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SYNTHESIS OF DESIGNED ACYLQUINIC ACID DERIVATIVES INVOLVED IN BLUE COLOR DEVELOPMENT OF HYDRANGEA AND THEIR CO-PIGMENTATION EFFECT

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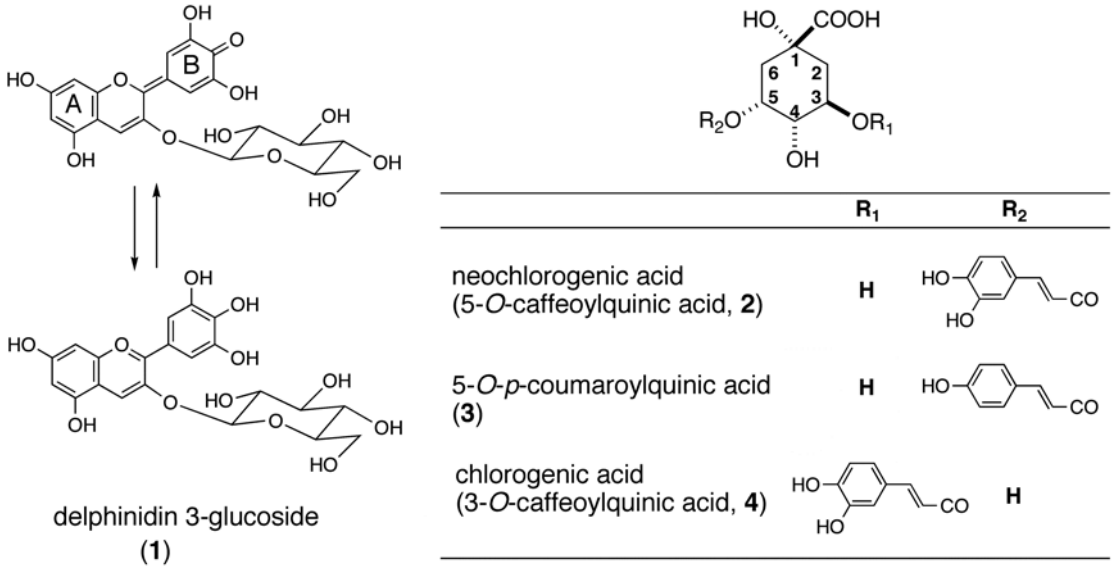
Abstract – The blue sepal color of hydrangea may be developed by an unstable supramolecular metal-complex pigment composed of delphinidin 3-glucoside (**1**), 5-*O*-caffeoylquinic acid (**2**) and 5-*O*-*p*-coumaroylquinic acid (**3**) as co-pigments and Al^{3+} in aqueous solution. To clarify the structure of blue pigment and the role of co-pigments, various acylquinic acid derivatives were designed and synthesized. As a result of reproduction experiment using the synthesized co-pigments, it was revealed that 1-COOH, 1-OH and 5-*O*-ester of quinic acid moiety was essential for blue color development. The aromatic plane of the 5-*O*-acyl moiety contributes to the stability of the blue complex.

INTRODUCTION

Hydrangea macrophylla originates in Japan and the sepal color of the wild type is blue. Red hydrangea was produced by European growers, then, imported to Japan. The most famous character of hydrangea is that the flower color (sepal color) of hydrangea changes readily.[1] Blue through purple to red sepals have one simple anthocyanin, delphinidin 3-glucoside (**1**, Scheme 1),[2-5] although the chromophores of blue flower pigments generally have a delphinidin nucleus and chromophores of red petals have pelargonidin or cyanidin chromophores.[6-8] Furthermore, the three major co-pigments, 5-*O*-caffeoyl[9] (**2**, neochlorogenic acid), 5-*O*-*p*-coumaroyl (**3**) and 3-*O*-caffeoyl (**4**, chlorogenic acid) quinic acids are also contained in all colored sepals.[2-5] A correlation between blue coloration and Al^{3+} has been suggested since the 1930s.[1,10-14] On blue hydrangea coloration Takeda et al. reported that stable blue solution was obtained by mixing **1**, **2**

or **3**, and Al^{3+}.[15-17] In 2003, we measured the vacuolar pH of the blue and red protoplasts prepared from both colored sepals of hydrangea using a pH-sensitive micro-electrode and found that the pHv of blue cells was 4.1 and that of red cells was 3.3.[18] Nevertheless, the chemical structure of blue pigment in hydrangea sepals is still unclear, because the blue pigment is unstable and can exist only in aqueous solutions. All purification and isolation experiments of the blue pigment have failed and the NMR spectrum of the blue solution was broad and unanalyzable. We studied the mechanism of blue color development of the hydrangea. To obtain structural information of the blue pigment, we designed and synthesized various co-pigment analogs.[19] We carried out reproduction experiments on the blue color by mixing them with **1** and Al^{3+} *in vitro*.[19] Here we report the essential structural part in the co-pigment for blue color development. We also discuss the molecular association of the anthocyanin and co-pigment for the blue sepal color of the hydrangea.



Scheme 1. Structure of anthocyanin and co-pigments of sepals of *Hyerangea macrophylla*.

RESULTS AND DISCUSSION

Design of co-pigment analogs and their synthesis

The results of our preliminary experiments suggest that not only the anthocyanin (**1**) but also co-pigments might interact with Al^{3+}. Therefore, to clarify the essential structural part of the co-pigments we designed several analogs. As shown in Figure 1, we focused on the following six structural parts; A: the position of acyl moiety whether 3-OH or 5-OH was essential, B: the necessity of the hydroxyl group(s) at the

aromatic acyl moiety, C: the effect of the length of the conjugate plane of the aromatic acyl moiety, D: the necessity of the 1-COOH of the quinic acid nucleus, E: the necessity of the 1-OH of the quinic acid nucleus, and F: the necessity of the 5-ester structure. The designed co-pigments are shown in Table 1. For examination of A, we compared **2** and **4**. For B, 5-*O*-cinnamoylquinic acid (**5**) was designed. For C, 5-*O*-benzyol (**6**), 5-*O*-naphtoyl (**7**) and 5-*O*-3',4'-dihydroxyphenylpropanoylquinic acid (**8**) were constructed. For D and E, methylester (**9**)[22] and 1-methoxyderivative (**10**) were planned. For F, 5-*O*-cinnamylquinic acid (**11**) was designed.

