 70% EtOH extract and $CHCl_3$ fraction of the tuber epidermis have anti-inflammatory and anti-colitis effects.[8,9] However, no detailed phytochemical investigations have been reported on the colored potato 'Jayoung' to date. In the present study, repeated chromatography of the CH_2Cl_2- and BuOH-soluble fractions from the 70% EtOH extract of the tuber epidermis of *S. tuberosum* L. cv Jayoung led to the isolation and characterization of four *p*-coumaroyl anthocyanins (**1**-**4**), four phenolic compounds (**5**-**8**), and three steroidal alkaloids (**9**-**11**). The compounds **5**-**11** were identified to be acetovanillone (apocynin) (**5**),[10] caffeic acid (**6**),[11] chlorogenic acid (**7**),[12] methyl chlorogenate (**8**),[13] solanidine (**9**),[14] α-solanine (**10**),[15] and α-chaconine (**11**)[16] by physical (mp, $[\alpha]_D$) and spectroscopic data (^{1}H-NMR, ^{13}C-NMR, 2D NMR, and MS) measurement and by comparison with published values (Figure 1). To our knowledge, this is the first report on the isolation of acetovanillone from *Solanum* spp. The structure elucidation of the anthocyanins **1**-**4** are described herein.

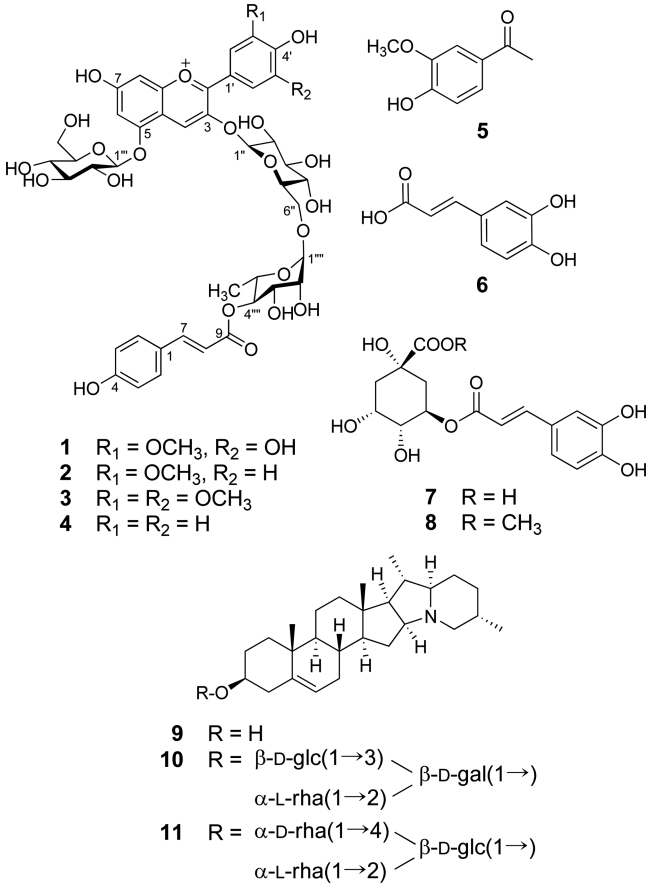

Figure 1. Chemical structures of **1**-**11** isolated from the tuber epidermis of *S. tuberosum* L. cv Jayoung.

Compound **1** was obtained as violet powder. The UV-vis spectrum of **1** revealed λ_{max} at 532 nm, suggesting that **1** is an anthocyanin. The ^{1}H-NMR and COSY spectra of **1** (Table 1) revealed two sets of 2H AB-type signals [δ_H 8.00 (1H, d,

[a]These authors contributed equally to this work.

