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ACYLATED PELARGONIDIN GLYCOSIDES FROM A RED POTATO

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Key Word Index—Tetraploid red potato tubers; *Solanum tuberosum*; Solanaceae; pelargonidin 3-acylrutinoside-5-glucoside; *p*-coumaric and ferulic acids.

Abstract—Two acylated pelargonidin glycosides were isolated from red tubers of a anthocyanin-rich tetraploid potato which was successfully selected from hybrid seedlings between cultivars of *Solanum tuberosum* and *S. andigena*. The major pigment was identified as pelargonidin 3-*O*-[(4″-*O*-(*trans*-*p*-coumaroyl)-α-L-6″-rhamnopyranosyl-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside] by chemical and spectral measurements, and another was determined to be pelargonidin 3-*O*-[(4″-*O*-(*trans*-feruloyl)-α-L-6″-rhamnopyranosyl-β-D-glucopyranoside]-*O*-[β-D-glucopyranoside] as a minor pigment. © 1997 Elsevier Science Ltd

INTRODUCTION

Earlier anthocyanin studies on the coloured potatoes, *Solanum andigena, S. phureja, S. tuberosum* and their hybrids, have been reported by Harborne [1–3] and Howard *et al.* [4]. To our knowledge 3-*p*-coumaroylrutinoside-5-glucoside, 3-rutinoside-5-glucosides and 3-rutinosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin were found in the coloured tubers of those plants. For the purpose of obtaining the anthocyanin-rich materials as food colorants, two the present authors (Y. U. and M. M.) have bred deep-coloured potatoes from the crosses among *S. tuberosum* cultivars. Recently, some anthocyanin-rich clones being tetraploid plants have been successfully selected from hybrid seedlings between cultivars of *S. tuberosum* and *S. andigena*. These plants contain two acylated pelargonidin glycosides one of which is new. This paper reports the structure elucidation of two acylated anthocyanins which were isolated from a deep-coloured clone, 90112-23.

RESULTS AND DISCUSSION

Two acylated anthocyanins, pigments **1** (major one) and **2** (minor one), were isolated from the red tubers of tetraploid red potato, 90112-23, with 40% acetic acid–MeOH, and purified by Amberlite × AD-7 column chromatography and preparative HPLC. Both pigments yielded pelargonidin, glucose, rhamnose and hydroxycinnamic acids (*p*-coumaric and ferulic acids) by acid hydrolysis, and also produced only one pigment as their deacyl anthocyanin by alkaline hydrolysis with NaOH under N_2 gas. This deacyl anthocyanin was determined to be pelargonidin 3-rutinoside-5-glucoside by analysis of HPLC and TLC with an authentic sample (Table 1).

The FAB mass spectrum of **1** gave $[M]^+$ peak at 887 *m/z* in good agreement with the mass calculated for $C_{42}H_{47}O_{21}$, which was composed of pelargonidin with two molecules of glucose, one of rhamnose and one of *p*-coumaric acid. The ^{1}H NMR spectrum (400 MHz) of **1** also supported the presence of pelargonidin and *p*-coumaric acid with *trans*-configuration in the olefinic part exhibiting large coupling constants (J = 15.4 Hz) (Table 2). The signals of the sugar moieties of **1** were observed in the region of δ 5.53–0.98. The signals of three anomeric protons appeared at δ 5.53 (*d*, J = 7.7 Hz, Glc A), 5.20 (*d*, J = 7.3 Hz, Glc B) and 4.69 (*s*, rhamnose). In the rhamnose moiety, one singlet proton (δ 4.69) and one doublet signal (δ 0.98, *d*, J = 4.7 Hz, -CH_3) suggested the existence of α-L-rhamnose. Two anomeric protons (δ 5.53 Glc A and 5.20 Glc B) with coupling constants of J = *ca* 7 Hz suggested that **1** had two molecules of β-D-glucopyranose. The other chemical shifts of these sugars

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Table 1. Chromatographic and spectral properties of anthocyanins from the red potato

