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Flower Colors and Their Anthocyanins in *Saintpaulia* Cultivars (Gesneriaceae)

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The flower colors and anthocyanin constitution of sixteen cultivars of *Saintpaulia* were surveyed to determine the relationship between their flower colors and anthocyanin components. Six anthocyanins were isolated from the flowers of these cultivars as major anthocyanins along with three minor ones, and their structures were identified by co-HPLC or chemical and spectroscopic techniques. Among them, a novel anthocyanin, pelargonidin 3-*O*-[6-*O*-(4-*O*-(acetyl)-α-rhamnopyranosyl)-β-glucopyranoside] (pelargonidin 3-acetyl-rutinoside; 8) was found in cultivars of 'Georgia' and 'Jessica' as a major anthocyanin. Regarding the flower color variation in these cultivars, the hue values (b*/a*) of these flower colors were responsible for the glycosidic positions in the anthocyanidin molecule and also the combination of anthocyanins. These flower colors were classified into six groups, A–F, based on the flower colors and anthocyanin components were arranged as follows. In violet-blue flowers of group A (hue values b*/a* = −2.61–−1.72, VB N89B–VB 94B) and purple-violet flowers of group B (−1.06 and −0.81, PV N82A and PV N80B), malvidin 3-acetyl-rutinoside-5-glucoside was the most effective major anthocyanin for flower colors. In purple-violet flowers (−0.69 and −0.53, PV N80B and PV N81A) of group C, peonidin 3-acetyl-rutinoside-5-glucoside was the most effective major anthocyanin for flower colors. In red-purple flowers (−0.44–−0.27, RP 73A–RP N74B) of groups D, pelargonidin 3-acetyl-rutinoside-5-glucoside, in red-purple flowers (−0.03 and −0.02, RP 60D and RP 71D) of group E, pelargonidin 3-acetyl-rutinoside, and in red-purple flowers (0.04 and 0.13, RP 61A and RP 71A) of group F, peonidin 3-acetyl-rutinoside were the most effective major anthocyanins for flower colors. From these results, the glucosylation of 5-OH in anthocyanidin 3-acetyl-rutinoside and an increase in the methylation of the B-ring in anthocyanidin were considered to have the most important effects on flower color variations in these *Saintpaulia* cultivars.

Key Words: acetic acid, acetylated anthocyanidin 3-glycosides, acetylated anthocyanidin 3-rutinoside-5-glucosides.

Introduction

The genus *Saintpaulia* Wendl. (Gesneriaceae), comprising approximately 20 species, has over 2000 cultivars (Huxley et al., 1992) with white, pink, red, red-purple, purple-violet, and violet-blue flowers. Many of these cultivars are widely cultivated as one of the most popular ornamental house plants bred from some wild species. The chemical structures of acetylated anthocyanins in the violet-blue flowers of *Saintpaulia* 'Thamires' and the red-purple ones of *S.* 'Tomoko' have previously been investigated (Tatsuzawa et al., 2012, 2015). As an extension of our study, we investigated floral anthocyanins in the 4 violet-blue, 4 purple-violet, and 8 red-purple flower cultivars of *Saintpaulia*. In the present study, we report the finding of a novel acetylated pelargonidin 3-rutinoside along with 8 known anthocyanins in these cultivars. In addition, we discuss the glycosidic positions in anthocyanidin molecules and the combination of anthocyanins for flower color variation in these cultivars.

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Materials and Methods

General procedures

Thin-layer chromatography (TLC) was performed on cellulose-coated plastic sheets (Merck, Darmstadt, Germany) using six mobile phases: BAW (*n*-BuOH/HOAc/H_2O, 4:1:2, v/v/v); BuHCl (*n*-BuOH/2N HCl, 1:1, v/v, upper layer); AHW (HOAc/HCl/H_2O, 15:3:82, v/v/v); 1% HCl for anthocyanins and organic acid,

BAW and ETN ($EtOH/NH_4OH/H_2O$, 16:1:3, v/v/v) for sugars with detection using UV light and aniline hydrogen phthalate spray reagent (AHP), and Forestal ($HOAc/HCl/H_2O$, 30:3:10, v/v/v) for anthocyanidins (Harborne, 1984).

