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THE FIRST SYNTHESIS OF 3-*O*-METHYLCYANIDIN AND THE EFFECT OF 3-*O*-SUBSTITUTION ON STABILITY UNDER ACIDIC CONDITIONS

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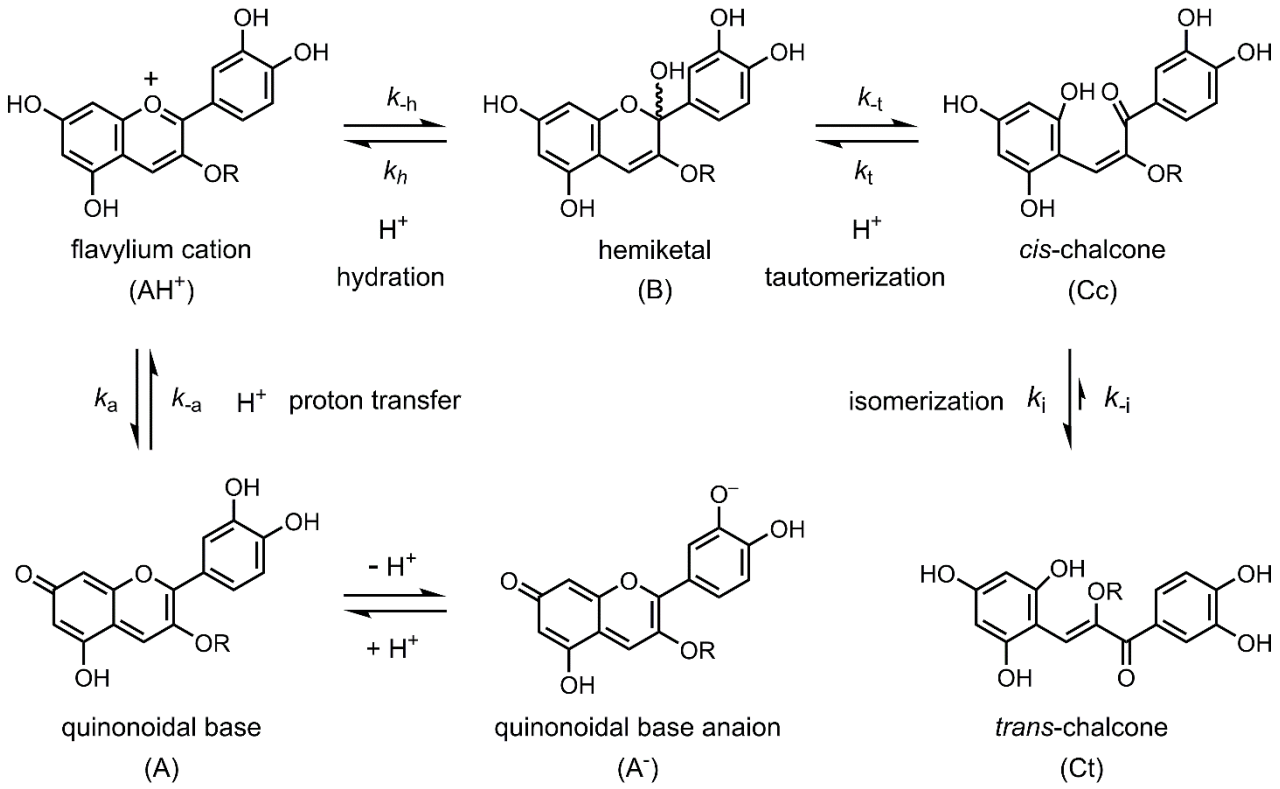
Abstract – The simplest and most common anthocyanin in nature is 3-*O*-glucosylcyanidin (**1**), and 3-*O*-glucosylation is believed to stabilize the chromophore. To clarify the effect of the glucose residue we compared the stability of **1** with its aglycone, cyanidin (**2**), and newly synthesized 3-*O*-methylcyanidin (**3**). In an aqueous solution at pH 1, **1** and **3** showed similar stabilities, and **2** was less stable than **1** and **3**, indicating that 3-*O*-substituion does enhance stability. We also analyzed the co-pigmentation effect of flavocommelin (**4**) and rutin (**5**), on the color and stability of 3-*O*-substituted cyanidins and cyanidin. The bathochromic shift of λvismax and stability of the color by addition of **4** was greater than that of rutin (**5**). **4** might stack closer and stronger to the anthocyanidin chromophore than **5**.

Dedicated to Professor Kiyoshi Tomioka on his 70th birthday

INTRODUCTION

Anthocyanins are plant pigments common in flowers, leaves, fruits, and roots of higher plants, and they exhibit a wide variety of colors from red and purple to blue.[1] These pigments have been used not only as safe food colorants[2] but also in several applications related to the prevention of life-style related diseases in humans such as hypertension, hyperlipidemia, arteriosclerosis, and diabetes.[3] Furthermore, studies on new materials for dye-sensitized solar cells have been carried out.[4]

The structures of anthocyanins vary in the number of hydroxy and methoxy groups in the chromophore,