Anthocyanin	R_f values (×100) BAW	BuHCl	1% HCl	AHW	Spectral data in 0.1% HCl–MeOH λ_{max} (nm)	E_{acyl}/E_{max} (%)	E_{440}/E_{max} (%)	$AlCl_3$	R_t (min)	FAB-MS $[M]^+$
1	41	12	33	61	512, 312, 290	84	31	0	31.0	887
2	47	8	26	54	512, 323, 288	91	33	0	31.4	917
deacyl pigment **1**	13	4	47	66	510, 288	—	28	0	16.3	—
deacyl pigment **2**	13	4	47	66	510,289	—	29	0	16.2	—
Pel 3-soph-5-Glc	12	5	55	69	507, 287	—	26	0	15.4	757
Pel 3-sam-5-Glc	11	10	45	67	507, 282	—	—	0	18.0	727

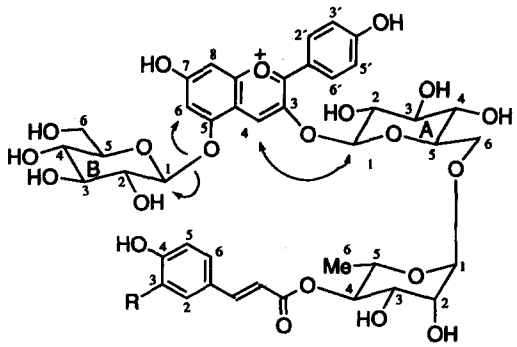
For key to abbreviations, see Experimental. Pel 3-soph-5-Glc = pelargonidin 3-sophoroside-5-glucoside, Pel 3-sam-5-Glc = pelargonidin 3-sambubioside-5-glucoside.

were assigned by analysis of ^{1}H-^{1}H COSY and negative NOE difference (DIFNOE) spectra [5] of **1** (Table 2). The H-4 of rhamnose (δ 4.85, *t*, J = 9.5 Hz) being shifted to a lower magnetic field was clearly assigned by this analysis, indicating that the OH-4 of rhamnose was acylated with *p*-coumaric acid. Irradiation of an anomeric proton (δ 5.53, Glc A) gave a negative NOE

Table 2. ^{1}H NMR data of acylated pelargonidin glycosides isolated from the red potato tubers (CF_3CO_2D-DMSO-d_6, 1:9 at 25°)

H	**1**	**2**
Pelargonidin		
4	8.98 *s*	8.93 *s*
6	6.99 *br s*	6.94 *br s*
8	7.04 *br s*	7.07 *br s*
2′, 6′	8.55 *m*†	8.52 *br d* (8.6)
3′, 5′	7.04 *m*†	7.48 *br d* (8.6)
Hydroxycinnamic acid		
2	] 7.37 *d* (7.7)	6.99 *br s*
6		6.96 *m*
3	] 6.76 *d* (7.7)	
5		6.72 *d* (7.7)
α	6.21 *d* (15.4)	6.22 *d* (15.8)
β	7.52 *d* (15.4)	7.49 *d* (15.8)
-CH_3		3.79 *s*
Sugar* (Glucose A)		
1	5.53	5.39
2	3.69	3.63
3	3.60	3.51
4	3.47	3.38–3.97
5	3.78	3.38
6a	3.69	3.63
6b	4.01	3.96
(Glucose B)		
1	5.20	5.11
2	3.69	3.63
3	3.60	] 3.40–3.60
4	3.53	
5	3.60	3.63
6a	3.83	3.72
6b	3.92	3.86
(Rhamnose)		
1	4.69	4.63
2	3.78	3.78
3	3.84	3.80
4	4.85	4.84
5	3.75	3.72
-CH_3	0.98 *d* (4.7)	0.95 *d* (5.6)

*Assigned by ^{1}H-^{1}H COSY.
† Assigned by DIFNOE.
Coupling constants (*J* in Hz) parentheses.



