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RECENT PROGRESS IN THE CHEMISTRY OF POLYACYLATED ANTHOCYANINS AS FLOWER COLOR PIGMENTS

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Abstract — It is recognized that the bluing effect and stabilization of flower colors will remarkably depend on the number of aromatic acids presented in the polyacylated anthocyanins. Although polyacyl functions would be considered to contribute strongly in the bluing of the flower color at the early stage of the investigation of polyacylated anthocyanin chemistry, the stabilization of flower color is considered to be another important objective by the presence of these aromatic acids at the present time. Since aromatic acids in the polyacylated anthocyanins are obviously linked with the sugar residues through the ester bonds, the polyacylated anthocyanins are classified into seven types by the substitution pattern of acyl functions, and the effect of the stacking structures of polyacyl anthocyanins on the stabilization of the flower color depending on the substitution pattern of acyl groups will be discussed. The physicochemical properties (NMR, UV-VIS spectra) in relation to the stability of the anthocyanins will also be discussed.

INTRODUCTION

Since the isolation of platyconin from the blue-violet flowers of *Platycodon grandiflorum* in 1971, more than 100 kinds of polyacylated anthocyanins having several molecules of aromatic acids in their structures were newly isolated. In these polyacylated anthocyanins, the aromatic acids, as acyl residues, play important roles in both stabilization and in the bluing of the flower colors. The first report for the presence of acylanthocyanin was appeared by Willstätter and Mieg in 1915, in which they mentioned that the flower

color pigment of *Delphinium* contained *p*-hydroxybenzoic acid as an acyl moiety in the acylanthocyanin structure.[1] In 1916, Willstätter and Weil also demonstrated that *p*-coumaric acid was present as the acyl unit of the pigment in pansy.[2] Until the middle of the 20th century, the occurrence of acylated anthocyanin with aromatic acid was still very rare.[3] In 1967, Harborne published his review article, in which only two kinds of plants, stock and red cabbage, were mentioned to contain the pigments, Matthiolanin and Rubrobrassicin, having more than two molecules of aromatic acids in their structures.[4]

After the appearance of this review, Saito *et al.* succeeded in the isolation of platyconin from the violet-blue flowers of bell-flower in 1971, which was proved to contain two molecules of caffeic acid, and this aromatic acid was supposed to contribute for forming the blue flower color and its stabilization in neutral conditions.[5,6] The detailed structure of platyconin was further determined by Goto *et al.*[7] After Saito's isolation work, the pigment, cinerarin, was found in the violet-blue flowers of *Senecio cruentus* by Yoshitama's group, [8,9] and also Heavenly Blue anthocyanin was isolated from *Ipomoea tricolor* by Ishikura's group,[10,11] and Asen's group,[12] independently. The structures of these pigments were afterwards determined by Goto's group.[13-17] These pigments having both three molecules of caffeic acid as the acyl function, were also proved to be stable under neutral aqueous condition.[8,11,16] Since these acylated anthocyanins isolated are structurally very interesting and also their stability in aqueous conditions is very attractive, much more research works have been devoted to figure out how the flower colors are stabilized and keep bluing.[18-29] As the results of such energetic research works, it was proposed by Goto's group[18] and Brouillard,[21,28,29] independently, that these aromatic acids contribute to keep the blue color of anthocycanins stable due to the formation of the sandwitch-like conformation depicted in Figure 1. The anthocyanins having more than two molecules of aromatic acids in their structures were called as polyacylated anthocyanins, and the characteristic interaction between the aromatic acid in the acyl residue and the aromatic moiety of aglycone was called as intramolecular co-pigmentation.[18,28,29]

In the last two decade, more than 100 kinds of new polyacylated anthocyanins were isolated and their structures were also determined based on spectroscopic and chemical degradation studies.[30-34]

In this review article, we would like to summarize recent progress in the chemistry of polyacylated anthocyanins as follows.

