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Structures and Antioxidant Activity of Anthocyanins in Many Accessions of Eggplant and Its Related Species

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Anthocyanins were detected in extracts from the peels of 123 accessions of eggplant (*Solanum melongena*) and its related species. Their anthocyanin profiles were classified into four types, including known Japanese eggplant type (type 1) and non-Japanese eggplant type (type 2). Although most of the eggplant accessions had one of the two known profiles, one accession had a novel profile (type 3). Two accessions of related species showed another novel profile (type 4). The major anthocyanins were identified as delphinidin 3-(*p*-coumaroylrutinoside)-5-glucoside (nasunin) (type 1), delphinidin 3-rutinoside (type 2), delphinidin 3-glucoside (type 3), and petunidin 3-(*p*-coumaroylrutinoside)-5-glucoside (petunidin 3RGc5G) (type 4). Delphinidin 3-caffeoylrutinoside-5-glucoside (delphinidin 3RGcaf5G) was isolated from the hybrid (F1) plants of a type 1 cultivar and a type 3 germplasm. Among the five purified anthocyanins, delphinidin 3RGcaf5G showed the highest radical-scavenging activities toward both 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and linoleic acid radical, followed in order by nasunin and petunidin 3RGc5G.

KEYWORDS: Eggplant; *Solanum*; anthocyanin; antioxidant activity; radical scavenging

INTRODUCTION

Anthocyanins are pigments widely distributed in plant foods and have attracted much attention because of their potentially beneficial effects in preventing diseases. Anthocyanins have been reported to have a variety of physiological functions such as antioxidative function (*1−3*), antimutagenic effects (*4*), anticancer effects (*5, 6*), and improvement of vision (*7, 8*). The antioxidative effects of flavonoids, including anthocyanins, on the oxidative modification of low-density lipoproteins (*9, 10*) might help slow the development of atherosclerosis, which can lead to coronary heart disease.

The purple pigments in eggplant (*Solanum melongena*) peels are known to be anthocyanins. It has been reported that the major anthocyanins in eggplant are delphinidin 3-(*p*-coumaroylrutinoside)-5-glucoside (nasunin) and delphinidin 3-rutinoside (tulipanin, D3R) (*11−13*). Furthermore, delphinidin 3-rutinoside-5-glucoside has been identified as a minor anthocyanin of non-Japanese type cultivars, containing D3R as a major component (*14*). Nasunin has been reported to have the strongest antioxidant activity among several anthocyanins (*15*) and to be a potent superoxide anion radical scavenger (*16, 17*). Dietary nasunin has been shown to have protective effects against paraquat-induced oxidative stress in rats (*18*).

Although the anthocyanin composition has been studied in several cultivars of eggplant (*13, 14*), that of many accessions of eggplant and its related species from various countries originally is not known. It is possible that those accessions might contain anthocyanins other than nasunin and D3R, including those with antioxidant activities higher than that of nasunin. This study primarily aimed to find eggplant anthocyanins with higher antioxidant activity compared to nasunin. For this purpose, we profiled anthocyanins in many accessions of eggplant and its related species and isolated and identified their anthocyanins. Moreover, we evaluated the radical-scavenging activity of the purified anthocyanins, using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and linoleic acid.

MATERIALS AND METHODS

Plant Materials. One hundred and twenty-three accessions of *Solanum* containing 110 accessions of eggplant and 13 accessions of its related species, maintained at the National Institute of Vegetable and Tea Science (NIVTS), were used in this investigation. The tested related species were *Solanum aculeatissimum*, *Solanum gilo*, *Solanum*

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Figure 1. *Solanum nigrum* BIR/S 0278.

integrifolium, *Solanum lacianiatum*, *Solanum mammosum*, *Solanum nigrum*, *Solanum nodiflorum*, *Solanum torvum* (one accession each), and *Solanum* sp. (five accessions). Seeds were sown in a greenhouse in late May, and the plants were grown in the field from the middle of July. The immature fruits were harvested in August, except for *S. nigrum* BIR/S 0278 and *Solanum* sp. BIR/S 0246, of which fully matured black fruits were harvested. Flower buds of the germplasm PI 286106 were emasculated and pollinated with the cultivar Nakate Shinkuro. The resultant F_1 plants were grown in a greenhouse, and their immature fruits were harvested. To identify the isolated anthocyanins, flowers of two wild *Petunia* taxa, *Petunia integrifolia* subsp. *integrifolia* var. *integrifolia* and *Petunia reitzii* (*19*−*21*), were kindly supplied by Dr. T. Ando and Dr. H. Watanabe, Chiba University.