1 : R=H
2 : R=OMe

Fig. 1. Anthocyanins from the red potato. Observed NOE's are indicated by arrows.

at H-4 of pelargonidin indicating Glc A linked with OH-3 of pelargonidin. Similarly irradiation of another anomeric proton (δ 5.20) of Glc B gave a negative NOE at H-6 of pelargonidin. Thus, Glc B was bonded with OH-5 of pelargonidin. By H_2O_2 degradation *p*-coumaroylrutinose was produced and detected by TLC [6]. Therefore **1** is pelargonidin 3-*O*-[(4″-*O*-(*trans*-*p*-coumaroyl)-α-L-6″-rhamnopyranosyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside], which was already reported and named as pelanin [1–4]. However this is a first report of its complete structure determination using modern methods of analysis.

The FAB mass spectrum of **2** gave a $[M]^+$ peak at 917 *m/z* in good agreement with the mass calculated for $C_{43}H_{49}O_{22}$ which was composed of pelargonidin with two molecules of glucose, one of rhamnose and one of ferulic acid. The structure of **2** was determined by analysis of ^{1}H NMR spectra including ^{1}H-^{1}H COSY and DIFNOE techniques. The ^{1}H NMR spectrum of **2** was superimposed on that of **1** except for the signals of the ferulyl moiety. Seven proton signals of pelargonidin and eight proton signals of ferulic acid were observed as shown in the Table 2. Signals of three anomeric protons of sugar moieties appeared at δ 5.39 (*d*, J = 7.3 Hz, Glc A), 5.11 (*d*, J = 7.3 Hz, Glc B) and δ 4.63 (*s*, rhamnose). The characteristic triplet peak in the down-field (δ 4.84, *t*, J = 9.5 Hz) was assigned to H-4 of rhamnose by analysis of ^{1}H-^{1}H COSY spectrum, indicating that the OH-4 of rhamnose is acylated. The structure of ferulic acid obtained by deacylation of **2** was confirmed as follows. Acetylation of this ferulic acid with acetic anhydride afforded its acetate, in the ^{1}H NMR spectrum of which the signal of H-5 was shifted in the down-field (δ 7.18) by comparison with that (δ 6.93) of authentic ferulic acid. This result suggested the presence of a free phenolic OH at the 4-position of its ferulic acid. Hence the structure of the acetate was unambiguously deduced as 4-acetoxy-3-methoxycinnamic acid. As irradiation at the anomeric proton (δ 5.39) of Glc A gave a negative NOE at H-4 of pelargonidin, Glc A is glycosylated with the OH-3 of pelargonidin. As the deacyl anthocyanin of **2** is essentially identical with that of **1**, the structure of **2** is pelargonidin 3-*O*-[(4″-*O*-(*trans*-feruloyl)-α-L-6″-rhamnopyranosyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside], which is a new anthocyanin [7, 8].

EXPERIMENTAL

Plant materials. The red tubers from deep-coloured tetraploidal potato, 90112-23, selected from hybrid seedlings between cultivars of *Solanum tuberosum* and *S. andigena*, were obtained from the plants growing in the experimental farm of Potato Breeding Laboratory, Hokkaido National Agricultural Experimental Station (Eniwa, Hokkaido).

General procedures. Pigment identifications were carried out by standard procedures involving H_2O_2 oxidation, deacylation with alkali and hydrolysis with acid [9]. TLC was carried out on microcrystalline cellulose using BAW (*n*-BuOH–HOAc–H_2O, 15:3:82), BuH (*n*-BuOH–2M HCl, 1:1), 1% HCl and HOAc–HCl (HOAc–HCl–H_2O, 15:3:82), for anthocyanins, BAW, IPB (iso-PrOH–*n*-BuOH–H_2O, 7:1:2), and IPW (*iso*-PrOH–H_2O, 4:1) for sugars, and BAW, EAA (EtOAc–HOAc–H_2O, 3:1:1), and EFW (Et_2O–HCO_2H–H_2O, 5:2:1) for cinnamic acids. Tosoh CCPM and CCPD pumping systems (dual mode) were used for analytical HPLC, which was run through an Inertsil ODS-2 column (4.6ϕ × 250 mm, GL Sciences) at 35° with a flow rate of 1 ml min^{-1} and monitoring at 320 nm. A linear-gradient elution was set over 60 min from 5 to 50% of solvent B (H_2O–MeOH–MeCN–HCO_2H, 8:5:5:2) in solvent A (H_2O–HCO_2H, 9:1). Prep. HPLC was run with a LC 8A pumping unit (Shimadzu) through a TSK gel PREP-ODS column (20ϕ × 250 mm, Tosoh) at a flow rate of 12 ml min^{-1} by isocratic elution, using a solvent (H_2O–AcOH–MeCN–CF_3CO_2H, 79.5:10:10:0.5) with monitoring at 320 mn. IR spectra were measured with a JEOL JMS-JX 102, ^{1}H NMR spectra with a JEOL JNM-GX-400 (400 MHz) and GSX-270 (270 MHz), using Me_4Si as a reference.