I. The distribution and structures of polyacylated anthocyanins in the plants.

II. The UV and VIS spectral properties in HCl-MeOH of polyacylated anthocyanins.

III. The UV and VIS spectral properties of polyacylated anthocyanins under neutral or weakly acidic condition.

IV. The ^{1}H-NMR spectra of polyacylated anthocyanins.

V. The stereostructures of polyacylated anthocyanins.

VI. Conclusion.

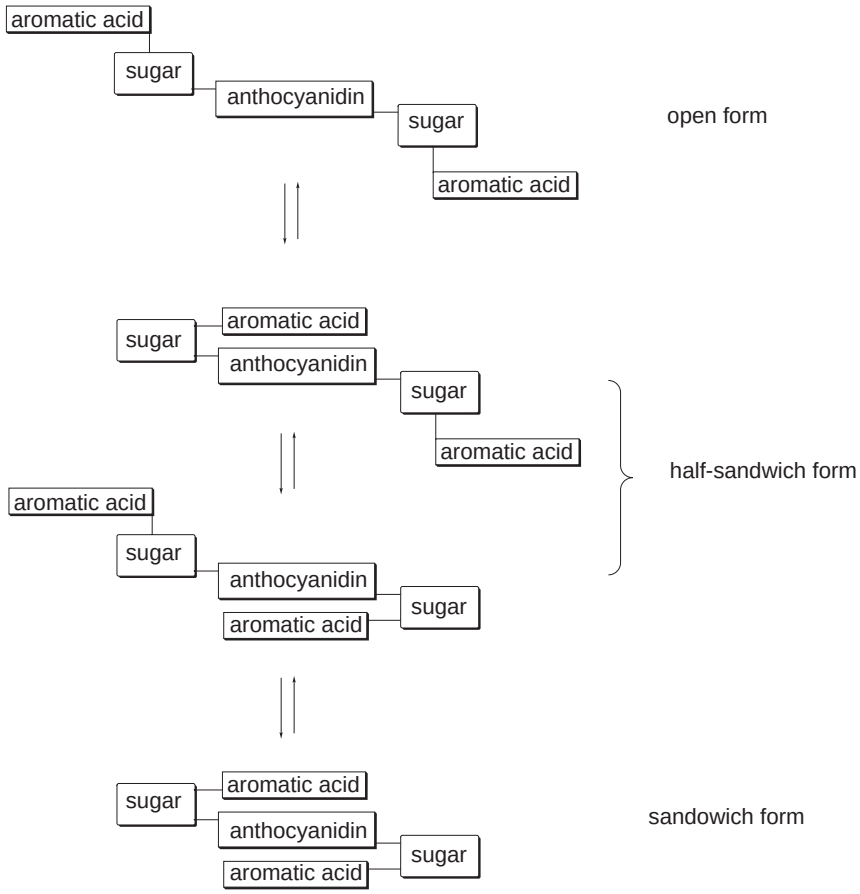


Figure 1. The conformations of polyacylated anthocyanin in solution.

I. The distribution and structures of polyacylated anthocyanins in the plants.

We classified the polyacylated anthocyanins recently isolated, into seven types of categories (1 - 7) by their structures, especially by their substitution position of the acyl-side chains in anthocyanidin, such as 1 = 3-polyacylated glycoside group, 2 = 7-polyacylated glycoside group, 3 = 3',5'-di-polyacylated glycoside groups, 4 = 7,3'-di-polyacylated glycoside groups, 5 = 3,5,3'-tri-polyacylated glycoside groups, 6 = 3,7,3'-tri-polyacylated glycoside group, and 7 = other miscellaneous groups as shown in Table 1. Regarding the aglycone in these polyacylated anthocyanins, pelargonidin, cyanidin, peonidin, petunidin,

malvidin, and delphinidin were well recognized. As aromatic acids, *p*-hydroxybenzoic acid and hydroxycinnamic acids, such as *p*-coumaric acid, caffeic acid, ferulic acid and sinapic acid, were well known, and these aromatic acids are bonded with sugars to make long side chain substituents. It is very interesting that none of the anthocyanin bonded aromatic acid directly with the hydroxy group of aglycone was isolated so far. These aromatic acids were bonded with sugars by making the ester bonds, and such sugars are attached with aglycone. Although various types of sugars are known to constitute polyacylated anthocycnins, a major sugar is glucose, and other types of sugars such as galactose, sophorose, xylosylglucose, rutinose, arabinosylglucose, xylosylgalactose, and laminalytriose are also recognized to be present in polyacylated anthocyanins. These structurally complex polyacylated pigments are commonly distributed in the more advanced families in higher plants except for the family of Ranunculaceae (Table 1).

Type 1. 3-Polyacylated glycoside group: Polyacyl functions in the sugars at the 3-position of aglycone.

Thirty-five anthocyanins belonging to this group have so far been isolated, and deacyl pigments obtained from alkaline hydrolysis of these anthocyanins were divided into the following two sub-groups: one is 3-glycoside and the other is 3,5-diglycoside.