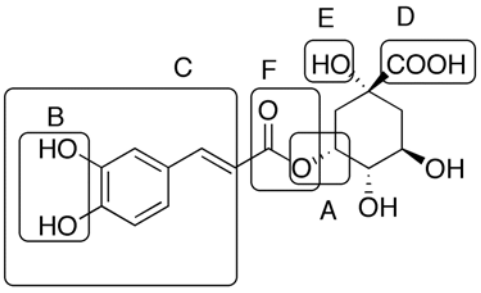


Figure 1. The modified structural parts of co-pigment for reproduction experiments.

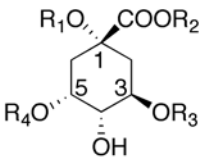
1-COOH of (-)-quinic acid (**12**) was converted to the methylester (**13**), then, the 3,4-dihydroxyl residues were protected according to the procedure of Montchamp et al.[20] **14** was dissolved in pyridine and treated by various acid chlorides to give 5-*O*-acyl derivatives. All the protecting groups were removed under acidic conditions (2N HCl-CH_3CN)[21] to give (**2**, **3**, **5**-**7**, Scheme 2).

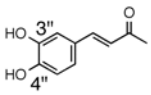
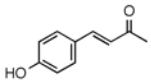
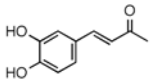
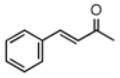
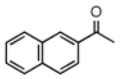
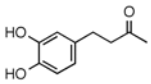
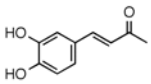
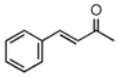
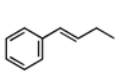
8 was obtained from **2** by hydrogenation under H_2/Pd-C in EtOH (quant.). **9**[22] was prepared by treatment of **2** with CH_2N_2/MeOH at 0°C (71%). **10** was synthesized from **14** as shown in Scheme 3. 5-OH of **14** was protected with TBDMS group,[23] then, the 1-OH was methylated with NaH/MeI in DMF. After removal of the TBDMS protecting group 5-OH was acylated using the same procedure described above to give **10** (3% from **14**, 5 steps).

5-*O*-Ether derivative **11** was synthesized by a reaction of **14** with cinnamyl bromide, then, the obtained compound was treated first under acidic conditions to remove 3,4-protecting groups and then under alkaline conditions to hydrolyze 1-COOMe (32% from **14**, 3 steps).

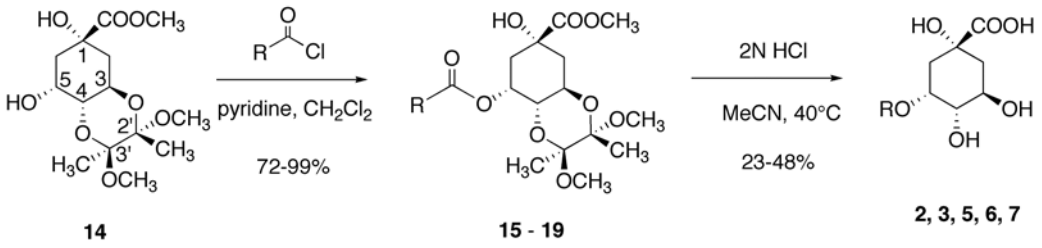
The problem in these synthetic routes is the poor yield of the last deprotecting reaction. The hydrolysis of the methylester of 1-COOH was competitive with the hydrolysis reaction of 5-ester even under acidic conditions. It might be caused by the 1,3-diaxial steric-hindrance between 1-OH and 5-*O*-ester.

Table 1. Structure of co-pigments and the co-pigmentation effect.

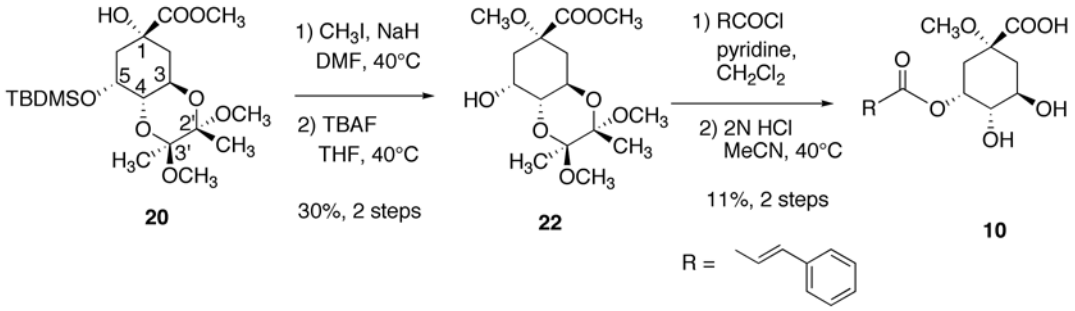


	R_1	R_2	R_3	R_4	co-pigmentation effect*
2	H	H	H		○
3	H	H	H		○
4	H	H		H	×
5	H	H	H		○
6	H	H	H		△
7	H	H	H		◎
8	H	H	H		△
9	H	CH_3	H		×
10	CH_3	H	H		×
11	H	H	H		×

*: co-pigmentation effect was examined by the color and the stability of the reproduction solution by mixing **1** : co-pigment : Al^{3+} = 1mM : 3 mM : 1 mM in buffer at pH 4.0. ◎: vary stable blue solution, ○: stable blue solution, △: Blue solution was obtained first, but after 1 day at rt small amount of precipitate appeared. ×: Blue-black precipitate quickly produced.



Scheme 2. Synthesis of 5-*O*-acylquinic acid derivatives (**2**, **3**, **5**-**7**).



Scheme 3. Synthesis of 1-*O*-methyl-5-*O*-cinnamoylquinic acid (**10**).

Reproduction of hydrangea blue color using synthetic co-pigments

Using the natural co-pigments and synthesized analogs we carried out reproduction experiments of the blue sepal color of hydrangea. First, we examined the analytical data of the colored blue cells of hydrangea sepals to determine the experimental conditions. The vacuolar pH value of blue cells was around 4.0.[18] Our preliminary analysis of colored protoplast revealed that the concentration of **1** in the colored vacuole was about 1-10 mM and the ratio of co-pigment, **2**, **3** and **4** to **1** was 1-10 eq. The content of Al^{3+} was varied depending on the sepal color[16,18,24] and the molar ratio of Al^{3+} to **1** in blue colored cells was ca. one equivalent or higher. Therefore, we determined the mixing conditions of the reproduction of the blue color to be **1** : co-pigment : Al^{3+} =1 mM : 1-5 eq. : 1/3-3 eq at pH 4. All the components were mixed in a buffer solution and UV/VIS spectrum and CD were measured in a quartz quvette (1.0 mm pathlength). The solution was stood at rt and the stability of the color was recorded.