Analytical HPLC was performed on an LC 10A system (Shimadzu, Kyoto, Japan), using a C18 (4.6 × 250 mm) column (Waters, Milford, MA, USA) at 40°C with a flow rate of 1 mL·min^{-1} and monitoring at 530 nm. The eluant by 5% HOAc-H_2O or 5% formic acid was applied as a linear gradient elution for 40 min from 20% to 85% solvent B (1.5% H_3PO_4, 20% HOAc, 25% MeCN in H_2O) in solvent A (1.5% H_3PO_4 in H_2O) with 5 min of re-equilibration at 20% solvent B, for anthocyanins, anthocyanidins, and hydroxycinnamic acids (method 1). The other eluant for acetic acid was applied as an isocratic elution of solvent A for 10 min and monitoring at 210 nm (Tatsuzawa et al., 2015) (method 2).

UV-Vis spectra of dried petals and purified anthocyanins were recorded on MPS-2450 (Shimadzu) in 0.1% HCl-MeOH (from 200 to 700 nm). Spectral absorption of the fresh flowers was directly measured on intact petals using a recording spectrophotometer operated as a double-beam instrument (Type MPS-2450) (Saito, 1967; Saito et al., 2015; Yokoi and Saito, 1973). Fast atom bombardment mass spectra (FABMS) were obtained in positive ion mode using a 1:1 mixture of dithiothreitol and 3-nitrobenzyl alcohol as a matrix with JMS-700 (JEOL Ltd., Tokyo, Japan). NMR spectra were recorded on JNM AL-400 (JEOL Ltd.) at 400 MHz for ^{1}H spectra and 100 MHz for ^{13}C spectra in DMSO-CF_3COOD (9:1). Chemical shifts are reported on the δ-scale from tetramethylsilane as the internal standard, and coupling constants (*J*) are in Hz. In addition, the alkaline and acid hydrolysis products of this anthocyanin producing deacylanthocyanin, aglycone, acid, and sugars were analyzed by TLC (Harborne, 1984). Authentic samples, glucose, rhamnose, and acetic acid were used with commercial standards (Wako Pure Chemical Industries, Ltd., Osaka, Japan); pelargonidin 3-rutinoside was obtained from orange-red flowers of *Alstroemeria* (Tatsuzawa et al., 2003).

Plant materials

The *Saintpaulia* cultivars were purchased from Royal Green Inc. (Gifu, Japan), Ozaki flower park Co., Ltd. (Tokyo, Japan), and Homac Co., Ltd. (Morioka, Japan). The fresh petals were collected in 2012 and 2013, dried overnight at 45°C, and kept at −20°C until use. The flower colors of these cultivars were recorded by comparing them directly with the Royal Horticultural Society Colour Chart (R.H.S. CC) 5th edition and their CIE L*a*b* chromaticity values were recorded on a CM-700d Spectro Color Meter (Konica-Minolta Co., Ltd., Tokyo, Japan) (Yokoi, 1975). Three flowers from each cultivar were measured and their average was obtained.

*Isolation and purification of pigment **8***

Dried petals of 'Georgia' (ca. 10 g) were immersed in 5% HOAc-H_2O (5 L) at room temperature for 12 h and extracted. The extract was passed through a Diaion HP-20 (Nippon Rensui Co., Tokyo, Japan) column (90 × 150 mm), on which pigments were absorbed. Next, the column was thoroughly washed with 5% HOAc-H_2O (20 L) and eluted with 5% HOAc-MeOH (500 mL) to recover the pigments. After concentration, the pigments were separated and purified by paper chromatography using BAW. The separated pigments were further purified by preparative HPLC, which was performed on a Waters C18 (19 × 150 mm, Waters) column at 40°C with a flow rate of 4 mL·min^{-1} and monitoring at 530 nm (Tatsuzawa et al., 2014a, b). The solvent used was as follows: a linear gradient elution for 15 min from 60% to 65% solvent B in solution A. The fraction was transferred to a Diaion HP-20 column, on which pigment was adsorbed. Pigment was eluted with 5% HOAc-MeOH (5:95, v/v) followed by the addition of excess Et_2O and then dried. The purified **8** (ca. 20 mg) was obtained as dried dark-red powder.

*Analyses of pigment **8***

The identification of anthocyanin was performed by standard procedures involving deacylation with acid, and both alkaline and acid hydrolysis (Harborne, 1984).