Table 1. The ^{1}H-NMR spectral data for **1** and **2**[a]

Position	δ_H mult., (*J* Hz)	
	1	**2**
Aglycone		
4	8.96 s	8.98 s
6	7.01 d (2.0)	7.02 br s
8	7.06 d (2.0)	7.07 br s
2'	8.00 d (2.4)	8.20 d (2.5)
5'		7.07 d (8.5)
6'	7.82 d (2.4)	8.27 dd (8.5, 2.5)
3'-OCH_3	4.00 s	4.00 s
3-O-β-Glucopyranoside		
1"	5.50 d (8.0)	5.49 d (7.5)
2"	3.71	3.70
3"	3.58	3.59
4"	3.47	3.51
5"	3.60	3.60
6A"	4.03	4.03
6B"	3.72	3.73
5-O-β-Glucopyranoside		
1"'	5.19 d (7.6)	5.20 d (7.5)
2"'	3.69	3.70
3"'	3.82	3.81
4"'	3.55	3.54
5"'	3.60	3.59
6A""	3.93	3.94
6B"'	3.79	3.77
6"-O-α-Rhamnopyranosyl		
1""	4.71 d (1.2)	4.71 d (1.0)
2""	3.79	3.80
3""	3.83	3.83
4""	4.90 t (9.6)	4.86 overlap
5""	3.76	3.75
6""	0.99 d (6.4)	0.99 d (6.5)
4""-E-p-Coumaroyl		
2/6	7.43 d (8.8)	7.39 d (8.5)
3/5	6.80 d (8.4)	6.79 d (9.0)
7	7.57 d (16.0)	7.54 d (15.5)
8	6.25 d (15.6)	6.23 d (16.0)

[a]The assignments were based on COSY, HSQC and HMBC experiments.

J = 2.4 Hz) and 7.82 (1H, d, *J* = 2.4 Hz); δ_H 7.06 (1H, d, *J* = 2.0 Hz) and 7.01 (1H, d, *J* = 2.0 Hz)]. The ^{1}H-NMR of **1** also showed two singlets at δ_H 8.96 (1H) and 4.00 (3H), indicating the aglycone of **1** as petunidin. The presence of a *trans*-*p*-coumaroyl moiety was suggested from the ^{1}H-NMR resonances at δ_H 7.57 (1H, d, *J* = 16.0 Hz), 7.43 (2H, d, *J* = 8.8 Hz), 6.80 (2H, d, *J* = 8.4 Hz), and 6.25 (1H, d, *J* = 15.6 Hz). The sugar region of the ^{1}H-NMR spectrum of **1** showed three anomeric proton signals at δ_H 5.50 (1H, d, *J* = 8.0 Hz), 5.19 (1H, d, *J* = 7.6 Hz), and 4.71 (1H, d, *J* = 1.2 Hz), in accordance with two glucose and one rhamnose units.

Comparison of the above with data in the literature[5,17] suggested that **1** was a *p*-coumaroyl-5-glucoside-3-rhamnosylglucoside derivative of petunidin. The downfield shift of C-6" (δ_C 65.9 ppm) in the HSQC spectrum of **1** showed the linkage between the 3-*O*-β-glucose and the rhamnose unit to be at the 6"-hydroxyl. The HMBC correlations (Figure 2) confirmed the assignments of all proton and carbon resonances and the location of the sugar units (C-3, C-5, C-6") and *p*-coumaroyl group (C-4""). Thus, **1** was identified as petanin {petunidin 3-*O*-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-α-rhamnopyranosy1)-β-glucopyranoside]-5-*O*-β-glucopyranoside}.[17]

Compounds **2-4** were obtained as a mixture and as purple powder. The UV-vis spectrum of the mixture showed λ_{max} at 520 nm, suggesting a mixture of anthocyanins. We found that the mixture was composed with three anthocyanins (ratio 8:1:1) by analysis of peak integration of the ^{1}H-NMR spectrum. API-ES-MS of the mixture also gave three molecular ion peaks at *m/z* 947, 917, and 887. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **2**, the major compound in the mixture, exhibited strong similarities

Table 2. The ^{13}C-NMR spectral data for aglycones of **1-4**[a]

Position	δ_c (ppm)			
	1	**2**	**3**	**4**
2	163.2	163.1	162.8	162.7
3	144.9	144.6	144.7	144.5
4	132.9	133.4	134.7	133.7
5	155.3	155.3	155.3	155.2
6	104.1	104.2	104.2	104.1
7	168.3	168.5	168.5	168.4
8	96.0	96.2	96.3	96.1
9	155.8	155.8	155.8	155.7
10	111.7	111.8	111.9	111.2
1'	118.4	119.4	118.1	119.5
2'	108.2	113.9	109.5	134.7
3'	148.5	148.2	148.4	116.7
4'	144.9	156.0	145.9	165.9
5'	146.4	116.5	148.4	116.7
6'	112.8	113.9	109.5	134.7
3'-OCH_3	55.8	55.5	55.9	
5'-OCH_3			55.9	

[a]The assignments were based on COSY, HSQC and HMBC experiments.