A mixture of **1** and Al^{3+} (1/3-3 eq) at pH 4.0 without co-pigment gave a blue colored solution first, but the color was unstable and a blue-black precipitate quickly appeared (Figure 2). The mixtures of **1** and **2**, **3**, or **4** without Al^{3+} at pH 4.0 showed only a red color even if 5 eq. of co-pigments was added (Figure 2). When mixing the three components, **1**, co-pigment (**2**, or **3**) and Al^{3+} blue solution which VIS spectrum and CD were identical to those of the blue protoplasts was obtained (Figure 2). To reproduce the stable blue solution more than 3 eq. of **2** or 2 eq. of **3** to **1** was needed, and less than the molar equivalent of the

co-pigment blue-black precipitate was gradually produced. In contrast to **2** and **3**, 3-*O*-caffeoylquinic acid (**4**) did not show such effect. The mixture of **1**, **4** and Al^{3+} gave a blue-black colored precipitate immediately.

The reproduction experiments using synthetic co-pigment analogs are shown in Figure 2. **1** (1 mM), co-pigment (3 mM) and Al^{3+} (1 mM) were mixed in a buffer solution at pH 4. 5-*O*-cinnamoylquinic acid (**5**) gave a stable blue solution the same as that of **2**. This result indicates that the hydroxyl groups at the aromatic moiety are not essential for the co-pigmentation effect. The co-pigment effect of 5-*O*-benzoylquinic acid (**6**), 5-*O*-naphtoylquinic acid (**7**), and 5-*O*-(3,4-dihydrioxyphenylpropanoyl)-quinic acid (**8**) were compared (Figure 2). Although all of them gave blue solutions, **7** gave the most stable blue color and **6** and **8** gave a small amount of precipitate within a few hours. The blue color with **7** was more stable than that obtained with the natural co-pigments, **2** and **3**. These results indicate that the large conjugate plane at 5-*O*-acyl moiety plays an important role in stabilizing the blue supramolecular complex. The methyl ester (**9**), the 1-methoxy derivative (**10**) and 5-*O*-cinnamylquinic acid (**11**) did not show any co-pigmentation effect: a blue-black precipitate quickly appeared.

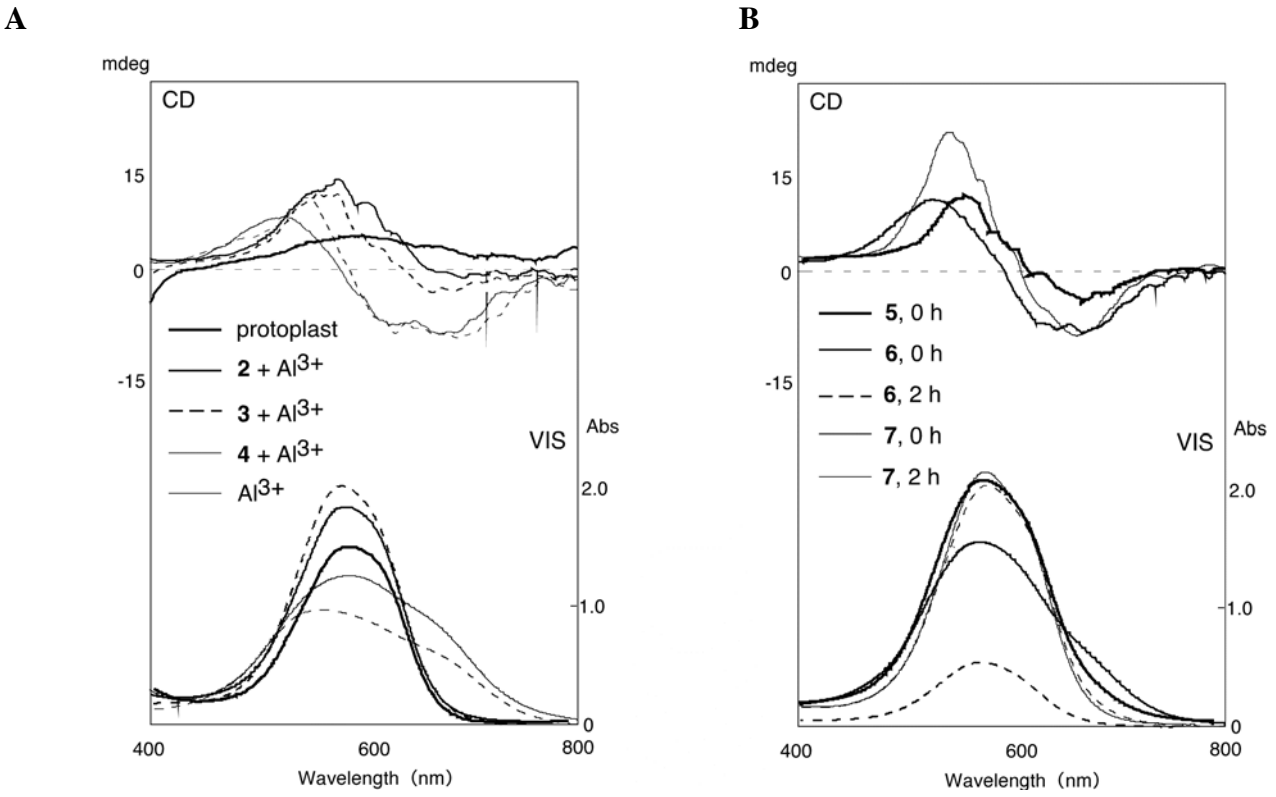


Figure 2. VIS spectra and CD of the blue hydrangea protoplast and the reproduction solution mixing with **1** (1 mM) and Al^{3+} (1 mM) with various co-pigment (3 mM) at pH 4.0 (cell length: 1.0 mm). A: Protoplasts suspension and mixtures of natural co-pigment, **2**, **3** or **4**. B: Mixtures of synthetic co-pigment, **5**, **6** or **7** and the stability of the blue color.

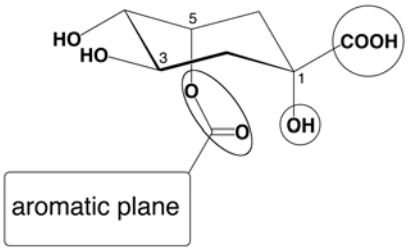


Figure 3. Essential structural part of co-pigment for blue color development of hydrangea. The circled structural parts are essential. The aromatic plane of the 5-*O*-acyl moiety contributes stabilization of the blue color.

Consideration on blue color development of hydrangea

The results of the reproducing experiments of hydrangea blue color are summarized in Table 1. It was concluded that the 5-*O*-ester, the 1-COOH and 1-OH in the co-pigment are essential to obtain blue solution (Figure 3). The dihydroxyl groups in the aromatic acyl moiety did not have any effect on formation of blue pigment. The aromatic plane of the 5-*O*-acyl moiety of quinic acid contributed to stabilizing the blue color.