*1. Pigment **8***

Dark-red powder: UV-VIS (in 0.1% HCl-MeOH): λmax 511, 432, 282sh, 270 nm, E_{440}/E_{max} = 44%, $AlCl_3$ shift 0; TLC: (R_f-values) BAW 0.57, BuHCl 0.64, 1% HCl 0.73, AHW 0.89; HPLC (method 1): *R*t (min) 26.8. ^{1}H NMR δ pelargonidin: 8.09 (*s*, H-4), 6.74 (*d*, *J* = 2.0 Hz, H-6), 6.97 (*d*, *J* = 2.0 Hz, H-8), 8.57 (*d*, *J* = 9.0 Hz, H-2' and 6'), 7.07 (*d*, *J* = 9.0 Hz, H-3' and 5'). Acetic acid: 1.94 (*s*, -CH_3). Glucose: 5.37 (*d*, *J* = 7.6 Hz, H-1), 3.51 (*t*, *J* = 8.3 Hz, H-2), 3.44 (*t*, *J* = 8.8 Hz, H-3), 3.30 (*t*, *J* = 9.1 Hz, H-4), 3.68 (*m*, H-5), 3.57 (*dd*, *J* = 5.8, 10.3 Hz, H-6a), 3.89 (*brd*, *J* = 10.3, H-6b). Rhamnose: 4.60 (*s*, H-1), 3.71 (*dd*, *J* = 1.5, 3.2 Hz, H-2), 3.65 (*dd*, *J* = 3.2, 9.8 Hz, H-3), 4.72 (*t*, *J* = 9.8 Hz, H-4), 3.55 (*m*, H-5), 0.89 (*s*, -CH_3). ^{13}C NMR δ pelargonidin: 162.6 (C-2), 144.3 (C-3), 136.1 (C-4), 156.7 (C-5), 103.1 (C-6), 169.6 (C-7), 94.8 (C-8), 158.4 (C-9), 112.7 (C-10), 119.8 (C-1'), 135.1 (C-2' and 6'), 117.5 (C-3' and 5'), 165.4 (C-4'). Acetic acid: 21.2 (-CH_3), 171.0 (COOH). Glucose: 102.5 (C-1), 73.8 (C-2), 76.9 (C-3), 70.1 (C-4), 76.3 (C-5), 66.7 (C-6). Rhamnose: 100.8 (C-1), 70.8 (C-2), 68.9 (C-3), 74.5 (C-4), 66.5 (C-5), 17.7 (-CH_3).

*Deacylanthocyanin and acetic acid of pigment **8***

Pigment **8** (ca. 0.5 mg) was dissolved in 2 N NaOH (1 mL) using a degassed syringe to stirred for 15 min. The solution was then acidified with 2 N HCl (1.1 mL). This solution was used for TLC and HPLC with authen-

tic pelargonidin 3-rutinoside from *Alstroemeria* (Tatsuzawa et al., 2003) (method 1) and acetic acid (Wako Pure Chemical Industries) (method 2).

*Aglycone, glucose, rhamnose, and acetic acid of pigment **8***

Acid hydrolysis of **8** (ca. 0.5 mg) was performed with 2 N HCl (1 mL) at 90°C for 2 h. The hydrolysates were used for the analysis of UV-vis, TLC, and HPLC with authentic pelargonidin (method 1), acetic acid (method 2), glucose, and rhamnose (Wako Pure Chemical Industries).

*Known anthocyanins (pigments **1**–**7** and **9**)*

Pigments **1**–**7** and **9** of *Saintpaulia* cultivars were easily identified to be pelargonidin 3-rutinoside-5-glucoside [*R*t (min) 14.9, method 1], peonidin 3-rutinoside-5-glucoside [*R*t (min) 16.8, method 1], malvidin 3-rutinoside-5-glucoside [*R*t (min) 18.0, method 1], pelargonidin 3-acetyl-rutinoside-5-glucoside [pelargonidin 3-(6-(4-(acetyl)-rhamnosyl)-glucoside)-5-glucoside; *R*t (min) 21.4, method 1], peonidin 3-acetyl-rutinoside-5-glucoside [peonidin 3-(6-(4-(acetyl)-rhamnosyl)-glucoside)-5-glucoside; *R*t (min) 22.6, method 1], malvidin 3-acetyl-rutinoside-5-glucoside [malvidin 3-(6-(4-(acetyl)-rhamnosyl)-glucoside)-5-glucoside; *R*t (min) 23.1, method 1], cyanidin 3-acetyl-rutinoside [cyanidin 3-(6-(4-(acetyl)-rhamnosyl)-glucoside); *R*t (min) 24.1, method 1], and peonidin 3-acetyl-rutinoside [peonidin 3-(6-(4-(acetyl)-rhamnosyl)-glucoside); *R*t (min) 28.1, method 1], respectively, when compared by HPLC with authentic samples obtained from *S.* 'Thamires' and *S.* 'Tomoko' (Tatsuzawa et al., 2012, 2015).