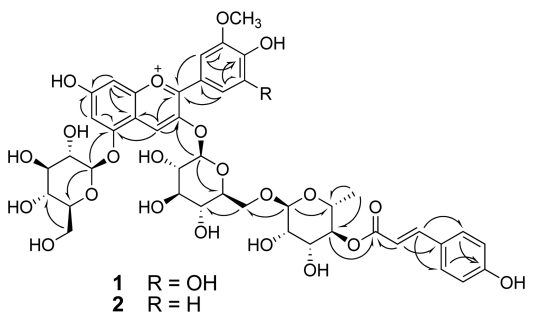

Figure 2. Key HMBC (H→C) correlations of **1** and **2**.

with those of **1** except for an anthocyanidin B-ring (Tables 1 and 2). In detail, the ^{1}H- and ^{13}C-NMR spectra of **2** revealed the 3H ABX system at δ_H 8.27 (1H, dd, J = 8.5, 2.5 Hz, H-6'), 8.20 (1H, d, J = 2.5 Hz, H-2'), and 7.07 (1H, d, J = 8.5 Hz, H-5'), in accordance with peonidin. Thus, the structure of **2** was determined to be peonanin {peonidin 3-*O*-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-α-rhamnopyranosy1)-β-glucopyranoside]-5-*O*-β-glucopyranoside}.[18]

The structure of **3**, one of the minor anthocyanins in the mixture, was determined to be malvanin {malvidin 3-*O*-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-α-rhamnopyranosy1)-β-glucopyranoside]-5-*O*-β-glucopyranoside} by analysis of ^{1}H- and ^{13}C-NMR data and by comparison with published values.[19] The presence of a molecular ion at *m*/*z* 947 in the API-ES-MS spectrum of the mixture confirmed this identification.

The structure of **4**, another minor anthocyanin in the mixture, was also identified as pelanin {pelargonidin 3-*O*-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-α-rhamnopyranosy1)-β-glucopyranoside]-5-*O*-β-glucopyranoside} by analysis of ^{1}H- and ^{13}C-NMR data, by comparison with published values,[20] and by a molecular ion at *m*/*z* 887 in the API-ES-MS spectrum.

To our knowledge, this is the first report on the characterization of **1**, **3**, and **4** in a Korean colored potato cultivar. Acylated anthocyanins like **1-4** have been found in the various colored potatoes such as Norwegian,[5] Japanese,[17] and Andean cultivars[22] as major pigments. Recently, it is reported that colored potatoes (Purple Majesty) are acute *in vivo* antioxidant source and hypotensive agent in human after supplementation to hypertensive subjects.[23] Although anthocyanins have many health-promoting benefits,[24] biological activity of **1-4** are little known to date. Meanwhile, there are some reports that the acylation of the saccharide moiety with *p*-coumarate or ferulate is considered the main reason for higher color stability of these antocyanins than non-acylated anthocyanins at most pH values.[3,5,21] Thus these *p*-coumaroly anthocyanins **1-4** are worthy of biological evaluation for their potential as new lead compounds.

Experimental

General Experimental Procedures. 1D and 2D NMR experiments were performed on a Bruker 400 MHz or Verian 500 MHz FT-NMR instrument with tetramethylsilane (TMS) or solvent residues as internal standard. Mass spectra were obtained using an Agilent 1200 series coupled to a 6120 Quadrupole LC/MS system. Silica gel (70-230 mesh and 230-400 mesh, Merck, Germany) was used for column chromatography (CC). Thin-layer chromatographic (TLC) analysis was performed on Kieselgel 60 F 254 plates (silica gel, 0.25 mm layer thickness, Merck, Germany); compounds were visualized by UV light (254 and 365 nm) and 20% (v/v) H_2SO_4 reagent (Aldrich). All solvents used for the chromatographic separations were distilled before use.