The precipitate obtained by the addition of **4**, or a small equivalent of **2** or **3**, to **1** and Al^{3+} were collected and the composition was analyzed. All of them were made by the same components, **1** and Al^{3+} (the molar ratio was ca. 3 : 2) without any co-pigments. Furthermore, the addition of Al^{3+} to the aqueous solution of **1** at pH 4.0 gave the same precipitate. These phenomena strongly indicate that Al^{3+} may chelete to the *ortho*-dihydroxyl groups at the B-ring of **1**,[17,19] but this blue **1**-Al^{3+} complex might be barely soluble in weakly acidic aqueous solution. 5-*O*-acylquinic acid derivatives have a co-pigmentation effect by a hydrophobic interaction between the anthocyanidin nucleus of **1** and the aromatic plane solubilizing the complex to stabilize the blue color in the solution.

This mechanism was indicated by CD measurements. The CD of the suspension of blue protoplasts and the solution obtained by mixing **1**, **2** and Al^{3+} were similar showing a single peak at 590 nm. The stable blue solutions obtained with **3**, **5** and **7** also showed the same CDs to that of **2**. However, the CD of the mixture with **4** gave a negative exiton-type Cotton effect around the λmax indicating the self-association of the chromophores of **1**. The mixture of **1** and Al^{3+} showed the same CD. Furthermore, the co-pigment analogs that did not give blue solution but precipitate expressed the negative exiton-type Cotton effect in CDs, too. These findings suggest that the hydrophobic aromatic acyl part may insert into the self-associated anthocyanidin nucleus of **1**-Al^{3+} complex and stack. At that time, some co-ordination between co-pigment and Al^{3+} may help the stacking.

Here, we propose a structure of the blue pigment of hydrangea that is caused by an Al^{3+}-complex coordinating of **1** at *ortho*-dihydroxyl groups of the B-ring.[17,19] The oxygen atoms at 1-COOH, 1-OH and the 5-ester residue may be essential for constructing a hydrogen bond network and/or for coordinating to

the Al^{3+}. The hydrophobic interaction between the aromatic acyl residue at the 5-position of the co-pigment and the nucleus of **1** may also stabilize the complex. This newly elucidated supramolecular complex with non-flavonoid co-pigment develops the blue hydrangea sepals. Further investigations are in progress.

EXPERIMENTAL

General

Melting points were recorded on a Yanagimoto MP-S3 apparatus and uncorrected. Optical rotations were recorded on a JASCO DIP-140 polarimeter. UV-VIS spectra were recorded on a JASCO V-560 spectrometer. IR spectra were obtained with a JASCO FT/IR-460 plus spectrometer. CD was recorded on a JASCO J-720 WN apparatus. NMR spectra were obtained with a JEOL JNM-A600 (^{1}H: 600 MHz, ^{13}C: 150 MHz), an ECA-500 (^{1}H: 500 MHz, ^{13}C: 125 MHz), a JNM-A400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), and a GSX-270 (^{1}H: 270 MHz, ^{13}C: 67.5 MHz) instrument in a 5-mm ϕ tube at variable temperature using $CDCl_3$ and CD_3OD as a solvent. Chemical shifts were reported as δ (ppm) with the CD_2HCl or CD_2HOD resonance as a standard and the coupling constant was expressed in Hz. EIMS and FABMS (glycerol, *m*-nitrobenzyl alchol and triethanolamine as a matrix) were recorded on a JEOL JMS-700 spectrometer. Analytical and preparative HPLC were conducted according to our procedure[25-27] using ODS-columns (Develosil ODS-HG-5, Nomura Chemicals) with aq. MeCN as an eluent. Column chromatography was performed Merck Kieselgel 60 and Fuji Silysia Chemical BW-300. Thin layer chromatography was performed on Merck Kieselgel 60 F_{254}.

Methyl (2'*S*,3'*S*)-3-*O*,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)quinate (14)[20]

According to the procedure of Montchamp *et al.*[20] (-)-quinic acid **12** (50 g, 0.26 mol) was converted to **14** (35.5 g, 0.11 mol, 42%). Colorless needles: mp 136.8-139.0ºC; $[\alpha]_D^{25}$ +113 (*c* 1.2, $CHCl_3$).

General procedure of 5-*O*-aylation to 14

Methyl (2'*S*,3'*S*)-3-*O*,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-5-*O*-naphthoylquinate (19)

To a solution of **14** (300 mg, 0.95 mmol) in dried pyridine (0.5 mL) was added naphtoyl chloride (209 mg, 1.11 mmol) in CH_2Cl_2 (0.5 mL) at 0°C, then the mixture was warmed to 30°C and stirred for 2.5 h. To the reaction mixture was added CH_2Cl_2 (5 mL) and washed with sat. aq. $NaHCO_3$ (3 mL x 2). The CH_2Cl_2 layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified with a silica-gel column chromatography (hexane-EtOAc = 2:1) to give **19** (430 mg, 0.91 mmol, 96%). Colorless plates (Hexane-EtOAc): mp 80-83ºC; $[\alpha]_D^{27}$ +71 (c 0.6, $CHCl_3$); IR (KBr) 2952, 1716, 1371, 1286, 1132 cm^{-1};

UV ($CHCl_3$) λmax nm (ε) 281 (12,400); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.21 (s, 3H), 1.28 (s, 3H), 2.05 (m, 2H), 2.19 (dd, *J* = 3.0, 15.5 Hz, 1H), 2.31 (d, *J* = 3.0, 15.5 Hz, 1H), 3.26 (s, 3H), 3.33 (s, 3H), 3.76 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.77 (s, 3H), 4.61 (dt, *J* = 6.5, 10.0 Hz, 1H), 5.52 (m, 1H), 7.53 (dt, *J* = 2.0, 8.0 Hz, 1H), 7.55 (dt, *J* = 2.0, 8.0 Hz, 1H), 7.84 (brd, *J* = 8.0 Hz, 1H), 7.85 (brd, *J* = 8.0 Hz, 1H), 7.92 (brd, *J* = 8.0 Hz, 1H), 8.10 (dd, *J* = 2.0, 8.0 Hz, 1H), 8.63 (brs, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 36.8, 38.8, 48.0, 53.2, 62.8, 70.3, 71.5, 74.6, 99.6, 100.0, 125.5, 126.5, 127.7, 128.0, 128.1, 129.4, 131.5, 132.5, 135.5, 166.4, 175.6; FABMS *m/z*: 475 $[M+H]^+$; Anal. Calcd for $C_{25}H_{30}O_9 \cdot 1/4\ H_2O$: C, 62.69; H, 6.42. Found: C, 62.79; H, 6.46.