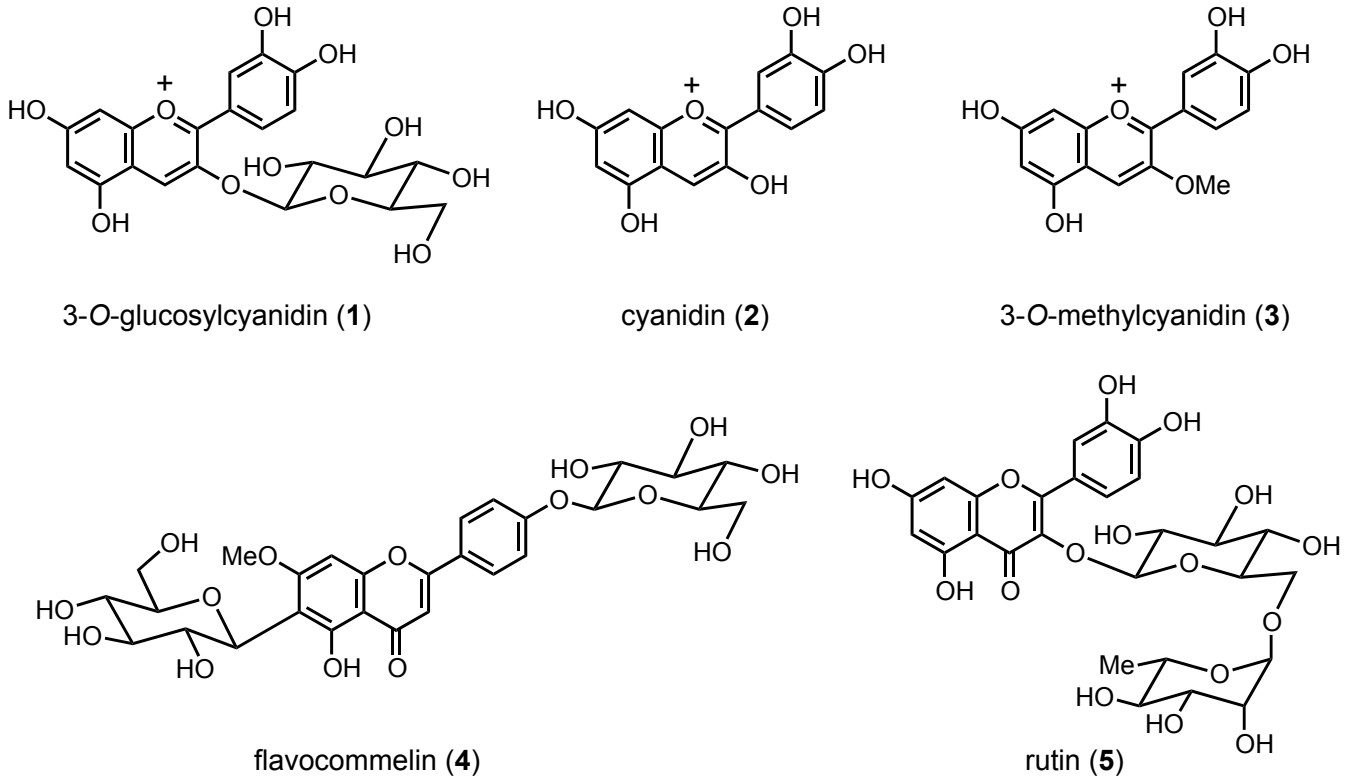


Scheme 1. The equilibrium of 3-*O*-substituted cyanidin in aqueous solution

anthocyanidin, and natural anthocyanins contain at least one sugar residue. The OH groups of the sugars are further substituted with sugars and/or acyl residues, and to date, more than 700 anthocyanins have been isolated.[1d] However, 3-*O*-monoglucosyl and 3,5-*O*-diglucosyl pigments without any acyl moieties are more widely distributed in plants. The main drawback of these simple anthocyanins for industrial applications is the instability of the color.

The structures of anthocyanins in aqueous solutions reach an equilibrium that depends on pH (Scheme 1).[5] The chemical form changes from a red flavylium cation (AH^+) under strong acidic conditions, to a purple quinonoidal base (A) in neutral solutions, and a blue quinonoidal base anion (A^-) under basic conditions. In weakly acidic to neutral pH conditions, hydration of AH^+ forms a colorless hemiketal (B), which can undergo ring-opening to give the *cis*-chalcone (Cc), and Cc can thermally isomerize to *trans*-chalcone (Ct).[6] To inhibit hydration, the anthocyanins can be present in several molecular stacking forms, such as intermolecular and intramolecular associations in plant vacuoles.[1b,1e,7] In addition to stabilization by molecular stacking, the attachment of sugars to the chromophore also has a stabilizing effect.[8] To clarify the function of the sugar substituents in simple anthocyanins, we compared the stability of 3-*O*-glucosylcyanidin (**1**), the most abundant anthocyanin in nature, with its aglycone, cyanidin (**2**),

and synthesized 3-*O*-methylcyanidin (**3**, Scheme 2). In weakly acidic environments the hydration reaction of these simple anthocyanins is too fast to analyze precisely, therefore, we tested their stabilities at pH 1. Furthermore, we studied the stabilizing effect of co-pigments, flavocommelin (**4**) and rutin (**5**), on these pigments (Scheme 2).



Scheme 2. Structures of studied anthocyanins, anthocyanidin and co-pigments

RESULTS AND DISCUSSION

Preparation of 3-*O*-glucosylcyanidin (1) and cyanidin (2)