Plant Material. The purple colored potato cultivar, "Jayoung" (*Solanum tuberosum* L. cv Jayoung, Solanaceae) was supplied by Highland Agriculture Research Center (HARC), the Korean National Institute of Crop Science, and the Rural Development Administration (RDA), Republic of Korea, in September 2012. A voucher specimen (KHP-2012-SOTU1) was deposited in the Lab. of Natural Product Medicine, College of Pharmacy, Kyung Hee University. The epidermis (< 5 mm thickness) from the fresh tubers of "Jayoung" were cut into small pieces and were freeze-dried.

Extraction and Isolation. The freeze-dried samples (7 kg) were extracted with 30 L of 70% EtOH three times by maceration. The extracts were combined and concentrated *in vacuo* to give a 70% EtOH extract (642 g). The 70% EtOH extract (641 g) was suspended in distilled water (2 L) and then successively extracted with *n*-hexane (3 × 2 L), CH_2Cl_2 (3 × 2 L), EtOAc (3 × 2 L), and *n*-butanol (3 × 2 L) to give *n*-hexane- (66.2 g), CH_2Cl_2- (5.1 g), EtOAc- (2.3 g), *n*-butanol- (61.1 g), and water-soluble fractions (506.0 g). The CH_2Cl_2-soluble extract was chromatographed over silica gel (230-400 mesh, ϕ 4.2 × 41.5 cm) as stationary phase with a CH_2Cl_2-MeOH gradient (from 95:5 to 85:15 v/v; final stage, MeOH 100%) as mobile phase to afford 14 pooled fractions (M1-M14). The fraction M4 (108 mg) was further fractionated using a Sephadex column (ϕ 2.5 × 75 cm) with CH_2Cl_2-MeOH mixture (1:1 v/v), yielding compound **5** (5.0 mg). The fraction M9 (125.7 mg) was subjected to a Sephadex column (ϕ 3.6 × 73.5 cm) with CH_2Cl_2-MeOH mixture (1:1 v/v) to give compound **9** (9.0 mg). Compound **10** (135.1 mg) was purified from the fraction M12 (408 mg) using a reversed phase column chromatography (CC) (YMC gel, ϕ 2.8 × 28.5 cm) with a MeOH-H_2O gradient (from 1:1 to 1:0 v/v) as mobile phase. A portion of the BuOH-soluble extract (20.2 g) was separated by Diaion HP20 CC (ϕ 5.0 × 59.0 cm), using gradient mixtures of a MeOH-H_2O (from 0:1 to 1:0 v/v) as mobile phases, affording 20 fractions (1B1-1B20). Compounds **6** (58.0 mg) and **7** (110.8 mg) were purified from the fraction 1B5 (570 mg) using a flash CC system with Redi Sep-C18 (48 g, MeOH-H_2O-formic acid = 15:85/1 → 30/70/1 v/v). Compound **8** (139.8 mg) was obtained from the fraction 1B9 (1.68 g) by using repeated silica gel CC. The fraction 1B12 (310 mg) was subjected to a Sephadex column (ϕ 3.2 × 37.8 cm) with MeOH to obtain compound **11** (38.8 mg). For the isolation of anthocyanins, a portion of the BuOH-soluble extract (10.26 g) was separated by Diaion HP20 CC (ϕ 4.4 × 48.8 cm), using gradient mixtures of a MeOH-H_2O-TFA (from 0:100:0.1 to 100:0:0.1 v/v) as mobile phases, affording five fractions (2B1-2B5). The fraction 2B4 (2.29 g) was further fractionated using a Sephadex column (ϕ 3.5 × 55.0 cm) with MeOH with 0.1% TFA, yielding seven fractions 2B4-1-2B4-7. An anthocyanin-rich fraction (2B4-4, 245.2 mg) was separated by using preparative HPLC with a gradient of MeCN-H_2O-TFA (15:85:0.1 to 35:65:0.1), resulting in the isolation of compound **1** (11.0 mg, violet powder) and a mixture of compounds **2-4** (28.0 mg, purple powder).