1-A) Anthocyanidin 3-glycoside: The first pigment belonging to this sub-group was isolated from the brownish-red flowers of *Ipomoea purpurea* (Convolvulaceae).[35] This plant contains six kinds of polyacylated anthocyanins, whose basic skeleton is cyanidin 3-sophoroside. Although the major pigment (**1**) of this plant was determined as cyanidin 3-*O*-[2-*O*-(6-*O*-(4-*O*-(6-*O*-(3-*O*-(β-D-glucosyl)caffeoyl)-β-D-glucosyl)caffeoyl)-β-D-glucosyl)-β-D-glucoside] (Ipomoea brownish-red anthocyanin-2), the precise structures of other two polyacylated pigments in this flower have not been determined yet.

However, the structures of dicaffeoylcyanidin 3-*O*-sophoroside (IBRA-6) and tri(glucosylcaffeoyl)cyanidin 3-*O*-sophoroside (IBRA-5) are assumed for these pigments. Moreover, as similar pigments, pelargonidin 3-*O*-[6-*O*-(3-*O*-(β-D-glucosyl)caffeoyl)-β-D-glucoside] and peonidin 3-*O*-[6-*O*-(3-*O*-β-D-glucosyl-caffeoyl)-β-D-glucoside] were so far isolated from the brownish-red and dusky-violet flowers of *Pharbitis nil* (Convolvulaceae),[36,37] which is a related plant to *Ipomoea purpurea.*

Table 1-1. New Polyacylated Anthocyanins with Aromatic Acids in Flowers

Acyl Type (Position)	Anthocyanin	Species	Family	Deacylanthocyanin	Aromatic Acid
1. 3-Type	Ipomoea brownish-red anthocyanin	*Ipomoea purpurea*	Convolvulaceae	Cyanidin 3-sophoroside	Caffeic acid
	Petunia dasky violet anthocyanin	*Petunia hybrida*	Solanaceae	Malvidin 3-rutinoside	Caffeic acid *p*-Coumaric acid
	Evolvulus anthocyanin	*Evolvulus pilosus*	Convolvulaceae	Delphinidin 3,5-diglucoside	Caffeic acid
	Triteleia anthocyanin	*Triteleia bridgesii*	Liliaceae	Cyanidin 3,5-diglucoside	*p*-Coumaric acid
	Heavenly Blue anthocyanin	*Ipomoea tricolor* *Pharbitis nil*	Convolvulaceae Convolvulaceae	Pelargonidin 3-sophoroside-5-glucoside	Caffeic acid
	Ipomoea blue-violet anthocyanins	*Ipomoea purpurea*	Convolvulaceae	Cyanidin 3-sophoroside-5-glucoside	Caffeic acid
	Pharbitis red anthocyanins	*Pharbitis nil*	Convolvulaceae	Pelargonidin 3-sophoroside-5-glucoside	Caffeic acid *p*-Coumaric acid
	Ipomoea red-purple anthocyanins	*Ipomoea purpurea*	Convolvulaceae	Pelargonidin 3-sophoroside-5-glucoside	Caffeic acid
	Brassica anthocyanins	*Brassica oleracea*	Cruciferae	Cyanidin 3-sophoroside-5-glucoside	Ferulic, Sinapic, & *p*-Coumaric acids
	Petunia anthocyanin	*Petunia* wild species	Solanaceae	Malvidin 3-rutinoside-5-glucoside (also Petunidin and Delphinidin)	Caffeic acid *p*-Coumaric acid
	Matthiola anthocyanins	*Matthiola incana*	Cruciferae	Cyanidin 3-sambubioside-5-glucoside (also Pelargonidin)	Caffeic, Ferulic, Sinapic, & *p*-Coumaric acids
	Sinapis anthocyanins	*Sinapis alba*	Cruciferae	Cyanidin 3-sambubioside-5-glucoside	Ferulic, Sinapic, & *p*-Coumaric acids