Quantitative analyses of pigments in the flowers

Dried petals (ca. 5 mg) from each cultivar were immersed in 5% HOAc or 5% formic acid (1 mL) at 25°C for 2 h and the pigments were extracted for HPLC analysis of anthocyanin distribution. In addition, as a similar process, dried petals (2.5 mg) of each cultivar were immersed in 0.1% HCl-MeOH (10 mL) at 4°C for 24 h and extracted for quantitative analysis of total anthocyanins and other flavonoids. The total anthocyanins and other flavonoids in these flowers were measured using a UV-Vis spectrophotometer (MPS-2450; Shimadzu). The main visible absorption maximum and its absorbance for anthocyanins and the main UV absorption maximum and its absorbance for other flavonoids of each cultivar were measured.

Results and Discussion

1. Analysis of flower colors of Saintpaulia cultivars

The flower colors of the sixteen *Saintpaulia* cultivars fell into three groups of violet-blue (Violet-Blue N89B–Violet-Blue N94B by the R.H.S. Colour Chart and chromaticity values of b*/a* = −2.61–−1.72 by CM-700d), purple-violet (Purple-Violet N80B–Purple-Violet N82A, b*/a* = −1.06–−0.53), and red-purple (Red-Purple 60D–Red-Purple N74C, b*/a* = −0.44–0.13) (Table 1).

Table 1. Flower color and spectral data of fresh flowers of *Saintpaulia* cultivars.

Group & No.		Cultivars	L*	b*/a*[z]	RHSCC[y]	λ max (nm)[x]			
A	1	Tamiko	56.35	–2.62	VB 94B	(624)	577	(546)	(511)
	2	Thamires (Solid blue)[w]	41.47	–1.75	VB N89B	(624)	577	(546)	(511)
	3	Taro	41.17	–1.74	VB N89B	(624)	577	(546)	(511)
	4	Maiko	31.92	–1.72	VB N89A	(624)	577	(546)	(511)
B	5	Seren	47.39	–1.06	PV N82A	(624)	(570)	542	(511)
	6	Thamires (Deep purple)[v]	50.85	–0.81	PV N80B	(624)	(570)	544	(511)
C	7	Dolly	46.14	–0.69	PV N80B			557	
	8	Akira	40.10	–0.53	PV N81A			555	
D	9	Thamires (Solid pink)[v]	59.28	–0.44	RP N74C		(580)	542	(511)
	10	Kaname	62.63	–0.34	RP N74C		(580)	540	(511)
	11	Indiana	63.16	–0.31	RP 73A		(580)	540	(511)
	12	Mina	56.35	–0.27	RP N74B		(580)	539	(511)
E	13	Georgia	53.03	–0.03	RP 60D			540	
	14	Jessica	53.03	–0.02	RP 71D			540	
F	15	Tomoko[u]	39.84	0.04	RP 61A			548	
	16	Ares	40.44	0.13	RP 71A			548	

[z] Hue (CIE).
[y] RHS Colour Chart 5th edition (The Royal Horticultural Society). VB = Violet-Blue, PV = Purple-Violet, RP = Red-Purple.
[x] ( ) = shoulder.
[w] Tatsuzawa et al. (2012).
[v] Sato et al. (2011).
[u] Tatsuzawa et al. (2015).

2. Light absorption spectral data of intact flowers of these cultivars

The absorption spectral curves of fresh petals of these cultivars were measured in the range of 400–700 nm and the results are given in Table 1. As shown in Table 1, the light absorption spectral data of these fresh petals fell into six types (Fig. 1) as follows: the first type exhibited marked shoulders at 624 and 546 nm, a weak shoulder at 511 nm, and one λmax at 577 nm, the second type exhibited marked shoulders at 624 and 570 nm, a weak shoulder at 511 nm, and one λmax in the region of 542–544 nm, the third type exhibited one λmax in the region of 555–557 nm, the fourth type exhibited weak shoulders at 580 and 511 nm and one λmax in the region of 539–542 nm, the fifth type exhibited one λmax at 540 nm, and the last exhibited one λmax at 548 nm.