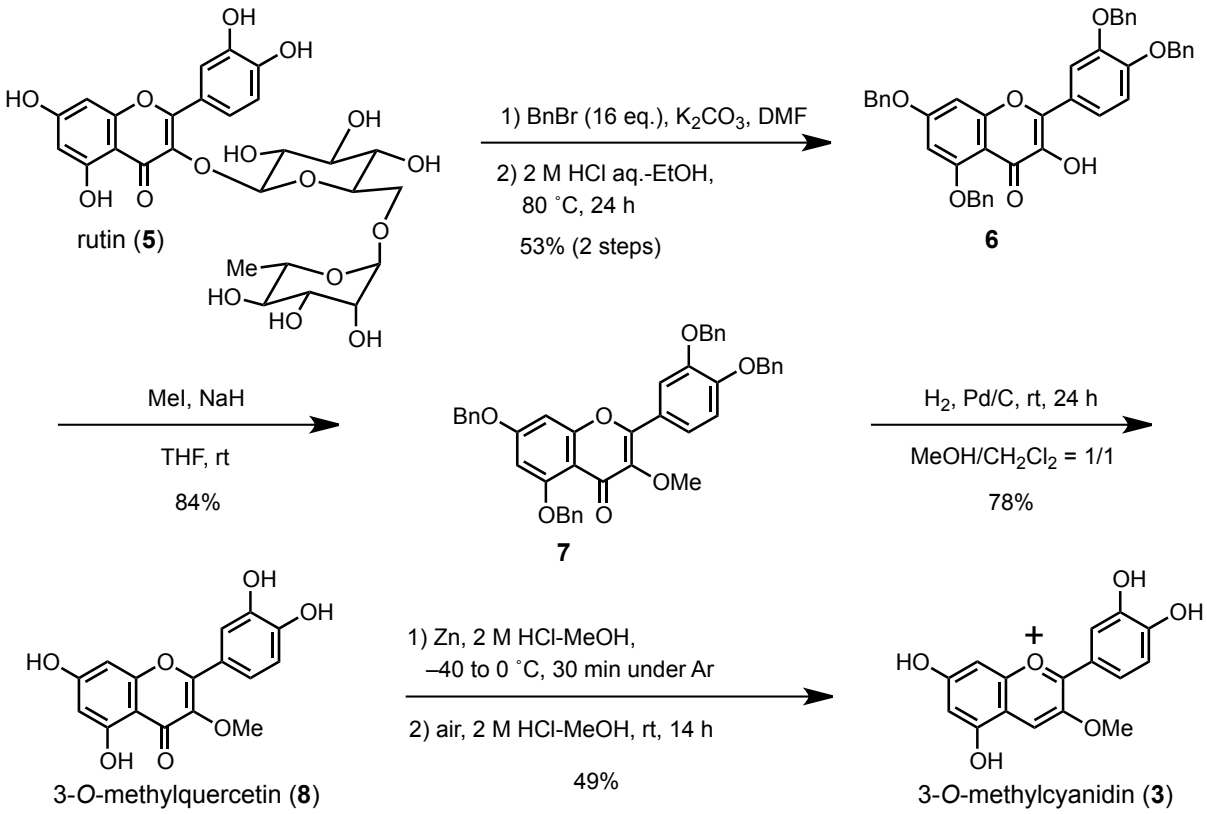
3-*O*-Glucosylcyanidin (**1**) is a major pigment in black soybean, *Glycine max*; thus, **1** was extracted and purified using our previously reported procedure.[9] Cyanidin (**2**) was obtained by acidic hydrolysis of **1**. **1** was dissolved in 6 M HCl aq.-MeOH and heated at 60 °C for 23 h, then at 80 °C for 3 h. Water was added to the resulting mixture, and then the reaction was purified by using Amberlite XAD-7 column chromatography to afford **2** as a dark red mass (yield: 88%)

Synthesis of 3-*O*-methylcyanidin

Calculated chemical properties of 3-*O*-methylcyanidin (**3**) have been reported,[10] but the compound has not been found in nature nor has it been synthesized until now. For the synthesis of **3**, we planned a retro-synthetic route from commercially available rutin (**5**). The phenolic OHs were protected, and then the rutinose residue was hydrolyzed. Next, the 3-OH group was methylated. After global deprotection, the

obtained 3-*O*-methylquercetin was converted to 3-*O*-methylcyanidin (**3**) by using a sequential Zn reduction-air oxidation procedure.[4e,11]

The synthesis of **3** was carried out as shown in Scheme 3. Benzylation of rutin (**5**) with 16 equivalents of BnBr and K_2CO_3 in DMF followed by hydrolysis with HCl aq.-EtOH afforded flavonol **6** in 53% yield over two steps.[12] Fewer equivalent of BnBr gave a mixture of 5,7,3',4'-*tetra*-benzylquercetin (**6**) and 7,3',4'-*tri*-*O*-benzylquercetin. The 5-hydroxyl group might be less reactive due to neighboring ortho-carboxyl group participation. Because the separation of **6** from 7,3'4'-*tri*-*O*-benzylquercetin is difficult, the completion of the benzylation is quite important for obtaining a high yield. Methylation of **6** with MeI and NaH in THF afforded **7** in 84% yield. Hydrogenation of **7** in a mixture of MeOH and CH_2Cl_2 for 24 h afforded 3-*O*-methylquercetin (**8**) in 78% yield.



Scheme 3. Synthesis of 3-*O*-methylcyanidin (**3**) from rutin (**5**)

With the requisite 3-*O*-methylquercetin (**8**) in hand, we converted **8** to 3-*O*-methylcyanidin (**3**) by using Zn powder and air oxidation.[4e,11] To a suspension of 3-*O*-methylquercetin (**8**) and Zn powder in anhydrous MeOH was added 2 M HCl-MeOH at –40 °C under Ar. The reaction mixture was gradually heated from –40 °C to 0 °C over 30 min to give flavenols (Figure 1-B). The MS spectroscopic data of these flavenols were consistent with the theoretical values (HR-ESI-Q-TOF-MS for flavenols: Calcd for $C_{16}H_{15}O_6$

$[M+H]^+$ 303.0863, Found 303.0865). Subsequently, the mixture of flavenols in 2 M HCl-MeOH was stirred at rt under air. When the reaction mixture was exposed to air for 14 h, the flavenols were completely consumed, and the desired 3-*O*-methylcyanidin (**3**) was afforded cleanly (Figure 1-C). After purification by silica gel column chromatography,[4e] pure 3-*O*-methylcyanidin (**3**) was obtained in 49% yield (Figure 1-D).