Petanin (1): Amorphous violet powder; UV (On-line HPLC-DAD) λ_{max} nm: 532; ^{1}H-NMR (0.1% CF_3COOD in CD_3OD, 400 MHz), see Table 1; ^{13}C-NMR (0.1% CF_3COOD in CD_3OD, 100 MHz), aglycone: see Table 2, *3-O-β-Glu*: δ 101.3 (C-1"), 73.3 (C-2"), 76.8 (C-3"), 69.9 (C-4"), 76.5 (C-

5"), 65.9 (C-6"), *5-O-β-Glu*: δ 101.4 (C-1'''), 73.4 (C-2'''), 76.2 (C-3'''), 69.6 (C-4'''), 77.3 (C-5'''), 60.8 (C-6'''), *6''-O-α-Rha*: δ 100.7 (C-1''''), 70.7 (C-2''''), 68.9 (C-3''''), 73.9 (C-4''''), 66.4 (C-5''''), 16.5 (C-6''''), *4'''-E-p-Cou*: δ 125.7 (C-1), 129.9 (C-2/C-6), 115.5 (C-3/C-5), 159.9 (C-4), 145.6 (C-7), 113.6 (C-8), 167.6 (C-9); APT-ES-MS (positive mode) *m/z* = 933 $[M]^+$.

Peonanin (2): Amorphous purple powder; UV (On-line HPLC-DAD) λ_{max} nm: 520; ^{1}H-NMR (0.1% CF_3COOD in CD_3OD, 500 MHz), see Table 1; ^{13}C-NMR (0.1% CF_3COOD in CD_3OD, 125 MHz), aglycone: see Table 2, *3-O-β-Glu*: δ 101.4 (C-1"), 73.3 (C-2"), 76.8 (C-3"), 69.8 (C-4"), 76.5 (C-5"), 65.9 (C-6"), *5-O-β-Glu*: δ 101.3 (C-1'''), 73.4 (C-2'''), 76.2 (C-3'''), 69.6 (C-4'''), 77.3 (C-5'''), 60.7 (C-6'''), *6''-O-α-Rha*: δ 100.7 (C-1''''), 70.7 (C-2''''), 68.9 (C-3''''), 73.9 (C-4"), 66.4 (C-5"), 16.5 (C-6"), *4'''-E-p-Cou*: δ 125.7 (C-1), 129.8 (C-2/C-6), 115.4 (C-3/C-5), 159.9 (C-4), 145.6 (C-7), 113.6 (C-8), 167.5 (C-9); APT-ES-MS (positive mode) *m/z* = 917 $[M]^+$.

Malvanin (3): ^{1}H-NMR (0.1% CF_3COOD in CD_3OD, 500 MHz): δ 9.00 (1H, s, H-4), 7.98 (2H, s, H-2'/H-6'); ^{13}C-NMR (0.1% CF_3COOD in CD_3OD, 125 MHz), aglycone: see Table 2; APT-ES-MS (positive mode) *m/z* = 947 $[M]^+$.

Pelanin (4): ^{1}H-NMR (0.1% CF_3COOD in CD_3OD, 500 MHz): δ 9.00 (1H, s, H-4), 8.59 (2H, d, *J* = 9.0 Hz, H-2'/H-6'), 7.06 (2H, d, *J* = 9.0 Hz, H-3'/H-5'); ^{13}C-NMR (0.1% CF_3COOD in CD_3OD, 125 MHz), aglycone: see Table 2; APT-ES-MS (positive mode) *m/z* = 887 $[M]^+$.

Acknowledgments. This research was supported by the "Cooperative Research Program for Agriculture Science & Technology Development", Rural Development Administration, Republic of Korea (Project No. PJ00914601). The NMR experiments were performed by the Korea Basic Science Institute (KBSI).

Supporting Information. The NMR spectra of **1-4** are available on request from the correspondence author.

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