3. Analysis of anthocyanin pigments

In a survey of the sixteen cultivars of *Saintpaulia* by HPLC analysis, 9 anthocyanin peaks were identified and six of them were observed as major ones (Table 2). Pigment (**1**–**9**) peaks were observed in extracts of the flowers of 16 cultivars by HPLC analysis. Among these pigments, **6** was dominantly distributed in violet-blue cultivars of 'Tamiko', 'Thamires' (Solid Blue), 'Taro', and 'Maiko' and in purple-violet cultivars of 'Seren' and 'Thamires' (Deep Purple); **5** was most dominantly distributed in purple-violet cultivars of 'Dolly' and 'Akira'; **4** was most dominantly distributed in red-purple cultivars of 'Thamires' (Solid Pink), 'Kaname', 'Indiana', and 'Mina'; **8** was most dominantly distributed in red-purple cultivars of 'Georgia' and 'Jessica'; and **9** was most dominantly distributed in red-purple cultivars of 'Tomoko' and 'Ares' (Table 2). HPLC data

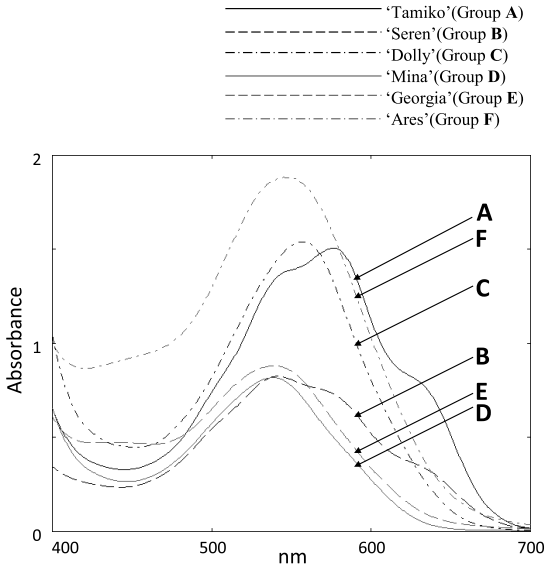

Fig. 1. Six absorption spectral curve types of fresh petals of *Saintpaulia* cultivars.

Table 2. Distribution of anthocyanins in *Saintpaulia*.

Group & No.[z]		Cultivars[z]	HPLC data of anthocyanins (as %)[y]								
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
A	1	Tamiko		0.8	1.1	0.4	4.9	86.4			
	2	Thamires (Solid blue)	0.2	0.1	1.1	7.9	6.7	77.9			
	3	Taro	0.1	0.5	1.3	2.6	9.0	81.4			
	4	Maiko	0.1	0.2	1.6	1.3	8.0	81.3			
B	5	Seren	0.1	2.2	3.8	3.2	24.9	50.4			
	6	Thamires (Deep purple)	0.7	0.2	1.6	27.5	13.1	51.3			
C	7	Dolly		0.7		0.3	85.1	0.1			
	8	Akira		1.2	0.5	0.7	66.9	17.2			1.0
D	9	Thamires (Solid pink)	1.2	0.5	0.3	50.0	27.4	10.1			
	10	Kaname	1.9	0.3	0.2	74.0	11.0	3.7			0.1
	11	Indiana	2.6			76.3	9.4	0.1			
	12	Mina	2.0	0.8	0.2	59.6	29.2	0.1			
E	13	Georgia				1.1	1.0	2.9	2.8	67.2	10.6
	14	Jessica				0.1	0.1	3.5	0.5	73.2	9.0
F	15	Tomoko				1.0	2.0	1.5	10.4	0.1	81.3
	16	Ares				0.4	0.4	0.4	19.4	0.8	66.6

[z] Group & No. and cultivars are the same as in Tables 1 and 3.
[y] Dried flowers (5 mg) were extracted with 5% HOAc (1 mL) at 25°C for 2 hours.
1: pelargonidin 3-rutinoside-5-glucoside.
2: peonidin 3-rutinoside-5-glucoside.
3: malvidin 3-rutinoside-5-glucoside.
4: pelargonidin 3-acetyl-rutinoside-5-glucoside.
5: peonidin 3-acetyl-rutinoside-5-glucoside.
6: malvidin 3-acetyl-rutinoside-5-glucoside.
7: cyanidin 3-acetyl-rutinoside.
8: pelargonidin 3-acetyl-rutinoside.
9: peonidin 3-acetyl-rutinoside.

of known pigments **1**–**7** and **9** are directly compared with authentic anthocyanins (Tatsuzawa et al., 2012, 2015) and are summarized in Materials and Methods.

The structure of **8** has not been reported until now. The identification of **8** was initially performed by acid and alkaline hydrolysis. From the results of acid hydrolysis, **8** was composed of pelargonidin, glucose, rhamnose, and acetic acid (see Materials and Methods). Moreover, from the results of alkaline hydrolysis, **8** was composed of pelargonidin 3-rutinoside and acetic acid (see Materials and Methods). From these results, **8** was assumed to be acetyl-pelargonidin 3-rutinoside.