Methyl (2'*S*,3'*S*)-5-*O*-(3'',4''-di-*O*-aceylcaffeoyl)-3-*O*,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-quinate (15)[21)]

From **14** (1.46 g, 4.6 mmol) 2.43 g of **15** was obtained (4.28 mmol, 94%). Colorless plates (Hexane-EtOAc): mp 95-104ºC; $[\alpha]_D^{26}$ +65 (*c* 1.3, $CHCl_3$).

Methyl (2'*S*,3'*S*)-5-*O*-(4''-*O*-aceyl-*p*-coumaroyl)-3-*O*,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)quinate (16)

From **14** (1.91 g, 5.96 mmol) 2.52 g of **16** was obtained (4.95 mmol, 83%). Colorless plates (Hexane-EtOAc): mp 90-94.5ºC; $[\alpha]_D^{26}$ +64 (*c* 1.1, $CHCl_3$); IR (KBr) 2952, 1767, 1734, 1713, 1371, 1203 cm^{-1} UV ($CHCl_3$) λmax nm (ε) 283 (19,200); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.28 (s, 3H), 1.29 (s, 3H), 2.02 (m, 2 H), 2.20 (dd, *J* = 3.0, 15.0 Hz, 1H), 2.23 (dt, *J* = 3.0, 15.5 Hz, 1H), 2.29 (s, 3H), 3.24 (s, 3H), 3.28 (s, 3H), 3.69 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.76 (s, 3H), 4.30 (dt, *J* = 6.0, 10.0 Hz, 1H), 5.34 (m, 1H), 6.43 (d, *J* = 16.0 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.53 (d, *J* = 8.0 Hz, 2H), 7.67 (d, *J* = 16.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.6, 17.8, 21.1, 36.6, 38.7, 47.9, 48.0, 53.2, 62.8, 69.8, 71.2, 74.6, 99.6, 100.1, 118.6, 122.0, 129.3, 132.3, 144.0, 152.0, 166.4, 169.1, 175.5; HR-FABMS Calcd for $C_{25}H_{33}O_{11}$ $[M+H]^+$ 509.2023, Found 509.2016.

Methyl (2'*S*,3'*S*)-5-*O*-cinnamoyl-3-*O*,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)quinate (17)

From **14** (1.00 g, 3.12 mmol) 1.10 g of **17** was obtained (2.36 mmol, 76%). Colorless plates (Hexane-EtOAc): mp 76-80ºC; $[\alpha]_D^{27}$ +83 (*c* 0.51, $CHCl_3$); IR (KBr) 2954, 1738, 1709, 1637, 1132 cm^{-1}; UV ($CHCl_3$) λmax nm (ε) 279 (26,200); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.26 (s, 3H), 1.28 (s, 3H), 2.00 (m, 2H), 2.10 (dd, *J* = 3.5, 15.5 Hz, 1H), 2.24 (dt, *J* = 3.0, 15.5 Hz, 1H), 3.26 (s, 3H), 3.29 (s, 3 H), 3.70 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.76 (s, 3H), 4.42 (td, *J* = 6.0, 10.0 Hz, 1H), 5.35 (m, 1H), 6.48 (d, *J* = 16.0 Hz, 1H), 7.35 (m, 3H), 7.52 (m, 2H), 7.70 (d, *J* = 16.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.6, 17.9, 36.6, 38.7, 47.9, 48.0, 53.2, 62.8, 69.8, 71.2, 74.6, 99.6, 100.1, 118.4, 128.2, 128.8, 130.2, 134.5, 145.2, 166.6, 175.4; FABMS *m/z*: 450 $[M]^+$; Anal. Calcd for $C_{23}H_{30}O_9 \cdot 1/5\ H_2O$: C, 60.84; H, 6.75. Found: C, 60.91; H, 6.65.

Methyl (2'*S*,3'*S*)-5-*O*-benzoyl-3-*O*,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)quinate (18)

From **14** (1.00 g, 3.12 mmol) 1.31 g of **18** was obtained (3.08 mmol, 99%). Colorless plates (Hexane-EtOAc): mp 98-103ºC; $[\alpha]_D^{27}$ +94 (*c* 0.55, $CHCl_3$); IR (KBr) 2952, 1736, 1703, 1284, 1132 cm^{-1}; UV ($CHCl_3$) λmax nm (ε) 277 (13,900); ^1H NMR ($CDCl_3$, 600 MHz) δ 1.17 (s, 3H), 1.23 (s, 3H), 1.99 (m, 2H), 2.11 (d, *J* = 3.0, 15.0 Hz, 1H), 2.21 (m, 1H), 3.21 (s, 3H), 3.24 (s, 3H), 3.69 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.72 (s, 3H), 4.48 (m, 1H), 5.43 (m, 1H), 7.38 (m, 2H), 7.48 (m, 1H), 8.02 (dd, *J* = 2.0, 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.6, 17.8, 36.8, 38.8, 47.9, 48.0, 53.2, 54.9, 62.8, 70.1, 71.4, 74.6, 99.5, 100.0, 128.3, 129.9, 130.7, 132.8, 166.2, 175.6; EIMS *m/z*: 409 $[M\text{-}Me]^+$; Anal. Calcd for $C_{21}H_{28}O_9 \cdot 1/3\ H_2O$: C, 58.60; H, 6.71. Found: C, 58.52; H, 6.69.

General procedure of deprotection

To the solution of **19** (120 mg, 0.25 mmol) in MeCN (3 mL), 2N HCl (0.9 mL) was added and the mixture was kept at 70° for 4 h. To the mixture, EtOAc (10 mL) was added and then extraction was performed with sat. aq. $NaHCO_3$ (10 mL). The aqueous layer was acidified with the addition of TFA (2 mL), then, extraction was performed with EtOAc (10 mL x 3). The EtOAc layer was dried up and purified with preparative HPLC (aq. MeCN) to give **7** as a colorless powder (37 mg, 0.11 mmol, 43%). Colorless powder (aq. MeOH): mp 184-188.5ºC; $[\alpha]_D^{27}$ –8.3 (*c* 0.32, MeOH); IR (KBr) 1701, 1288, 1231, 1120, 1131 cm^{-1}; UV (MeOH) λmax nm (ε) 280 (11,600); ^1H NMR (CD_3OD, 500 MHz) δ 2.04 (m, 1H), 2.23 (m, 3H) 3.73 (dd, *J* = 3.5, 8.5 Hz, 1H), 4.29 (brt, 3.5 Hz, 1H), 5.57 (brs, 1H), 7.58 (m, 2H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.98 (brd, *J* = 8.0 Hz, 1H), 8.08 (dd, *J* = 1.5, 8.0 Hz, 1H), 8.68 (brs, 1H),; ^{13}C NMR (CD_3OD, 125 MHz) δ -11.6, 10.9, 36.9, 41.8, 68.4, 74.0, 75.2, 101.3, 126.5, 127.4, 128.8, 129.0, 129.3, 130.4, 132.3, 134.0, 137.0, 168.1, 195.2; HR-FABMS Calcd for $C_{18}H_{19}O_7$ $[M+H]^+$ 347.1131, Found 347.1142.