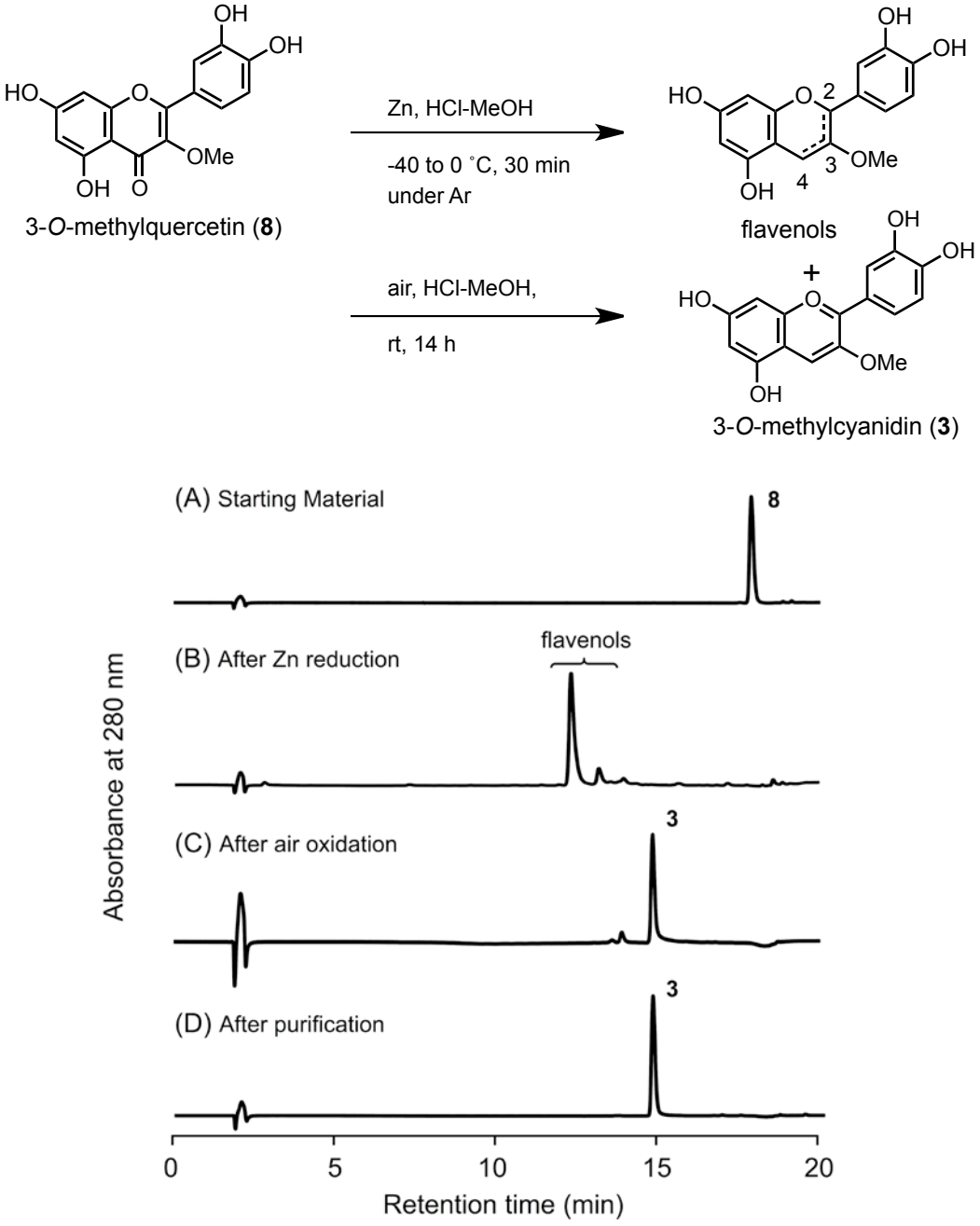


Figure 1. HPLC chromatograms of the transformation of 3-*O*-methylquercetin (**8**) to 3-*O*-methylcyanidin (**3**). (A): The starting material **8**. (B): The reaction mixture of the Zn reduction after 30 min. (C): The reaction mixture of the air oxidation after 14 h. (D): Compound **3** after purification by silica gel column chromatography.

The effect of 3-*O*-substitutions on the stability

Because we would like to focus the stability of **1**-**3** from the perspective of hydration to the flavylium cation form, the experiments were carried out in a buffered solution at pH 1. The stock solution of anthocyanins and anthocyanidin (**1**-**3**) was diluted with the buffer solution to give a final concentration of 0.05 mM, and the obtained pigment solution was kept at rt in the dark, and the UV-Vis spectrum of each solution was measured. The effects of the co-pigments were studied by adding 1 to 10 equivalents of **4** or **5** to **1**-**3**. The time-dependent change in the absorbance (λvismax) was recorded for 20 days, and the results are summarized in Table 1 and Figure 2A.