The detailed structure of **8** was determined by analysis of its high-resolution FAB mass spectra (HR-FABMS) and NMR spectra as follows.

The molecular ion $[M]^+$ of **8** was observed at *m/z* 621 ($C_{29}H_{33}O_{15}$) using FABMS, indicating that **8** is composed of pelargonidin with one molecule each of glucose, rhamnose, and acetic acid. These elemental components of **8** were confirmed by measuring its HR-FABMS (calc $C_{29}H_{33}O_{15}$: 621.1819. Found: 621.1802).

The structure of **8** was further elucidated on the basis of analysis of its ^1H and ^{13}C NMR spectra, including 2D COSY, 2D NOESY, HMQC, and HMBC spectra, according to the process described previously (Tatsuzawa et al., 2015).

The chemical shifts of seven aromatic protons of the pelargonidin moiety with their coupling constants were assigned on the basis of the analysis of the 2D COSY spectrum (see Materials and Methods). The signals of two anomeric protons of sugar moieties in **8** appeared at δ 5.37 (*d*, *J* = 7.6 Hz, Glucose) and δ 4.60 (*s*, Rhamnose), and the chemical shifts of other sugar protons were assigned by analysis of the 2D COSY spectrum with their coupling constants (see Materials and Methods), indicating that the glucose residues of **8** must be β-glucopyranose. In the rhamnose moiety, the singlet signal corresponds to an anomeric proton (δ 4.60, *s*) and doublet signals of methyl protons (δ 0.89, *d*, *J* = 6.6 Hz) at C-5 suggested the presence of α-rhamnopyranose. The signal of the anomeric proton of glucose correlated with that of the C-3 carbon (δ 144.3) of pelargonidin in the HMBC spectrum and also to the signal of the H-4 proton (δ 8.90) in the NOESY spectrum of pelargonidin. These characteristic features revealed that the OH-3 position of pelargonidin is glycosylated by glucose. The signal of the anomeric proton of rhamnose correlated with that of the C-6 carbon (δ 66.7) in the HMBC spectrum and to the signal of H-6a and b protons (δ 3.57 and 3.89) in the NOESY spectrum of glucose. Therefore, rhamnose was bonded with glucose at OH-6 of glucose forming rutinose in the pigment. The proton signal of the H-4 of rhamnose (δ 4.72, *t*, *J* = 9.8 Hz) was shifted downfield (see Materials and Methods), indicating that the OH-4 of rhamnose is acylated with acetic acid. This linkage was further confirmed by HMBC correlation (Fig. 2). Consequently, the structure of **8** was elucidated to be pelargonidin 3-*O*-[6-*O*-(4-*O*-(acetyl)-α-rhamnopyranosyl)-β-glucopyranoside] (Fig. 2), which is a new anthocyanin in plants (Andersen and Jordheim, 2006; Harborne and Baxter, 1999; Veitch and Grayer, 2008, 2011).

4. Distribution of anthocyanins and their flower colors in 16 cultivars of Saintpaulia

Recently, we have reported the intermolecular co-pigmentation effect of anthocyanin and flavon to form flower colors for violet-blue flower cultivars of *Saintpaulia* (Tatsuzawa et al., 2012). In the present study, this effect was roughly confirmed by calculating the ratio of absorbance of λmax of the ultraviolet region (F) to that of the visible region (A) (the F/A value). We found that the F/A values of 4 violet-blue flower cultivars were 1.7–6.4 (Table 3). Therefore, it was considered that intermolecular co-pigmentation was effective in this range for violet-blue flower cultivars. In the 4 purple-violet and 8 red-purple flower cultivars, the F/A values were 1.7–6.2 (Table 3), and this range is almost equal to that of violet-blue flower cultivars. From the aforementioned data, a comparative study of the purple-violet and red-purple flower colors of *Saintpaulia* was possible using the distribution pattern of acetylated anthocyanins, except for the intermolecular co-pigmentation effect.