5-*O*-Caffeoylquinic acid (2)

From **15** (1.55 g, 2.73 mmol) 228 mg of **2** was obtained (0.64 mmol, 23%). Colorless plates (EtOH): mp 217-220ºC; $[\alpha]_D^{27}$ +11 (*c* 0.24, MeOH).

5-*O*-*p*-Coumaroylquinic acid (3)

From **16** (2.45 g, 4.89 mmol) 531 mg of **3** was obtained (1.57 mmol, 32%). Colorless plates (hot water): mp 195-199ºC; $[\alpha]_D^{28}$ +5.0 (*c* 0.37, MeOH).

5-*O*-Cinnamoylquinic acid (5)

From **17** (68 mg, 0.15 mmol) 19 mg of **5** was obtained (0.06 mmol, 40%). Colorless plates (aq. MeOH): mp 194-195.5ºC; $[\alpha]_D^{25}$ –5.7 (*c* 0.52, MeOH); IR (KBr) 3465, 3338, 1710, 1678, 1290 cm^{-1}; UV (MeOH) λmax nm (ε) 277 (30,800); ^1H NMR (CD_3OD, 270 MHz) δ 1.98 (m, 1H), 2.20 (m, 3H), 3.67 (dd, *J* = 3.0,

9.0 Hz, 1H), 4.18 (dt, *J* = 4.0, 9.0 Hz, 1H), 5.39 (m, 1H), 6.59 (d, *J* = 16.0 Hz, 1H), 7.30-7.70 (m, 5H), 7.75 (d, *J* = 16.0 Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 36.7, 41.6, 68.3, 73.4, 74.8, 119.6, 129.2, 130.0, 131.4, 136.0, 146.2, 168.4; FABMS *m/z*: 323 $[M+H]^+$; Anal. Calcd for $C_{16}H_{18}O_7$: C, 59.62; H, 5.63. Found: C, 59.37; H, 5.63.

5-*O*-Benzoylquinic acid (6)

From **18** (0.96 g, 2.26 mmol) 0.32 g of **6** was obtained (1.08 mmol, 48%). Colorless plates (hot EtOH): mp 204-208.5ºC; $[\alpha]_D^{25}$ –20 (*c* 0.70, MeOH); IR (KBr) 3484, 1732, 1698, 1291, 1122 cm^{-1}; UV (MeOH) λmax nm (ε) 229 (16,600); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.96 (m, 1H), 2.20 (m, 3H), 3.67 (dd, *J* = 3.0, 9.0 Hz, 1H), 4.25 (dt, *J* = 4.0, 9.0 Hz, 1H), 5.50 (m, 1H), 7.44 (m, 2H), 7.57 (m, 1H), 8.08 (m, 2H); ^{13}C NMR (CD_3OD, 67.5 MHz, rt) δ 36.8, 41.9, 68.2, 73.8, 75.2, 129.3, 130.9, 132.1, 134.0, 168.0; FABMS *m/z*: 297 $[M+H]^+$; Anal. Calcd for $C_{14}H_{16}O_7$: C, 56.76; H, 5.44. Found: C, 56.72; H, 5.55.

5-*O*-(3,4-Dihydroxyphenylpropanoyl)quinic acid (8)

To **2** (0.28 g, 0.80 mmol) were added dried EtOH (3 mL) and 5% Pd-C (40 mg). The mixture was stirred under H_2 atmosphere at rt for 7 h, then filtered with cerite. The filtrate was dried *in vacuo* to give **8** (0.29 g, 0.80 mmol, quant.). Colorless powder: mp 172.5-175ºC; $[\alpha]_D^{25}$ –26 (*c* 0.41, MeOH); IR (KBr) 1729, 1688, 1520, 1280, 1208 cm^{-1}; UV (MeOH) λmax nm (ε) 282 (14,400); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.91 (dd, *J* = 10.0, 13.0 Hz, 1H), 2.07 (m, 3H), 2.60 (m, 2H), 2.78 (m, 2H), 3.58 (dd, *J* = 3.5, 9.0 Hz, 1H), 4.05 (ddd, *J* = 3.5, 9.0, 13.0 Hz, 1H), 5.23 (m, 1H), 6.53 (dd, *J* = 2.0, 8.0 Hz, 1H), 6.66 (m, 2H); ^{13}C NMR (CD_3OD, 125 MHz, rt) δ 31.2, 36.5, 37.4, 68.3, 73.1, 74.5, 78.3, 78.7, 115.4, 116.3, 116.4, 120.5, 123.7, 133.8, 144.5, 165.2, 171.7, 174.7; HR-FABMS Calcd for $C_{16}H_{19}O_9Na_2$ $[M-H+2Na]^+$ 401.0824, Found 401.0832.

Methyl 5-*O*-caffeoylquinate (9)[22)]

A solution of **2** (123 mg, 0.35 mmol) in MeOH (3 mL) was cooled to 0°C and added CH_2N_2 (8.5 mL) using a dropping funnel, then the mixture was warmed to rt and stirred at the temperature for 2 h. The mixture was evaporated *in vacuo*, then the residue was purified with prep. TLC (CH_2Cl_2-MeOH = 1:4) to give **9** (94 mg, 0.25 mmol, 71%). Pale yellow plates (aq. MeOH): mp 164-168ºC; $[\alpha]_D^{25}$ +10 (*c* 0.87, MeOH).

Methyl (2'*S*,3'*S*)-5-*O*-*t*-butyldimethylsilyl-3-*O*,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)quinate (20)[23)]

A solution of **14** (300 mg, 0.94 mmol) in dried DMF (9 mL) was stirred under argon atmosphere, then, to the solution were added imidazole (192 mg, 2.8 mmol) and TBDMSCl (425 mg, 3.8 mmol). After the reaction mixture was allowed to stand at rt for 18 h, sat. aq. NH_4Cl (10 mL) was added to the mixture and

extracted with EtOAc (10 mL x 4). The EtOAc layer was dried over $MgSO_4$ and evaporated. The residue was purified with silicagel column chromatography (hexane-EtOAc = 2:1) to give **20** (396 mg, 0.91 mmol, 97%). Colorless solid (Hexane-EtOAc): mp 114.5-118ºC; $[\alpha]_D^{27}$ +67 (*c* 0.36, $CHCl_3$).