Table 1. The color and stability of **1**, **2** and **3** and their degradation kinetics parameter at pH 1

pigment	co-pigment	eq. to pigment	λmax (nm)[a]	Abs[a]	stability (%)[b]	k (d^{-1})	$t_{1/2}$ (d)	R^2
1	-	-	510	1.14	97	0.001	525	98
1	**4**	1	511	1.11	97	0.001	537	99
1	**4**	5	516	1.04	98	0.001	686	95
1	**4**	10	520	0.97	99	0.001	785	71
1	**5**	1	512	1.04	96	0.002	431	95
1	**5**	5	516	0.98	96	0.002	453	78
2	-	-	515	1.41	5	0.144	5	99
2	**4**	1	517	1.36	6	0.134	5	99
2	**4**	5	521	1.31	13	0.093	7	99
2	**4**	10	526	1.25	20	0.072	10	99
2	**5**	1	516	1.38	4	0.158	4	100
2	**5**	5	521	1.24	7	0.123	6	99
3	-	-	516	1.07	90	0.006	122	93
3	**4**	1	519	1.04	86	0.006	123	91
3	**4**	5	525	0.96	92	0.004	177	81
3	**4**	10	532	0.92	95	0.003	217	81
3	**5**	1	519	1.06	91	0.005	142	86
3	**5**	5	525	0.97	89	0.005	154	81

a: The data at day 0. b: Calculated from the ratio of abs at day 20 to abs at day 0.

The λvismax of 3-*O*-glucosylcyanidin (**1**) was 510 nm and those of cyanidin (**2**) and 3-*O*-methylcyanidin (**3**) were 515 nm and 516 nm, respectively (Table 1). The difference might be due to the hydrophobicity of the pigment. As shown in Figure 2A and Table 1, 3-*O*-glucosylcyanidin (**1**) and 3-*O*-methylcyanidin (**3**)

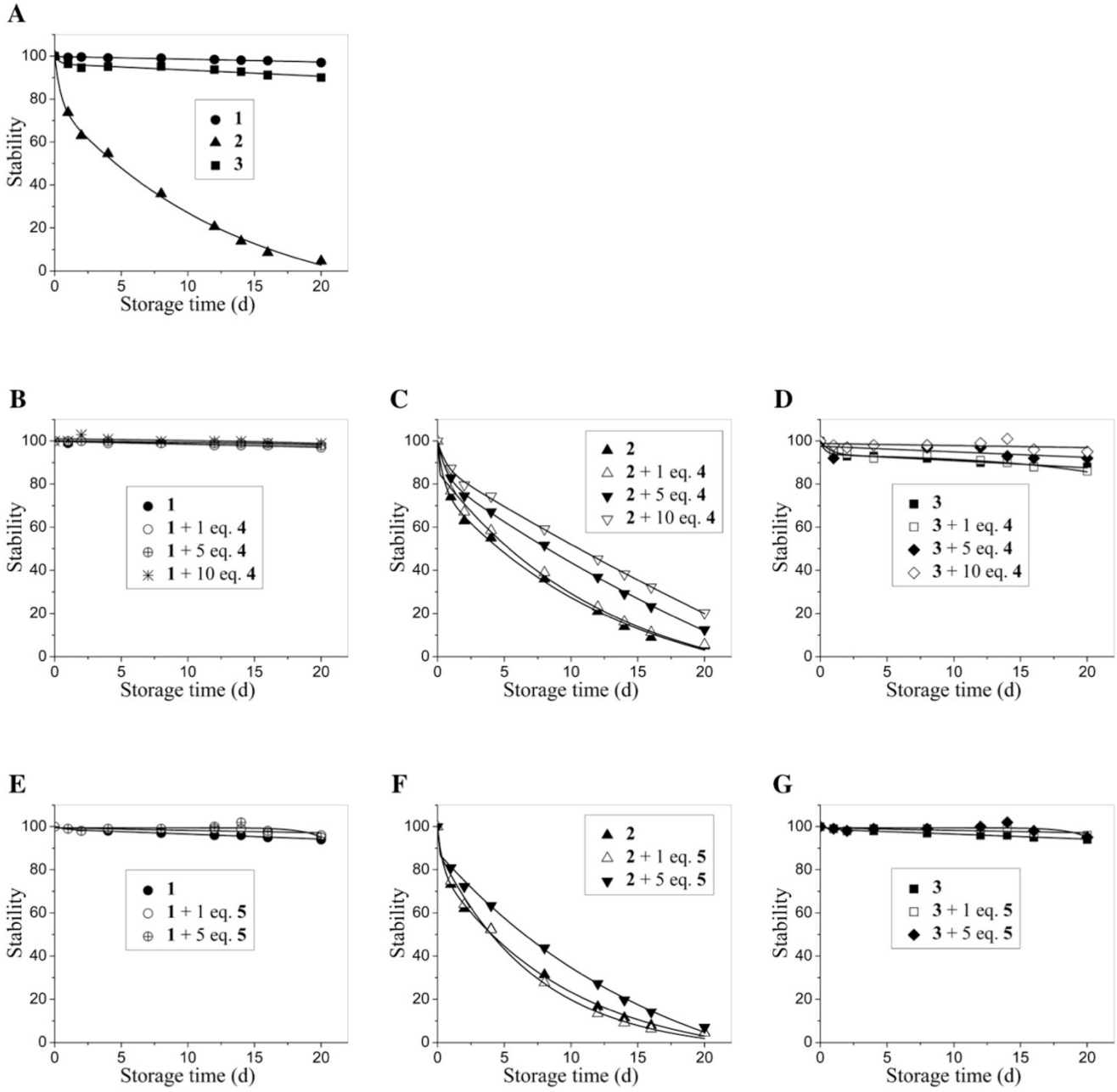


Figure 2. Stability of 3-*O*-glucosylcyanidin (**1**), cyanidin (**2**) and 3-*O*-methylcyanidin (**3**) w/wo flavocommelin (**4**) or rutin (**5**) in aq. buffered solution at pH 1 (200 mM KCl aq.-HCl, cell length: 10 mm, rt). The stability was indicated as the ratio of absorbance (A_t/A_0) at λvismax. A: pigment only, B, C, D: pigment with 1-10 eq. of **4**, E, F, G: pigment with 1 and 5 eq. of **5**.