Reagents. Nasunin and D3R were kindly supplied by Dr. Y. Sakata and Dr. F. Hashimoto, Kagoshima University. Delphinidin 3-glucoside (D3G) was purified from bilberry (*Vaccinium myrtillus*, a wild-type blueberry) according to the previous method (*22*). Briefly, bilberry extract (Myltoserect, Indena Co. Ltd.) (10 g) was dissolved in 1% trifluoroacetic acid (TFA) aqueous solution (10 mL) and subjected to low-pressure liquid chromatography. Anthocyanins were recovered in the fraction eluted with 30% MeOH containing 1% TFA (yield = 3.3 g). The anthocyanin fraction was further chromatographed over MCI-gel CHP-20 (45 mm × 450 mm) (Mitsubishi, Tokyo, Japan) with H_2O containing increasing amounts of MeOH (0:1→1:0) to give 11 fractions. The D3G fraction was further separated by Sephadex LH-20 chromatography (25 mm × 260 mm). D3G was purified by HPLC using a Develosil ODS-HG5 column (20 mm × 250 mm, Nomura Chemical Co. Ltd., Seto, Japan) and 20% MeOH containing 0.1% TFA as the elution solvent. The structure of D3G was assigned by extensive 1D and 2D nuclear magnetic resonance (NMR) as well as by tandem mass spectrometry (MS) analysis. NMR spectra were recorded on a JEOL ECA500 spectrometer, and chemical shifts are reported as δ (parts per million) with tetramethylsilane (TMS) as an internal standard. Other reagents used were all of analytical or HPLC grade.

Extraction and HPLC Analysis. Four round sections 12 mm in diameter were prepared from each peel and freeze-dried. Each section was dipped into 3 mL of 5% formic acid and left in the darkness at room temperature for 24 h. After centrifugation at 5000*g* and 4 °C for 5 min, the supernatants were filtered through a 0.20 μm filter. Anthocyanins in the extracts (20 μL) were analyzed by high-performance liquid chromatography (HPLC) under the following conditions: column, TSKgel ODS-80Ts (4.6 mm × 250 mm, Tosoh, Tokyo, Japan); solvent, linear gradient of 0−40% acetonitrile in 10% formic acid for 35 min; flow rate, 1.0 mL/min; column temperature, 35 °C; and monitoring, UV−vis 240−600 nm using a photodiode array detector. Flowers of two wild taxa of *Petunia* were also extracted with 5% formic acid, and the extracts were used for cochromatography with purified anthocyanins.

Isolation of Anthocyanins. Peels (50−100 g) of the eggplants Nakate Shinkuro, Yalova, and PI 286106 and of the F_1 plants of Nakate Shinkuro and PI 286106 and the fruits (50 g) of *S. nigrum* BIR/S 0278 (**Figure 1**) were freeze-dried and extracted with 5% formic acid according to the methods described above. The extracts were subjected to a nonionic polymeric adsorbent (Amberlite XAD-7, Rohm and Haas, Philadelphia, PA) column (20 mm × 300 mm), which was prewashed with 1% formic acid. After elution with 1% formic acid, anthocyanins were eluted with formic acid/H_2O/MeOH (1:49:50), and the fraction was concentrated to the aqueous phase in vacuo. The aqueous residue was dissolved in 10% formic acid and purified on a CAPCELL Pak UG column (20 mm × 250 mm, Shiseido, Tokyo, Japan) held at 35 °C using a linear gradient of 10−30% acetonitrile in 10% formic acid for 30 min with a flow rate of 10.0 mL/min and monitoring at UV−vis 240−600 nm using a photodiode array detector.