Methyl (2'*S*,3'*S*)-5-*O*-*t*-butyldimethylsilyl-1-*O*-methyl-3-*O*,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-quinate (21)

To a solution of **20** (400 mg, 0.92 mmol) in dried DMF (9 mL) under argon atmosphere was added NaH (91 mg, 2.3 mmol) and the mixture was stirred at rt. To the mixture MeI (0.6 mL, 9.2 mmol) was added, then stirred at rt for 15 h, then, warmed to 40°C and allowed to stand at the temperature for 0.5 h. After cooling to 0°C, sat. aq. NH_4Cl (20 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (30 mL x 3). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified with silicagel column chromatography (hexane-EtOAc = 3:1) to give **21** (413 mg, 0.96 mmol, quant.). Colorless powder (aq. MeOH): mp 47.5-53ºC; $[\alpha]_D^{27}$ +84 (*c* 0.78, $CHCl_3$); IR (KBr) 2955, 1734, 1246, 1142, 1130, 1117 1050 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 0.05 (s, 3H), 0.13 (s, 3H), 0.90 (s, 9H), 1.25 (s, 3H), 1.27 (s, 3H), 1.72(t, *J* = 10.0 Hz, 1H), 1.88 (dd, *J* = 3.0, 9.0 Hz, 1H), 2.10-2.40 (m, 2H), 3.20 (s, 3H), 3.22 (s, 3H), 3.23 (s, 3H), 3.38 (dd, *J* = 3.0, 9.0 Hz, 1H), 3.73 (s, 3H), 4.13 (m, 1H), 4.27 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ -4.9, -4.6, 17.6, 17.8, 18.1, 25.6, 25.8, 33.2, 38.3, 47.4, 47.6, 52.2, 52.7, 61.9, 68.2, 73.2, 80.5, 99.0, 99.6, 173.9; FABMS *m/z*: 449 $[M+H]^+$; Anal. Calcd for $C_{21}H_{40}O_8Si$: C, 56.22; H, 8.99. Found: C, 56.42; H, 9.29.

Methyl (2'*S*,3'*S*)-1-*O*-methyl-3-*O*,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)quinate (22)

To a solution of **22** (432.0 mg, 0.96 mmol) in dried THF (12 mL) was added 1 M TBAF in THF (2.9 mL, 2.9 mmol) under argon atmosphere. The solution was stirred at rt for 10 h, then warmed to 40°C and allowed to stand at the temperature for 5 h. After cooling to 0°C, sat. aq. NH_4Cl (20 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (30 mL x 3). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified with column chromatography (hexane-EtOAc = 1:1) to give **22** (97 mg, 0.29 mmol, 30%). Colorless powder (hexane-EtOAc): mp 88.5-95ºC; $[\alpha]_D^{28}$ +140 (*c* 0.28, $CHCl_3$); IR (KBr) 2955, 1728, 1128 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.27 (s, 3H), 1.32 (s, 3H), 1.83 (m, 2H), 2.33 (m, 2H), 3.23 (s, 6H), 3.29 (s, 3H), 3.51 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.73 (s, 3H), 4.10 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.8, 17.9, 25.8, 33.8, 36.1, 47.8, 47.9, 52.5, 62.0, 68.2, 68.3, 73.0, 81.7, 99.6, 100.2, 172.3; HR-FABMS Calcd for $C_{15}H_{26}O_8Na$ $[M+Na]^+$ 357.1525, Found 357.1534.

Methyl (2'*S*,3'*S*)-5-*O*-cinnamoyl-1-*O*-methyl-3-*O*,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)quinate (23)

From **22** (97 mg, 0.29 mmol) 132 mg of **22** was obtained (0.28 mmol, 98%). Colorless powder hexane-EtOAc): mp 53-60ºC; $[\alpha]_D^{27}$ +48 (*c* 0.36, $CHCl_3$); IR (KBr) 2952, 1734, 1712, 1636, 1450 1129 cm^{-1}; UV (CH_2Cl_2) λmax nm (ε) 279 (20,000); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.20 (s, 3H), 1.23 (s, 3H), 1.77 (t, *J* = 12.0 Hz, 1H), 2.02 (dd, *J* = 4.0, 15.0 Hz, 1H), 2.28 (m, 1H), 2.59 (dd, *J* = 3.0, 15.0 Hz, 1H), 3.10 (s, 3H), 3.18 (s, 3H), 3.25 (s, 3H), 3.67 (dd, *J* = 2.0, 10.0 Hz, 1H), 3.67 (s, 3H), 4.29 (dt, *J* = 4.0, 10.0 Hz, 1H), 5.36 m, 1H), 6.43 (d, *J* = 16.0 Hz, 1H), 7.30 (m, 3H), 7.48 (m, 2H), 7.65 (d, *J* = 16.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 17.6, 17.8, 31.0, 37.1, 47.9, 52.5, 62.5, 69.2, 71.3, 80.2, 99.5, 100.0, 118.5, 128.1, 128.9, 130.2, 134.6, 145.0, 166.7, 173.0; HR-FABMS Calcd for $C_{24}H_{33}O_9$ $[M+H]^+$ 465.2125, Found 465.2132.

5-*O*-Cinnamoyl-1-*O*-methylquinic acid (10)

From **23** (168 mg, 0.36 mmol) 14 mg of **22** was obtained (0.04 mmol, 11%). Colorless powder (aq. MeOH): mp 89-92ºC; $[\alpha]_D^{25}$ –19 (*c* 0.96, MeOH); IR (KBr) 1702, 1637, 1450, 1311, 1280, 1189 cm^-; UV (MeOH) λmax nm (ε) 277 (23,000); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.86 (dd, *J* = 10.0, 13.0 Hz, 1H), 2.17 (dd, *J* = 4.0, 15.0 Hz, 1H), 2.26 (m, 1H), 2.37 (m, 1H), 3.19 (s, 3H), 3.65 (dd, *J* = 3.5, 8.0 Hz, 1H), 4.08 (dt, *J* = 4.0, 9.0 Hz, 1H), 5.36 (m, 1H), 6.55 (d, *J* = 16.0, 1H), 7.40 (m, 3H), 7.60 (m, 2H), 7.72 (d, *J* = 16.0, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 31.8, 40.0, 52.5, 68.1, 73.0, 74.5, 119.2, 129.2, 130.0, 131.5, 135.8, 146.4, 168.3; HR-FABMS Calcd for $C_{17}H_{20}O_7$ $[M]^+$ 336.1209, Found 336.1198.