The sixteen cultivars of *Saintpaulia* used in this study were classified into six groups, **A**–**F**, depending on their flower colors and anthocyanin components (Tables 1 and 2; Fig. 3). In group **A**, the flowers of cultivars were violet-blue (VB N89B–VB 94B) on the R.H.S. CC and their hue values (b*/a*) = −2.61–−1.72 by CM-700d. As their major anthocyanin, **6** (average value of four cultivars: 81.8%) was identified to be malvidin 3-acetyl-rutinoside-5-glucoside. In group **B**, the flowers were purple-violet (PV N82A and PV N80B) with hue values = −1.06 and −0.81. In this group, **6** (average

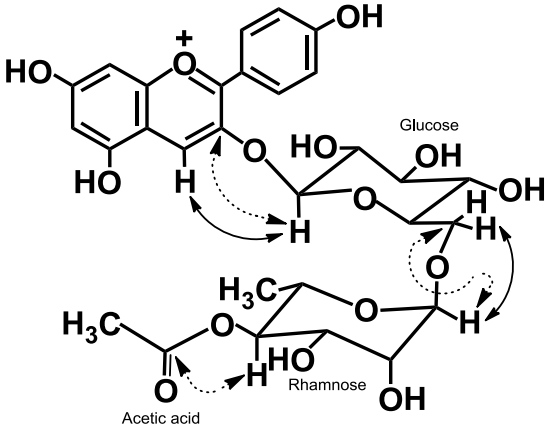

Fig. 2. New anthocyanin (pigment **8**) from the flowers of *Saintpaulia* cultivars. Observed main NOE correlations are indicated by arrows. Observed main HMBC correlations are indicated by dotted arrows.

Table 3. Absorption maxima and their absorbances of *Saintpaulia* cultivars in 0.1% HCl-MeOH[z].

Group & No.[y]		Cultivars[y]	Visible (Vis) region		Ultraviolet (UV) region		UV/Vis
			λ max (nm)	ABS(A)[x]	λ max (nm)	ABS(F)[w]	F/A[v]
A	1	Tamiko	539	0.169	323	1.083	6.4
	2	Thamires (Solid blue)	536	0.383	324	1.073	2.8
	3	Taro	539	0.378	323	0.994	2.6
	4	Maiko	539	0.545	323	0.949	1.7
B	5	Seren	533	0.380	325	0.665	1.8
	6	Thamires (Deep purple)	520	0.130	321	0.803	6.2
C	7	Dolly	528	0.273	330	0.981	3.6
	8	Akira	530	0.278	328	0.476	1.7
D	9	Thamires (Solid pink)	511	0.245	324	1.116	4.6
	10	Kaname	511	0.442	327	0.855	1.9
	11	Indiana	511	0.188	326	0.951	5.1
	12	Mina	511	0.354	325	1.147	3.2
E	13	Georgia	514	0.195	322	0.964	4.9
	14	Jessica	514	0.268	323	1.066	4.0
F	15	Tomoko	531	0.542	329	1.294	2.4
	16	Ares	531	0.311	329	0.720	2.3

[z] Dried flowers (2.5 mg) were extracted with 0.1% HCl-MeOH (10 mL) at 4°C for 24 hours. UV-Vis spectra of extracts (1.0 mL) were recorded on an MPS-2450 from 200 to 700 nm.
[y] Group & No. and cultivars are the same as in Tables 1 and 2.
[x] ABS(A): absorbance of λ max of visible region.
[w] ABS(F): absorbance of λ max of ultraviolet region.
[v] F/A: ABS(F)/ABS(A).