Instrumental Analysis of Purified Anthocyanins. High-resolution fast-atom bombardment mass spectrometry (HR-FAB-MS) was recorded on a JEOL JMS-700 system. The matrix was *m*-nitrobenzyl alcohol/dithiothreitol (1:1). The ^1H and ^{13}C NMR spectra were obtained by a JEOL JMN-EX270 system in MeOH-d_4/TFA-*d* (9:1) containing TMS as an internal standard. Chemical shifts in ^1H NMR spectra are referenced to TMS (δ = 0.00 ppm).

Antioxidative Assay. The radical-scavenging activity (RSA) was assayed using DPPH and linoleic acid. The assay of DPPH RSA was carried out according to the method reported by Brand-Williams et al. (*23*) with some modification. All reactions were conducted in 96-well microplates. The sample solution (10 μL) containing the test compound at concentrations of 0−7.5 mM in 70% MeOH containing 1% formic acid was added with 190 μL of DPPH working solution consisting of 0.4 mM DPPH in EtOH/ 400 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 6.1)/H_2O (4:1:3). The mixture was held for 20 min at room temperature. The decrease in the absorbance of DPPH at 535 nm was measured using a microplate reader. The absorbance of each sample was measured against a blank of 70% MeOH containing 1% formic acid without DPPH. All experiments were performed in triplicate. DPPH RSA is expressed as concentration values yielding a 50% reduction in absorbance of DPPH radicals (IC_{50}). Linoleic acid RSA was measured as follows: 25 μL of 2 mM anthocyanins or 3-*tert*-butyl-4-hydroxyanisole (BHA) in 70% MeOH containing 1% formic acid was mixed with 1.0 mL of 0.2 M phosphate buffer (pH 7.0), 0.375 μL of distilled water, and 1.0 mL of 2.5% (w/v) linoleic acid in ethanol. As a blank, 25 μL of 70% MeOH containing 1% formic acid was used. Peroxidation was initiated by the addition of 50 μL of 0.1 mM 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) and was carried out at 37 °C for 3 h in the dark. Peroxides in the resulting reaction solution were determined according to the thiocyanate method (*24*). Briefly, 100 μL of reaction solution was added with 4.7 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate, and 0.1 mL of 20 mM $FeCl_2$ in 3.5% HCl, and the absorbance at 500 nm was measured after 3 min. Linoleic acid RSA is expressed in terms of preoxidation inhibitory ratio (percent) relative to that of BHA and was calculated according to the following equation: linoleic acid RSA (%) = [(absorbance of blank − absorbance of samples)/(absorbance of blank − absorbance of BHA)] × 100.

Statistical Analysis. To evaluate the differences in the antioxidant activity among the purified anthocyanins, statistical analysis was performed using Student's *t* test. A probability of $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Anthocyanin Profiles of the Tested Accessions. Among the tested 123 accessions, anthocyanins were detected in 105 accessions (101 accessions of eggplant and 4 accessions of related species) having a peel color of reddish purple, purple, dark purple, or brown. In all accessions with a green or white peel color, no anthocyanins were detected. The HPLC profiles of anthocyanins from the peels of the 105 accessions were classified into the four types shown in **Figure 2**. All of the profiles were simple, and each contained one major anthocyanin. Among them, types 1 and 2 have been previously reported as the anthocyanin profiles of Japanese type eggplant and non-Japanese type eggplant, respectively (*13*), whereas types 3 and 4 were novel profiles.