were very stable under acidic conditions, and 97% and 90%, respectively, of the red colors of each solution remained after 20 days of storage. In contrast, cyanidin (**2**) was unstable, and the solution became colorless (5%) after 20 days, which is consistent with a previous studiy.[8a]

The degradation kinetics constant (k) and half-life ($t_{1/2}$) of 3-*O*-substituted cyanidins showed the same trend. The 3-*O*-substitution of the cyanidin chromophore reduced the k value and increased the $t_{1/2}$ to 0.001 d^{-1} and 525 d, respectively, for **1** and 0.006 d^{-1} and 122 d, respectively, for **3**. Compound **2** was

found to have the highest degradation rate ($k = 0.144\ d^{-1}$ and $t_{1/2} = 5$ d). The instability of **2** might suggest that the *cis*-chalcone form of **2**, obtained by hydration, easily isomerizes to the 3-keto structure by keto-enol tautomerization. In contrast, 3-*O*-glycosyl and 3-*O*-methycyanidin cannot generate such structures. This may be the major reason for the enhanced stability of 3-*O*-substitution, and the difference between the stabilities of **1** and **3** may be due to the steric hinderance and the number of hydrogen bonds of the 3-*O* residue.

The effect of co-pigments on the stability

It is well known that anthocyanin is stabilized with co-existence of other compounds such as flavonoids, amino acids, and organic acids. This phenomenon, co-pigmentation, causes bathochromic shifts with hyperchromic effects of anthocyanins.[1b,1e,7,10,13] Therefore, we also studied the stability of **1**-**3** with co-pigments. Flavocommelin (**4**) is a unique co-pigment found in commelinin, a metalloanthocyanin in blue dayflower, *Commelina communis*.[14] **4** is freely soluble in water and exhibits a very strong co-pigmentation effect.[14c,1b] Rutin (**5**) is commercially available and commonly used as a co-pigment;[13c,15] thus, we compared the effects of **4** and **5** in this study. The effects of different equivalents of **4** and **5** on the stabilities of **1**-**3** are displayed in Table 1 and Figure 2B-G. Because of its poor solubility in water, the effect of 10 eq. of rutin (**5**) could not be studied. **4** and **5** caused obvious bathochromic shifts in the pigments; the addition of 5 eq. of **4** or **5** to **1** and **2** cause an approximately 5 nm shift to longer wavelength, and a 10 nm shift was seen following the addition of 5 eq. of **4** or **5** to **3**. When 10 eq. of **4** was added to **3**, a larger shift (15 nm) was observed. The absorbance of each solution was slightly decreased (Table 1), which is consistent with Quina and coworker's observation that increasing the molar ratio of ferulic acid added to pelargonin produced a clear redshift and a small decrease in absorbance under highly acidic conditions (pH 0.25).[15b]

Concerning the stability of the color, the addition of 5 and 10 eq. of **4** and **5** showed a co-pigmentation effect (Table 1, Figure 2B-G), but the addition of 1 eq. did not cause the typical effect. The effect of **4** was dose-dependent, and the $t_{1/2}$ values of **1** and **3** were increased by 50% and 75%, respectively, by the addition of 10 eq. of **4**. In contrast to the 3-*O*-substituted pigments **1** and **3**, the more unstable cyanidin (**2**) was also stabilized by the addition of **4** (Figure 2C). In the case of **2**, **4** was found to have a dose-dependent stabilizing effect (Figure 2C). The stabilizing effect of **5** was lower than that of **4** (Figure 2F). Based on the k and $t_{1/2}$ values, the co-pigment effect of **5** was less than half a strong as that of **4**. The difference in the co-pigment effects of **4** and **5** may be due to their structural difference; the glycosyl residues at 6 and 4' in **4** gave it greater water-solubility and made its aromatic chromophore stack more tightly with the anthocyanidin. The 3-*O*-rutinose residue in **5** is larger than the sugar residue in **4** and inhibit efficient hydrophobic interactions with the anthocyanidin chromophore.

In conclusion, we synthesized 3-*O*-methylcyanidin (**3**) and studied the effect of 3-*O*-substitution on its stability in an aqueous acidic solution at pH 1. Compared with cyanidin (**2**), 3-*O*-methylation has a stabilizing effect comparable to that of 3-*O*-glucoside, indicating that 3-*O*-substitution plays an important role on inhibiting hydration and/or irreversible decolorization. In this study, the co-pigmentation effects of flavocommelin (**4**) and rutin (**5**) on these three cyanidin-based pigments were compared. Flavocommelin (**4**) showed a stronger co-pigmentation effect than that of rutin (**5**). This might be due to the sterics of the compound and its water solubility.

EXPERIMENTAL

General Experimental Procedures.