Table 1-2. New Polyacylated Anthocyanins with Aromatic Acids in Flowers

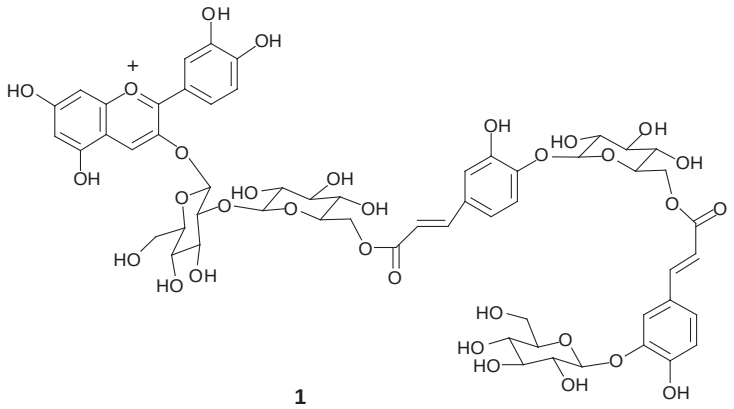
Acyl Type (Position)	Anthocyanin	Species	Family	Deacylanthocyanin	Aromatic Acid
2. 7-Type	Armeniaca anthocyanins	*Consolida armeniaca*	Ranunculaceae	Delphinidin 3,7-di-glucoside Delphinidin 3-glucoside-7-sophoroside	*p*-Hydroxybenzoic acid
	Senecio pink anthocyanins	*Senecio cruentus*	Compositae	Pelargonidin 3,7-di-glucoside	Caffeic acid
	Delphinium red anthocyanin	*Delphinium hybridum*	Ranunculaceae	Pelargonidin 3-rutinoside-7-glucoside + Pelargonidin 3,7-diglucoside	*p*-Hydroxybenzoic acid
	Platyconin	*Platycodon grandiflorum*	Campanulaceae	Delphinidin 3-rutinoside-7-glucoside	Caffeic acid
	Campanin	*Campanula medium* *C. isophylla* *C. carpatica*	Campanulaceae	Delphinidin 3-rutinoside-7-glucoside	*p*-Hydroxybenzoic acid
	Rubrocampanin	*Campanula medium*	Campanulaceae	Pelargonidin 3-rutinoside-7-glucoside	*p*-Hydroxybenzoic acid
	Monodeacylcampanin	*Campanula isophylla* *C. carpatica* *C. poskarschyana*	Campanulaceae	Delphinidin 3-rutinoside-7-glucoside	*p*-Hydroxybenzoic acid
	Violdelphin	*Aconitum chinense* *Delphinium hybridum* *Campanula isophylla,* *C. carpatica,* *C. poskarschyana*	Ranunculaceae Campanulaceae	Delphinidin 3-rutinoside-7-glucoside	*p*-Hydroxybenzoic acid
	Cyanodelphin	*Delphinium hybridum*	Ranunculaceae	Delphinidin 3-rutinoside-7-laminaritrioside	*p*-Hydroxybenzoic acid

Table 1-3. New Polyacylated Anthocyanins with Aromatic Acids in Flowers

Acyl Type (Position)	Anthocyanin	Species	Family	Deacylanthocyanin	Aromatic Acid
3. 3' or 3', 5'-Type	Ternatin A ~ D Preternatin A3 and C4	*Clitoria ternatea*	Leguminosae	Delphinidin 3,3',5'-triglucoside	*p*-Coumaric acid
4. 7, 3'-Type	Cinerarin	*Senecio cruentus*	Compositae	Delphinidin 3,7,3'-triglucoside	Caffeic acid
	Rubrocinerarin	*Senecio cruentus* *Gynura aurantiaca*	Compositae Compositae	Cyanidin 3,7,3'-triglucoside	Caffeic acid
	Laeliocattleya anthocyanins	*Cattleya walkeriana* *Laelia pumila*	Orchidaceae Orchidaceae	Cyanidin 3,7,3'-triglucoside	Caffeic, Ferulic & *p*-Coumaric acids
	Bletilla anthocyanins 1-8	*Bletilla striata*	Orchidaceae	Cyanidin 3,7,3'-triglucoside	Caffeic acid *p*-Coumaric acid
	Phalaenopsis anthocyanins	*Phalaenopsis hybrids*	Orchidaceae	Cyanidin 3,7,3'-triglucoside	Ferulic and Sinapic acids
	Dendrobium anthocyanin	*Dendrobium* "Pramot"	Orchidaceae	Cyanidin 3,7,3'-triglucoside	*p*-Hydroxybenzoic acid
	Ceanothus anthocyanin	*Ceanothus papillosus*	Rhamnaceae	Delphinidin 3-rutinoside-7,3'-diglucoside	*p*-Coumaric acid