As shown in **Table 1**, 101 eggplant accessions contained anthocyanins among the tested 110 accessions, and most of them

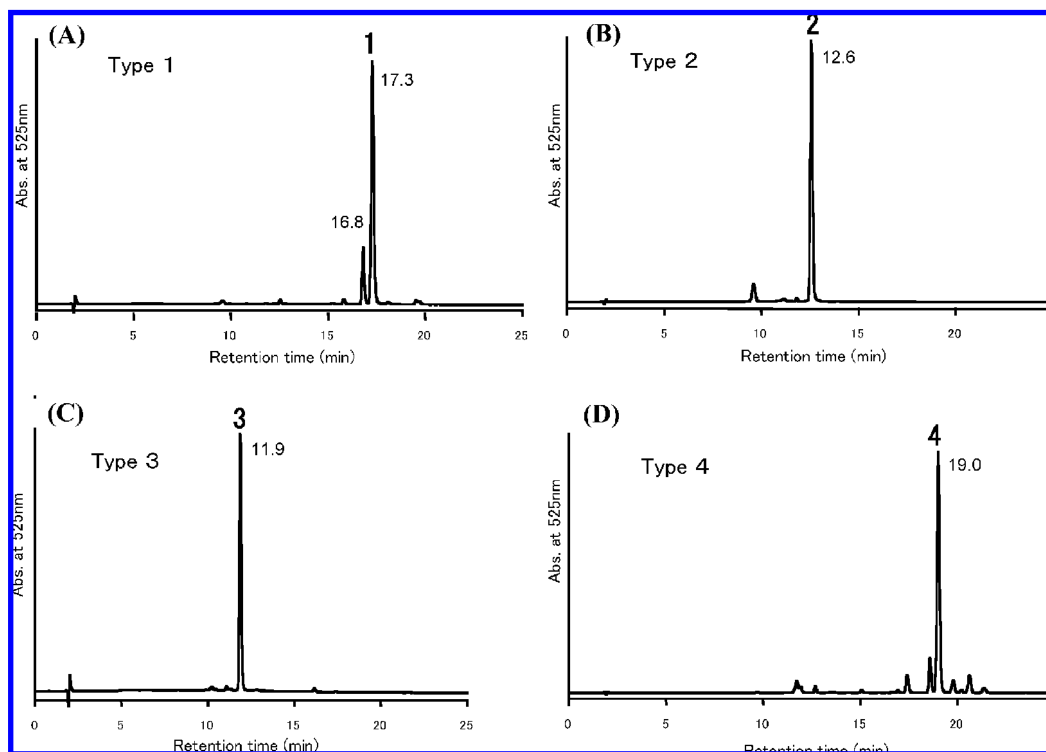

Figure 2. Four types of HPLC anthocyanin profiles in the peels of tested accessions of eggplant and its related species: (**A**) *Solanum melongena* Nakate Shinkuro; (**B**) *S. melongena* Yalova; (**C**) *S. melongena* PI 286106; (**D**) *Solanum nigrum* BIR/S 0278. Conditions: column, TSKgel ODS-80Ts (4.6 mm × 250 mm); eluent, linear gradient of 0−40% acetonitrile in 10% formic acid for 35 min; flow rate, 1.0 mL/min; column temperature, 35 °C.

Table 1. Anthocyanin Profile Type of the Tested Eggplant Accessions

anthocyanin profile	no. of accessions
type 1	57
type 2	43
type 3	1
ND[a]	9
total	110

[a] ND, not detectable.

had one of the two known profiles, type 1 or 2. However, only one eggplant accession, PI 286106, had the novel type 3 profile. Among the 13 related species, anthocyanins were detected in four accessions. Whereas *Solanum* sp. Yaseinasu 1 and *S. torvum* 130 had type 1 and type 2 profiles, respectively, *S. nigrum* BIR/S 0278 and *Solanum* sp. BIR/S 0246 showed the novel type 4 profile.

F_1 plants of Nakate Shinkuro (type 1) and PI 286106 (type 3) showed the type 1 anthocyanin profile (**Figure 3**). On the profile of F_1 plants, the anthocyanin with a retention time of 16.1 min (**Figure 3**) had significantly higher peak area ratios (14−19%) compared to those of Nakate Shinkuro (0.3%) (**Figure 2A**).