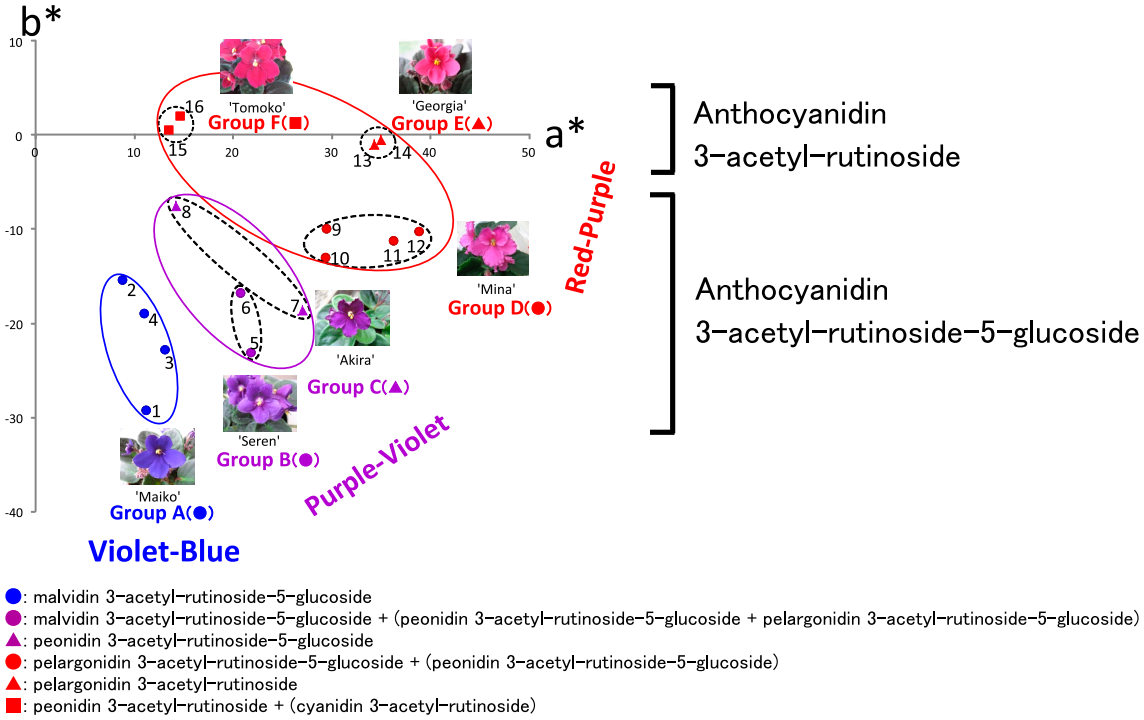

Fig. 3. Distribution of flower colors in *Saintpaulia* cultivars. CIE L*a*b* chromaticity diagram. Group and number are the same as in Tables 1–3.

value of two cultivars: 50.9%) was observed as their major anthocyanin, along with **4** (pelargonidin 3-acetyl-rutinoside-5-glucoside) and **5** (peonidin 3-acetyl-rutinoside-5-glucoside) as the 2nd and 3rd most abundant anthocyanins. In group **C**, the flowers were purple-violet (PV N80B and PV N81A) with hue values = −0.69 and −0.53. The major anthocyanin of this group was **5** (average value of two cultivars: 76.0%). In group **D**, the flowers were red-purple (RP 73A–RP N74B) with hue values = −0.44–−0.27. The major anthocyanin of this group was **4** (average value of four cultivars: 65.0%), along with **5** as the 2nd most abundant anthocyanin. In group **E**, the flowers were red-purple (RP 60D and RP 71D) with hue values = −0.03

and −0.02. As their major anthocyanin, **8** (average value of two cultivars: 70.2%) was identified to be pelargonidin 3-acetyl-rutinoside. In group **F**, the flowers were red-purple (RP 61A and RP 71A) with hue values = 0.04 and 0.13. As their major anthocyanin, **9** (average value of two cultivars: 74.0%) was identified to be peonidin 3-acetyl-rutinoside, along with **7** (cyanidin 3-acetyl-rutinoside) as the 2nd most abundant anthocyanin.

The flower colors of group **B** were very similar to those of group **C** (Fig. 3); however, their anthocyanin constitutions were clearly distinguished by HPLC data (Table 2). The purple-violet flower colors of group **B** were produced by **6**, which coexisted with **4** and **5**; however, the purple-violet flower colors of group **C** were mainly produced by **5** (Table 2).

The flower colors of groups **D** and **E** were the same red-purple by R.H.S. CC (Table 1; Fig. 3) and included the same pelargonidin-based anthocyanin as the major component; however, their anthocyanin constitutions were clearly distinguished by the HPLC data (Table 2). Moreover, groups **C** and **F** included the same peonidin-based anthocyanin as the major component; however, their anthocyanin constitutions were clearly distinguished by the HPLC data (Table 2). When the major anthocyanin structures of groups **C**, **D**, **E**, and **F** are compared, the major anthocyanin structures of groups **E** and **F** are 5-OH free anthocyanins (anthocyanidin 3-acetyl-rutinoside); in contrast, the anthocyanin structures of groups **D** and **C** are not 5-OH free anthocyanins (anthocyanidin 3-acetyl-rutinoside-5-glucoside) (Fig. 3). The absorption spectral curves of fresh petals of these cultivars are shown in the region of 400–700 nm in Figure 1. In the region of 400–500 nm of the absorption curve, [**E** and **F** is higher than that of group **D** and **C**, respectively] (Fig. 1). Therefore, it was thought that the flower colors of groups **E** and **F** were more duskish and reddish than those of groups **D** and **C**, respectively, similar to the duskish and reddish appearance of morning glory (Saito et al., 1998; Yoshida et al., 2003) (Table 1; Fig. 3).

From these results, the glucosylation of 5-OH in anthocyanidin 3-acetyl-rutinoside and an increase in the methylation of the B-ring in anthocyanidin are considered to be responsible for the bathochromic shifts of the absorption maxima in spectral curves of intact flowers (Fig. 1). This was thought to be the most important effect on flower colors in these *Saintpaulia* cultivars.

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