Isolation of the pigments. The red tubers of a tetraploid red potato, 90112-23, (8.75 kg) were cut into small strips and immersed in 40% AcOH–MeOH (18 l), then the extract was concd. The concd extract was adsorbed to a Amberlite × AD-7 column (65ϕ × 300 mm), washed with H_2O and eluted with 5% AcOH–MeOH. The eluted extract was evapd to dryness and the residue was recrystallised with 5% AcOH–MeOH and Et_2O. The red powder (7.0 g) obtained by filtration was subjected to ODS CC with a solvent H_2O–AcOH–MeOH (14:1:5), and the crude pigment (1.2 g) was pptd from the eluted fr. Furthermore, from 100 mg of this crude pigment, pigment **1** (75 mg) and **2** (8 mg) was isolated, respectively by prep. ODS-HPLC.

Deacylation. Each pigment **1** and **2** (*ca* 10 mg) was dissolved in 5% NaOH (1 ml) and stirred under N_2 at room temp. After 1 hr, the reaction mixt. was acidified to pH 1 with 5% HCl. The resultant mixt. was

extracted with Et_2O, and the combined organic layer was dried over $MgSO_4$ and evapd to give the carboxylic acids (each *ca* 1.8 mg). The aq. layer was used as the deacylated pigment soln.

Acetylation. The carboxylic acid (ferulic acid), (*ca* 1.8 mg) obtained by deacylation of pigment **2** was dissolved in Ac_2O (1 ml), stirred for 1 hr at 50°C, and evapd to give 4-acetoxy-3-methoxycinnamic acid (*ca* 1.8 mg). IR spectral data. **1**, IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1680 (C=O), 1635 (C=C), 1610 (C=C); **2** IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1680 (C=O), 1640 (C=C), 1610 (C=C).

Ferulic acid. NMR (270 MHz), δ_H ($CDCl_3$): 7.70 (1H, *d*, J = 15.9 Hz, β-H), 7.10 (1H, *dd*, J = 1.8 and 7.9 Hz, 6-H), 7.05 (1H, *d*, J = 1.8 Hz, 2-H), 6.93 (1H, *d*, J = 7.9 Hz, 5-H), 6.29 (1H, *d*, J = 15.9 Hz, α-H), 3.94 (3H, *s*, OMe).

4-*Acetoxy*-3-*methoxycinnamic acid.* NMR (270 MHz), δ_H ($CDCl_3$): 7.75 (1H, *d*, J = 15.9 Hz, β-H), 7.17 (1H, *dd*, J = 1.8 and 7.6 Hz, 5-H), 7.13 (1H, *d*, J = 1.8 Hz, 2-H), 7.08 (1H, *d*, J = 7.6 Hz, 6-H), 6.38 (1H, *d*, J = 15.9 Hz, α-H), 3.88 (3H, *s*, OMe), 2.32 (3H, *s*, Ac).

p-*Coumaroylrutinose.* **1** was dissolved in H_2O and oxidized with H_2O_2 [6, 9]. The resulting soln was chromatographed in BAW and a band containing the acylated sugar was cut out, eluted and purified by TLC. The authentic *p*-coumaroylrutinose was similarly obtained by H_2O_2 degradation of malvidin 3-*p*-coumaroylrutinoside-5-glucoside isolated from the purple-violet flowers of *Petunia hybrida.* TLC-R_f values: 63 (BAW), 67 (EAA), 61 (EFW).

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