Identification of Anthocyanins. Anthocyanins **1**−**5**, shown in **Figures 2** and **3**, were extracted from the peels of representative accessions with each anthocyanin profile and purified successfully by a XAD-7 column and preparative HPLC. Purified anthocyanins **1** and **2** are major compounds in known profiles, and their retention times in HPLC analysis agreed with those of authentic nasunin and D3R (**Figure 4**), respectively, which had been already identified. Furthermore, only one peak was obtained in HPLC analysis by both the co-injection of anthocyanin **1** and nasunin and the co-injection of anthocyanin **2** and D3R. From these results, anthocyanins **1** and **2** were identified as nasunin and D3R, respectively. Ichiyanagi

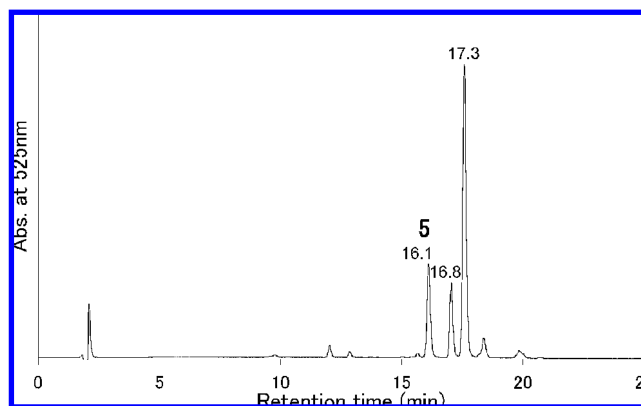

Figure 3. HPLC chromatogram of anthocyanins from the peel of an F_1 plant of *Solanum melongena* Nakate Shinkuro and *S. melongena* PI 286106. Conditions: column, TSKgel ODS-80Ts (4.6 mm × 250 mm); eluent, linear gradient of 0−40% acetonitrile in 10% formic acid for 35 min; flow rate, 1.0 mL/min; column temperature, 35 °C.

et al. (*25*) reported that eggplant nasunin consisted of cis−trans isomers, because it has a *p*-coumaroyl moiety. On the basis of that report, anthocyanin **1** was thought to be nasunin containing *p-trans*-coumaric acid, and a minor compound with a retention time of 16.8 min was thought to be the cis-isomer of anthocyanin **1**.

The UV−vis λ_{max} values of anthocyanin **3** were at 540 and 279 nm. Purified anthocyanin **3** was analyzed by HR-FAB-MS and $^1H/^{13}C$ NMR spectroscopy: HR-FAB-MS, *m/z* 465.1042 $[M]^+$ (calcd for $C_{21}H_{21}O_{12}$, 465.1033); ^1H NMR [270 MHz, MeOH-d_4/TFA-*d* (9:1)] δ 8.98 (1H, s, 4-*H*), 7.78 (2H, s, 2′-*H*, 6′-*H*), 6.87 (1H, broad d, J = 2.0 Hz, 8-*H*), 6.67 (1H, d, J = 2.0 Hz, 6-*H*), 5.32 (1H, d, J = 7.8 Hz, G1-*H* (G, glycosyl portion)], 3.95 (1H, dd, J = 2.7, 10.8 Hz, G6-*H*), 3.79−3.71 (2H, m), 3.63−3.46 (3H, m); ^{13}C NMR spectral data, see **Table 2**. These data were in good agreement with those of D3G