Melting points were measured on a Yanaco Mp-S3 micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO FT/IR 6100 spectrometer, ultraviolet-visible (UV-Vis) absorption spectra on a JASCO V-560 spectrophotometer (cell length: 10 mm), and ^{1}H and ^{13}C NMR spectra on a JEOL JNM-ECA-500 spectrometer. Chemical shifts are reported in ppm on the δ scale relative to $CDCl_3$ (δ = 7.26 for ^{1}H NMR), $CDCl_3$ (δ = 77.0 for ^{13}C NMR), CD_3OD (δ = 3.31 for ^{1}H NMR), and CD_3OD (δ = 49.0 for ^{13}C NMR) as internal references. Signal patterns are indicated as s, singlet; d, doublet; m, multiplet. Elemental analysis was performed on YANACO MT-6 elemental analyser. High-resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-QII (ESI) spectrometer. Analytical thin-layer chromatography (TLC) was performed on Merck precoated analytical plates, 0.25 mm thick, silica gel 60 F254. The pH was adjusted to 1.0 by the pH meter model (HORIBA, Ltd). High performance liquid chromatography (HPLC) was performed with a system equipped with JASCO PU-1585 pumps, an MD-2018 detector, HG-1580-32 mixer, and DG-2080-53 degasser, JASCO 860-CO, JASCO. An ODS column (Develosil C30, 2.6 μm, 2.0 i.d × 150 mm, NOMURA CHEMICAL) was eluted at 40 °C with linear gradient elution (0.2 mL/min) over 20 min from 10% to 90% aq. MeCN solution containing 0.5% trifluoroacetic acid (TFA). Flash silica gel chromatography was performed using silica gel PSQ60B (Fuji Silysia Chemical). All reagents were purchased from commercial sources. All reactions were performed under an argon (Ar) atmosphere.

Preparation of pigments 1 and 3, and co-pigments 4 and 5

3-*O*-Glucosylcyanidin (**1**) was isolated from black soy bean (*Glycine max*) and purified by a combination of XAD-7 column chromatography and ODS-LC according to our procedure as TFA salt.[9] Cyanidin (**2**) was obtained by acidic hydrolysis of **1**. 3-*O*-Glucosylcyanidin (**1**, 20 mg, 35.6 μmole) was dissolved in 4 mL of 6 M HCl aq. The mixture was heated at 60 °C for 23 h, then the reaction temperature was raised to 80 °C and kept for 3 h. The reaction mixture was diluted with 10 mL of water, then, poured into an

Amberlite XAD-7 column (20 mm i.d. × 200 mm), which was pre-washed with 0.5% TFA aq. The column was washed with 60 mL of 0.5% TFA aq., then eluted with 50 mL of 0.5% TFA-MeOH. The red eluents were collected and evaporated under reduced pressure to be obtained pure **2** as a TFA salt (12.6 mg, 88%). Flavocommelin (**4**) was purified from blue petals of dayflower (*Commelina communis*) as reported previously.[14b] Rutin (**5**) was purchased from Nacalai Tesque Inc.

Synthesis of 3-*O*-methylcyanidin (3)

5,7,3',4'-Tetra-O-benzylquercetin (**6**)

To a solution of rutin (**5**) (5.00 g, 8.19 mmol) and K_2CO_3 (18.0 g, 131 mmol) in DMF (60 mL) was added BnBr (16 mL, 131 mmol) at rt. After stirring for 3 h at 60 ºC, 2 M NaOH aqueous solution (10 mL) was added to the reaction mixture at rt. After stirring for 5 min, the reaction mixture was concentrated under reduced pressure. The residue was added with saturated aq. $NaHCO_3$ (60 mL) and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in EtOH (6.7 mL) and 2 M HCl aq. (33.3 mL) at rt. After stirring for 24 h at 80 ºC, the reaction mixture was neutralized by 2 M NaOH and the solvents were evaporated. The residue was purified by flash silica gel column chromatography (20% AcOEt/Hexane to 50% AcOEt/Hexane) to give **6** as a yellow solid (2.86 g, 53%). mp 53–54 ºC, IR (KBr) 3450, 3030, 1618, 1510, 1452, 1258, 1197, 1166, 1015 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.12 (2H, s), 5.23 (2H, s), 5.24 (2H, s), 5.26 (2H, s), 6.48 (1H, d, J = 2.0 Hz), 6.59 (1H, d, J = 2.0 Hz), 7.03 (1H, d, J = 9.0 Hz), 7.3–7.6 (20H, m), 7.76 (1H, dd, J = 9.0, 2.0 Hz), 7.88 (1H, d, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 70.6, 70.7, 70.9, 71.5, 93.7, 97.5, 106.7, 114.2, 121.2, 124.3, 126.6, 127.2, 127.5, 127.6, 127.8, 127.9, 128.4, 128.5, 128.7, 128.8, 135.6, 136.1, 136.8, 137.1, 137.7, 141.9, 148.6, 150.1, 158.7, 159.4, 163.2, 171.7; ESI-TOF HRMS Calcd. For $C_{43}H_{35}O_7$ $[M+H]^+$, 663.2377; Found: 663.2378.

5,7,3',4'-Tetra-O-benzyl-3-O-methylquercetin (**7**)

To a solution of 7,5',3',4'-tetra-*O*-benzylquercetin (**6**) (200 mg, 0.3 mmol) and NaH (72 mg, 1.8 mmol, 60% oil dispersion) in THF (10 mL) was added MeI (188 μL, 3 mmol) at rt. After stirring for 24 h at this temperature, the reaction was quenched with saturated aq. $NaHCO_3$ and extracted with AcOEt. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (33% AcOEt/Hexane) to give **7** as a white solid (171 mg, 84%). mp 79–80 ºC, IR (KBr) 2924, 1636, 1603, 1511, 1445, 1355, 1268, 1216, 1024 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.73 (3H, s), 5.10 (2H, s), 5.23 (2H, s), 5.26 (4H, s), 6.47 (1H, d, J = 2.0 Hz), 6.53 (1H, d, J = 2.0 Hz), 7.02 (1H, d, J = 8.5 Hz), 7.3–7.5 (18H, m), 7.6–7.7 (3H, m), 7.79 (1H, d, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 59.9, 70.4, 70.7, 70.9, 71.3, 93.8, 97.9, 110.0, 113.8,

115.0, 122.0, 123.8, 126.6, 127.1, 127.2, 127.6, 127.8, 128.0, 128.4, 128.5, 128.6, 128.7, 135.7, 136.3, 136.7, 137.0, 141.3, 148.3, 150.7, 152.4, 158.6, 159.7, 162.7, 173.8; ESI-TOF HRMS Calcd. For $C_{44}H_{37}O_7$ $[M+H]^+$, 677.2534; Found: 677.2533.