Methyl (2'*S*,3'*S*)-5-*O*-cinnamyl-3-*O*,4-*O*-(2',3'-dimethoxybutane-2',3'-diyl)quinate (24)

To a solution of **14** (1.00 g, 3.12 mmol) in dried DMSO (15 mL) was added NaH (230 mg, 5.6 mmol) and cinnamyl bromide (0.7 mL, 4.7 mmol) under argon atmosphere at 15°C. The solution was stirred at the temperture for 15 min, then warmed up to rt and allowed to stand at the temperature for 10 min. To the mixture was added sat. aq. NH_4Cl (20 mL) and the mixture was extracted with EtOAc (15 mL x 3). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified with silicagel column chromatography (hexane-EtOAc = 2:1) to give **24** (0.86 g, 1.97 mmol, 63%). Colorless powder (hexane-EtOAc): mp135.5-138ºC; $[\alpha]_D^{26}$ +120 (*c* 0.36, $CHCl_3$); IR (KBr) 3500, 1727, 1131 cm^{-1}; UV ($CHCl_3$) λmax nm (ε) 255 (17,800); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.13 (s, 3H), 1.18 (s, 3H), 1.75 (t, 13.0 Hz, 1H), 1.83 (m, 1H), 2.01 (m, 1H), 2.07 (m, 1H), 3.08 (s, 3H), 3.10 (s, 3H), 3.45 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.59 (s, 3H), 3.88 (m, 1H), 4.17 (m, 2H), 4.33 (ddd, *J* = 1.0, 6.0, 13.0 Hz, 1H), 6.17 (m, 1H), 6.43 (d, *J* = 16.0 Hz, 1H), 7.20-7.50 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.7, 17.8, 34.4, 36.7, 47.8, 47.9, 52.5, 62.2, 66.4, 68.4, 73.1, 81.6, 99.6, 100.2, 124.8, 126.6, 127.9, 128.5, 133.1, 136.4, 172.4; HR-FABMS Calcd for $C_{23}H_{32}O_8Na$ $[M+Na]^+$ 459.1995, Found 459.1982.

Methyl 5-*O*-cinnamylquinate (25)

To a solution of **24** (397 mg, 0.91 mmol) in EtOAc (10 mL) was added TFA (1 mL) and the mixture was allowed to stand at 40°C for 7.5 h. To the reaction mixture was added EtOAc (10 mL) and the mixture was washed with sat. aq. $NaHCO_3$ (5 mL x 2). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified with silicagel column chromatography (hexane-EtOAc = 1:4) to give **25** (132 mg, 0.41 mmol, 45%). Colorless powder (hot MeOH): mp 54-59ºC; $[\alpha]_D^{26}$ –25 (*c* 0.50, $CHCl_3$); IR (KBr) 2925, 1734, 1450, 1250, 1124, 1086, 1070 cm^{-1}; UV (CH_2Cl_2) λmax nm (*ε*) 255 (11,300); ^1H NMR ($CDCl_3$, 600 MHz) *δ* 1.84 (dd, *J* = 11.0, 13.0 Hz, 1H), 1.96 (dd, *J* = 3.0, 15.0 Hz, 1H), 2.30 (m, 2H), 3.49 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.78 (s, 3H), 4.07 (m, 1H), 4.10 (m, 1H), 4.26 (ddd, *J* = 1.0, 6.0, 12.0 Hz, 1H), 4.37 (ddd, *J* = 1.0, 6.0, 12.0 Hz, 1H), 6.26 (dt, *J* = 6.0, 16.0 Hz, 1H), 6.61 (brd, *J* = 16.0 Hz, 1H), 7.20-7.40 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) *δ* 34.8, 41.1, 52.8, 67.7, 71.4, 75.6, 75.9, 124.4, 126.6, 128.1, 128.6, 134.0, 136.1, 174.1; HR-EIMS Calcd for $C_{17}H_{22}O_6$ $[M]^+$ 322.1416, Found 322.1413.

5-*O*-Cinnamylquinic acid (11)

To a solution of **25** (99 mg, 0.31 mmol) in CH_3CN (2 mL) at 0°C was added aq. 0.5 N NaOH (1 mL) and stirred at rt for 5.5 h. The reaction mixture was poured into the column (Amberlist 15E, 2.5 cmϕ × 18 cm) and the column was eluted with aq. 50% MeCN to give **11** (96 mg, 0.31 mmol, quant.). Colorless powder (aq. MeOH): mp 59-60ºC; $[\alpha]_D^{25}$ ±0.0 (*c* 1.0, MeOH); IR (KBr) 2927, 1718, 1593, 1387, 1117, 1076, 1047 cm^{-1}; UV (MeOH) λmax nm (*ε*) 252 (27,800); ^1H NMR (CD_3OD, 600 MHz) *δ* 1.94 (dd, *J* = 9.0, 14.0 Hz, 1H), 2.10 (dd, *J* = 3.0, 14.0 Hz, 1H), 2.21 (m, 1H), 2.33 (m, 1H), 3.47 (dd, *J* = 3.0, 8.0 Hz, 1H), 3.96 (dt, *J* = 3.0, 10.0 Hz, 1H), 4.03 (m, 1H), 4.11 (dd, *J* = 6.0, 11.0 Hz, 1H), 4.22 (dd, *J* = 6.0, 11.0 Hz, 1H), 6.33 (dt, *J* = 6.0, 16.0 Hz, 1H), 6.62 (d, *J* = 16.0 Hz, 1H), 7.20-7.50 (m, 5H); ^{13}C NMR (CD_3OD, 125 MHz, rt) *δ* 36.9, 38.1, 66.7, 68.3, 70.5, 76.2, 126.8, 127.5, 128.7, 129.6, 133.7, 138.2; HR-FABMS Calcd for $C_{16}H_{19}O_6Na_2$ $[M-H+2Na]^+$ 353.0977, Found 353.0969.

Reproduction of blue solution

To a buffer solution (100 mM AcOH-AcONa, pH 4.0) was added aq. solution of co-pigment, Al^{3+} and **1** at the final concentration of them to be 3 mM, 1 mM, 1 mM, respectively, them UV/VIS spectrum and CD were recorded in a quartz cell (path length: 1.0 mm) at 25°C.

Analysis of blue-black precipitate

The precipitate was collected by centrifugation (20,000 x *g*, 10 min) and the precipitate was washed with water twice. The residue was dissolved in 0.5% aq. HNO_3 and analyzed by HPLC (for **1**-**4**) and GFAAS (for Al).

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