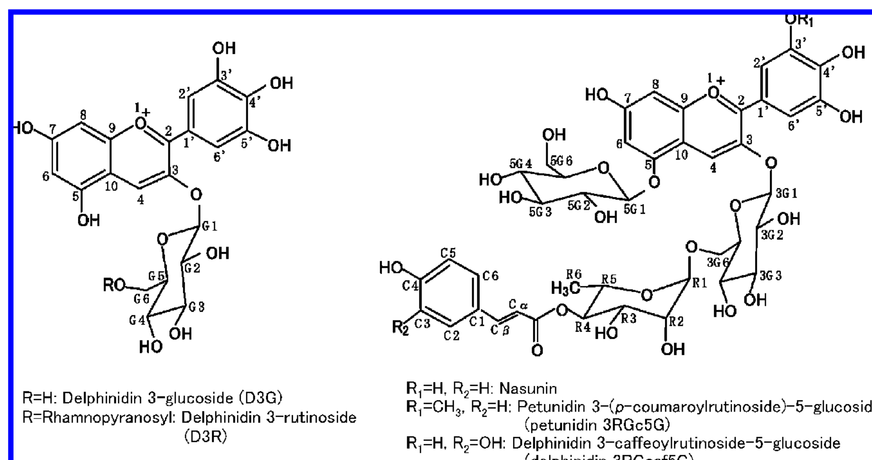

Figure 4. Structures of nasunin, petunidin 3-(*p*-coumaroylrutinoside)-5-glucoside, and delphinidin 3-caffeoylrutinoside-5-glucoside.

(**Figure 4**) reported previously (*26*). The same retention times were obtained in HPLC analysis of purified anthocyanin **3** and authentic D3G, and only one HPLC peak was observed on the cochromatography of purified anthocyanin **3** with authentic D3G. From these results, anthocyanin **3** was identified as D3G.

The UV−vis λ_{max} values of anthocyanin **4** were at 540, 310, and 283 nm. Purified anthocyanin **4** was analyzed by HR-FAB-MS and $^1H/^{13}C$ NMR spectroscopy: HR-FAB-MS, *m/z* 933.2757 $[M]^+$ (calcd for $C_{43}H_{49}O_{23}$, 933.2665); ^1H NMR [270 MHz, MeOH-d_4/TFA-*d* (9:1)] δ 8.96 (1H, s, 4-*H*), 7.96 (1H, d, J = 2.2 Hz, 2′-*H*), 7.82 (1H, d, J = 2.2 Hz, 6′-*H*), 7.58 [1H, d, J = 15.7 Hz, Cβ-*H* (C, *p*-coumaroyl portion)], 7.42 (2H, d, J = 8.6 Hz, C2-*H*, C6-*H*), 7.04 (1H, s, 6-*H* or 8-*H*), 7.03 (1H, s, 6-*H* or 8-*H*), 6.83 (2H, d, J = 8.6 Hz, C3-*H*, C5-*H*), 6.25 (1H, d, J = 15.7 Hz, Cα-*H*), 5.51 [3G1-*H* (3G, 3-glycosyl portion) signals were hidden under the residual proton signals of NMR solvent], 5.21 [1H, d, J = 7.6 Hz, 5G1-*H* (5G, 5-glycosyl portion)], 4.93 [1H, t, J = 9.6 Hz, R4-*H* (R, rhamnosyl portion)], 4.74 (1H, s, R1-*H*), 4.07 (1H, broad d, J = 11.1 Hz, 3G6-*H*), 4.00 (3H, s, OCH_3), 3.98 (1H, d, J = 12.4 Hz, 5G6-*H*), 3.89−3.47 (13H, m), 1.02 (3H, d, J = 6.2 Hz, R6-*H*); ^{13}C NMR spectral data, see **Table 2**. These data were in good agreement with those of petunidin 3-(*p*-coumaroylrutinoside)-5-glucoside (petunidin 3RGc5G) (**Figure 4**) reported previously (*21*). The $^1H/^{13}C$ NMR data (**Table 2**) of anthocyanin **4** also coincided with those of petunidin 3RGc5G (*27*). The HPLC peak of anthocyanin **4** overlapped with that of petunidin 3RGc5G in an extract from flowers of *P. integrifolia* subsp. *integrifolia* var. *integrifolia* (*19*−*21*) on the cochromatography of anthocyanin **4** and the extract. From these results, anthocyanin **4** was identified as petunidin 3RGc5G.