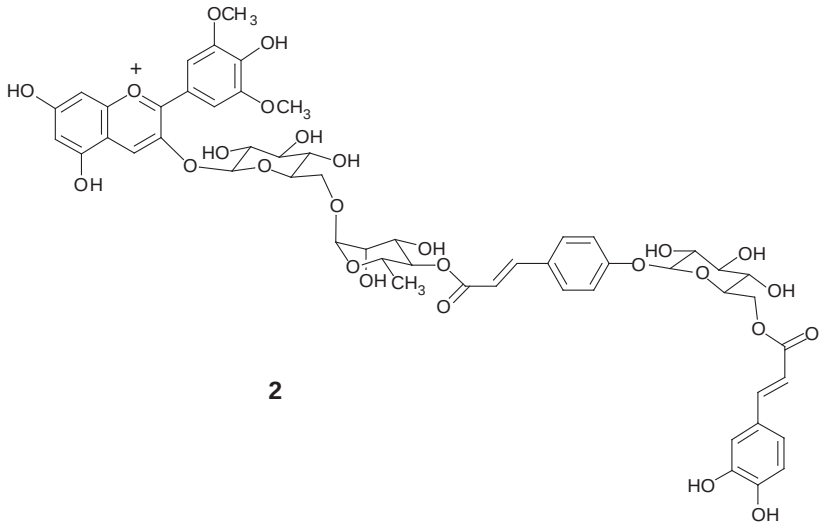
Table 1-4. New Polyacylated Anthocyanins with Aromatic Acids in Flowers

Acyl Type (Position)	Anthocyanin	Species	Family	Deacylanthocyanin	Aromatic Acid
5. Poly-mono-acyl-type	Alatanin A	*Dioscorea alata*	Dioscoreacea	Cyanidin 3-glucoside (1-3) glucosyl(1-6)glucoside-7,3'-diglucoside	Sinapic acid
	Lobelinins	*Lobelia erinus*	Lobeliaceae	Delphinidin 3-rutinoside-5,3',5'-triglucoside	Caffeic, Ferulic & *p*-Coumaric acids
	Lobelia red anthocyanin	*Lobelia erinus*	Lobeliaceae	Cyanidin 3-rutinoside-5,3'-diglucoside	Caffeic, Ferulic & *p*-Coumaric acids
	Zebrinin	*Zebrina pendula*	Commelinaceae	Cyanidin 3-arabinosyl-glucoside-7,3'-di-glucoside	Caffeic acid Ferulic acid
	Tradescantia anthocyanin	*Tradescantia pallida* *T. reflex*	Commelinaceae Commelinaceae	Delphinidin 3-arabinosyl-glucoside-7,3'-di-glucoside (also Cyanidin)	Caffeic acid Ferulic acid
6. Poly-mono-acyl-type	Gentiodelphin	*Gentiana makinoi*	Gentianaceae	Delphinidin 3,5,3'-tri-glucoside	Caffeic acid
	Gentiocyanins A ~ C	*Gentiana cv.*	Gentianaceae	Cyanidin 3,5,3'-tri-glucoside	Caffeic acid
	Albireodelphins	*Gentiana cv.*	Gentianaceae	Delphinidin 3,5,3'-tri-glucoside	Caffeic acid *p*-Coumaric acid
7. Diester of Malonic acid	Eichhornia anthocyanin	*Eichhornia crassipes*	Pontederiaceae	Delphinidin 3-gentiobioside	Malonic acid
	Anemone anthocyanin	Anemone coronaria	Ranunculaceae	Pelargonidin 3-xylosyl-galactoside	Glucosylcaffeic acid Tartaric acid
	Agapanthus anthocyanin	*Agapanthus praecox*	Liliaceae	Delphinidin 3,7-diglucoside	*p*-Coumaric acid Succinic acid
	Anemone blue-violet anthocyanins	*Anemone coronaria*	Ranunculaceae	Delphinidin 3-xylosyl-galactoside-7-glucoside-3'-glucuronide	Caffeic acid Tartaric acid



1

Very recently, a pigment having malvidin as its aglycone, malvidin 3-*O*-[6-*O*-(4-*O*-(4-*O*-(6-*O*-caffeoyl-β-D-glucosyl)-*p*-coumaryl)-α-L-rhamnosyl)-β-D-glucoside] (Petunia dasky-violet anthocyanin) (**2**), was isolated from the dusky violet flowers of *Petunia integrifolia* subsp. *inflata*[38] (Solanaceae). Deacyl compound of this pigment was malvidin 3-rutinoside. Commonly, the pigments in flowers of *Petunia* genus, *Ipomoea. purpurea,* and *Pharbitis*. *nil* are composed from anthocyanidin 3,5-glycoside as described in the sections 1-B, and the pigments with 3-glycoside are very rare in these plants. Therefore, these flower pigments may be formed by the loss of anthocyanin 5-glucosyltransferase, which plays a key role in glycosidation of the hydroxy group at the 5-position of the aglycone.