3-O-Methylquercetin (**8**)

To a solution of 5,7,3',4'-tetra-*O*-benzyl-3-*O*-methylquercetin (**7**) (100 mg, 148 μmol) in CH_2Cl_2 (5 mL) and MeOH (5 mL) was added 5% Pd/C (30 mg). The suspension was stirred for 24 h at rt under 1 atm of hydrogen. The reaction mixture was filtrated through a Celite pad and the filtrate was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (80% AcOEt/Hexane) to give **8** as a pale yellow solid (36.7 mg, 78%). mp 200–201 ºC (decomposed), IR (KBr) 3398, 1651, 1606, 1497, 1361, 1294, 1215, 1165 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 3.78 (3H, s), 6.18 (1H, d, J = 2.0 Hz), 6.37 (1H, d, J = 2.0 Hz), 6.89 (1H, d, J = 8.5 Hz), 7.52 (1H, dd, J = 8.5, 2.0 Hz), 7.61 (1H, d, J = 2.0 Hz); ^{13}C NMR ($CDOD_3$, 125 MHz) δ 60.5, 94.7, 99.7, 105.9, 116.4, 116.5, 122.3, 122.9, 139.5, 146.4, 149.9, 158.0, 158.4, 163.1, 165.9, 178.0; ESI-TOF HRMS Calcd. For $C_{16}H_{12}O_7Na$ $[M+Na]^+$, 339.0475; Found: 339.0471.

3-O-Methylcyanidin (**3**)

To a suspension of 3-*O*-methylquercetin (**8**) (100 mg, 316 μmol) and Zn powder (1.0 g) in anhydrous MeOH (10 mL) was added 2 M hydrogen MeOH solution (5 mL) at –40 ºC and the reaction mixture was stirred. After the temperature was raised gradually from –40 ºC to 0 ºC for 30 min, Zn was removed by suction filtration. The filtrate solution was stirred under air for 14 h. After the reaction solution was added with water containing 0.5% trifluoroacetic acid (TFA) (100 mL), MeOH was removed under reduced pressure. The residual aqueous solution was purified by XAD-7 (9% MeCN/H_2O with 0.5% TFA, 17% MeCN/H_2O with 0.5% TFA, 60% MeCN/H_2O with 0.5% TFA). After MeCN and MeOH were removed under reduced pressure, the residual aqueous solution was extracted with AcOEt with 0.5% TFA. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (80% AcOEt/Hexane with 0.5% TFA) to give **3** as a TFA salt containing one H_2O molecule (dark red solid) (67 mg, 49%). mp 239–240 ºC (decomposed); IR (KBr) cm^{-1}; 3428, 1635, 1335, 1281, 1178 cm^{-1}; UV-Vis (0.1% HCl-MeOH) λmax nm (ε): 536 nm (20600), 280 nm (13100); ^1H NMR (10% TFA-*d*-CD_3OD, 500 MHz) δ 4.16 (3H, s), 6.64 (1H, s), 6.85 (1H, s), 6.98 (1H, d, J = 8.5 Hz), 7.99 (1H, d, J = 2.0 Hz), 8.10 (1H, dd, J = 8.5, 2.0 Hz), 8.70 (1H, s); ^{13}C NMR (10% TFA-*d*-$CDCl_3$, 125 MHz) δ 58.2, 113.1, 117.5, 118.3, 121.4, 128.0, 130.7, 147.5, 148.6, 156.0, 156.7, 158.4, 163.7, 169.1; ESI-TOF HRMS Calcd. For $C_{16}H_{13}O_6$ $[M+H]^+$, 301.0707; Found: 301.0707. Anal. Calcd for $C_{18}H_{15}F_3O_9$: C, 50.01: H, 3.50. Found: C, 50.02; H, 3.65.

Measurement of UV-Vis spectra and stability of anthocyanins

The stock solution of anthocyanins **1**-**3** were prepared with concentration of 5 mM in MeOH. The stock solutions of co-pigment **4** and **5** were prepared dissolving in water and EtOH, respectively, with concentration of 50 mM. To a buffered solution of pH 1 (200 Mm KCl-HCl), anthocyanin was added with the final concentration of anthocyanin to be 0.05 mM. When co-pigment (0 to 10 eq. to anthocyanin) was added, stock solution of the co-pigment was added at first, then, the stock solution of the anthocyanin was put into the mixture. Just after mixing, UV-Vis spectrum (200 to 800 nm) was measured in a quartz cell (path length: 1 mm) at rt. After measurement, each solution was stored in dark at rt, then measured UV-Vis repeatedly with the interval indicated in the main text.

Kinetic analysis:

The degradation kinetics of the studied anthocyanin compound during storage were determined by modeling the first order rate reaction,[8a,15e,16] $A_t/A_0 = e^{-kt}$ where A_0 and A_t are the original absorbance at the time of preparation and absorbance at the time t, respectively, and the k is the reaction rate constant that can be obtained from the slope of the straight line. The half-life time ($t_{1/2}$) was calculating from (ln 2)/k.

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