Purified anthocyanin **5** from F_1 plants of Nakate Shinkuro and PI 286106 was analyzed by HR-FAB-MS but not by $^1H/^{13}C$ NMR, because the amount isolated was not enough for $^1H/^{13}C$ NMR. The HR-FAB-MS analysis of anthocyanin **5** gave a molecular ion at *m/z* 935.2648, corresponding to $C_{39}H_{51}O_{26}$ (calcd 935.2669). The UV−vis λ_{max} values of this anthocyanin were at 541, 326, and 280 nm. These data agreed with those of delphinidin 3-caffeoylrutinoside-5-glucoside (delphinidin 3RGcaf5G) (**Figure 4**) isolated from flowers of *P. reitzii* (*21*). The HPLC peak of anthocyanin **5** overlapped with that of delphinidin 3RGcaf5G in an extract from flowers of *P. reitzii* (*19*−*21*) on the cochromatography of purified anthocyanin **5** and the extract. From these results, anthocyanin **5** was identified as delphinidin 3RGcaf5G.

D3G is a common glycoside of delphinidin and had been found in *Phaseolus* legumes (*27*−*29*), black currant (*30, 31*), camu-camu (*32*), and others. Petunidin 3RGc5G had been

Table 2. ^{13}C NMR Spectra for Anthocyanins **3** and **4**

anthocyanin **3**		anthocyanin **4**	
position	δ	position	δ
delphinidin		petunidin	
2	164.3	2	164.2
3	146.0	3	146.4
4	136.5	4	134.6
5	159.3	5	157.3
6	103.6	6	105.9
7	170.5	7	170.0
8	95.3	8	97.7
9	157.8	9	156.9
10	113.5	10	113.4
1′	120.3	1′	119.9
2′	112.8	2′	109.8
3′	147.7	3′	150.1
4′	144.8	4′	147.9
5′	147.7	5′	146.4
6′	112.8	6′	114.5
		OMe	57.4
3-*O*-glucosyl		3-*O*-glucosyl	
1	103.9	1	102.9
2	74.9	2	74.9
3	78.3	3	78.4
4	71.2	4	71.6
5	79.0	5	77.9
6	62.5	6	67.5
		rhamnosyl	
		1	102.3
		2	72.3
		3	70.6
		4	75.6
		5	68.0
		6	18.0
		5-*O*-glucosyl	
		1	103.1
		2	75.0
		3	78.1
		4	71.2
		5	78.9
		6	62.4
		p-coumaroyl	
		COO	169.3
		α	115.2
		β	147.3
		1″	127.4
		2″,6″	131.4
		3″,5″	117.1
		4″	161.5

Table 3. DPPH Radical-Scavenging Activity of the Isolated Anthocyanins

anthocyanin	activity[a] (mM)
nasunin	0.468
delphinidin 3-rutinoside	0.975
delphinidin 3-glucoside	1.120
petunidin 3RGc5G[b]	0.514
delphinidin 3RGcaf5G[c]	0.425

[a] Concentration yielding a 50% reduction in the absorbance of DPPH at 535 nm (IC_{50}). [b] Petunidin 3-(*p*-coumaroylrutinoside)-5-glucoside. [c] Delphinidin 3-caffeoylrutinoside-5-glucoside.

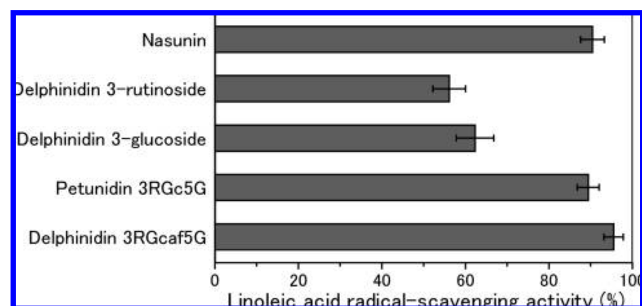

Figure 5. Linoleic acid radical-scavenging activity of the isolated anthocyanins (2 mM) as measured by the thiocyanate method: petunidin 3RGc5G, petunidin 3-(*p*-coumaroylrutinoside)-5-glucoside; delphinidin 3RGcaf5G, delphinidin 3-caffeoylrutinoside-5-glucoside. The activity is expressed in terms of preoxidation inhibitory ratio (percent) relative to that of 2 mM BHA. Each value is the mean ± standard deviation ($n = 3$).

isolated from tubers and shoots of the purple potato (*26, 33*) and flowers of Japanese garden iris (*34−37*), in addition to flowers of native taxa of *Petunia* (*19−21*). However, these three anthocyanins were isolated and identified from eggplant or its related species for the first time in this study.

Radical-Scavenging Activity (RSA). The scavenging activities of five purified anthocyanins toward DPPH radical and linoleic acid radical are shown in **Table 3** and **Figure 5**, respectively. In both systems, delphinidin 3RGcaf5G showed the highest activity, followed in order by nasunin and petunidin 3RGc5G. The activity of D3R and D3G was weak. It is very interesting that the RSA of delphinidin 3RGcaf5G was higher than that of nasunin, which had been reported to have high scavenging activity toward linoleic acid radicals and superoxide anion radicals (*15−17*). Delphinidin 3RGcaf5G has a caffeoyl group instead of a *p*-coumaroyl group contained in the structure of nasunin. Previous studies on the structure−antioxidant activity relationships of phenolic acids have demonstrated the importance of having at least two hydroxyl groups, especially the catechol group (*o*-dihydroxyl group) on the phenyl ring for their antiradical efficacy (*38−41*). The antioxidant efficiency of monohydroxylcinnamates, such as *p*-coumaroyl acid, was increased when a second hydroxyl group was introduced in the ortho position, as in caffeic acid. The results in this study are consistent with these previous observations.

The slightly lower RSA of petunidin 3RGc5G compared to nasunin could be explained by the smaller number of hydroxyl groups on the B ring of its aglycone moiety. It has been reported that flavonoids with three hydroxyl groups on the B ring, such as delphinidin and myricetin, had high antioxidant activity and that the loss of one hydroxyl group decreased activity slightly (*41*). The weak activity of D3R and D3G could be explained by the absence of an acyl group such as a caffeoyl or coumaroyl group, needed for effective radical scavenging.

Although delphinidin 3RGcaf5G has higher RSA than other anthocyanins isolated from eggplant, its content in type 1 eggplants is much lower than nasunin, and this may limit its potential health benefits. The ratios of delphinidin 3RGcaf5G to total anthocyanins in F_1 plants grown in a greenhouse were higher compared to those of F_1 plants cultivated in the field (data not shown). This anthocyanin was present in all accessions with a type 1 anthocyanin profile, and its content in Nakate Shinkuro grown in the field was approximately 3 times higher than those of F_1 plants grown in the field, although there was no significant difference in the ratio to total anthocyanins between F_1 plants and Nakate Shinkuro.

In conclusion, the 123 tested accessions of eggplant and its related species could be classified into four types containing two major ones based on their anthocyanin profiles. We found novel anthocyanin profiles, types 3 and 4, and the presence of three anthocyanins, D3G, petunidin 3RGc5G, and delphinidin 3RGcaf5G, in eggplant and its related species for the first time in this study. The data on RSA of isolated anthocyanins have demonstrated that Japanese type eggplant is superior to non-Japanese type eggplant and a type 3 eggplant accession with respect to the antioxidative function. Moreover, very interestingly, delphinidin 3RGcaf5G was shown to have higher RSA compared to nasunin, a known eggplant anthocyanin with high RSA. It is a novel and important finding in the field of antioxidants in foods that delphinidin 3RGcaf5G, possessing high antioxidative activity, has been isolated from eggplant as a food, as the presence of this anthocyanin was known in only flowers of *P. reitzii*. The results obtained in this study are also useful for the cultivation and breeding of eggplant accessions with higher antioxidative functions. To find eggplant cultivation methods that increase the content of delphinidin 3RGcaf5G, it is necessary to clarify the effects of cultivation conditions on its content.

ABBREVIATIONS USED

DPPH, 1,1-diphenyl-2-picrylhydrazyl; TFA, trifluoroacetic acid; TMS, tetramethylsilane; RSA, radical-scavenging activity; BHA, 3-*tert*-butyl-4-hydroxyanisole; Abs, absorbance.

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