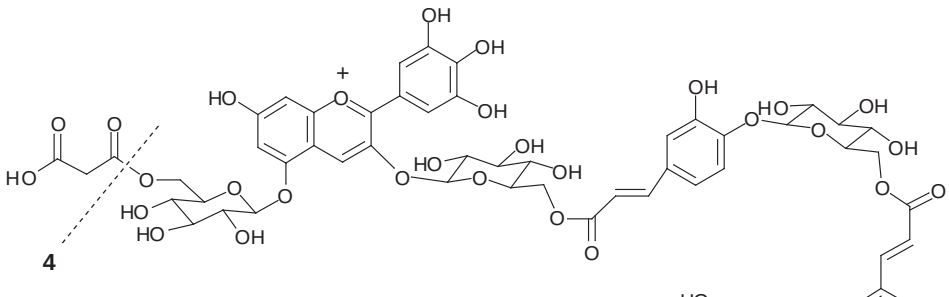


2

1-B) Anthocyanidin 3,5-diglycoside: The basic glycoside structure belonging to this sub-group was 3,5-diglycoside and the polyacyl functions were usually attached only to the sugars at the 3-position. The pigments of this sub-group were further divided into the following four types by the structure of the sugars.

1-B-1) Glucose as the sugar residue at the 3-position.

From the blue flowers of *Evolvulus pilosus* (Convolvulaceae), we have isolated a blue pigment, so called Evolvulus anthocyanin 1 (EA-1), whose structure was determined as delphinidin 3-*O*-[6-*O*-(4-*O*-(6-*O*-(3-*O*-β-D-glucosylcaffeoyl)-β-D-glucosyl)caffeoyl)-β-D-glucoside]-5-*O*-[6-*O*-(malonyl)-β-D-glucoside] (**3**).[39]



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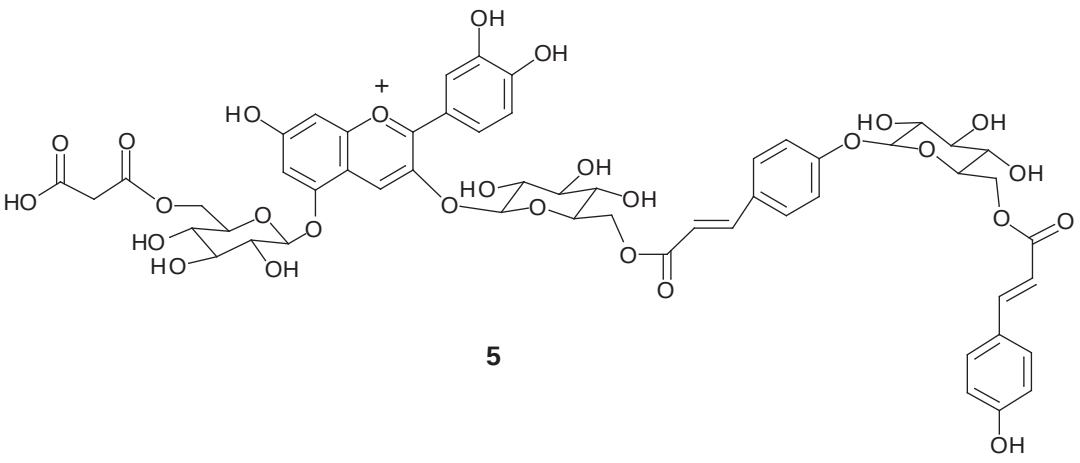
3

3: Evolvulus anthocyanin 1 (EA-1)
4: Evolvulus anthocyanin 2 (EA-2)

Deacyl compound of this pigment was delphinidin 3,5-*O*-di-β-D-glucoside. The substitution pattern of the long side chain in this pigment was very similar to that of polyacylated pigments from *Ipomoea* and *Pharbitis* plants. The observed difference of the side chains between this pigment and the other pigments, such as *Ipomoea* and *Pharbitis* pigments, is that there is no branched substituent at the 2-OH position of 3-glucoside in this pigment. In addition to this pigment, another pigment, Evolvulus anthocyanin 2, was also isolated from this plant, and has the same side chain at the 3-position of delphinidin. Its structure was determined as the demalonyl derivative of Evolvulus anthocyanin 1 (**4**).



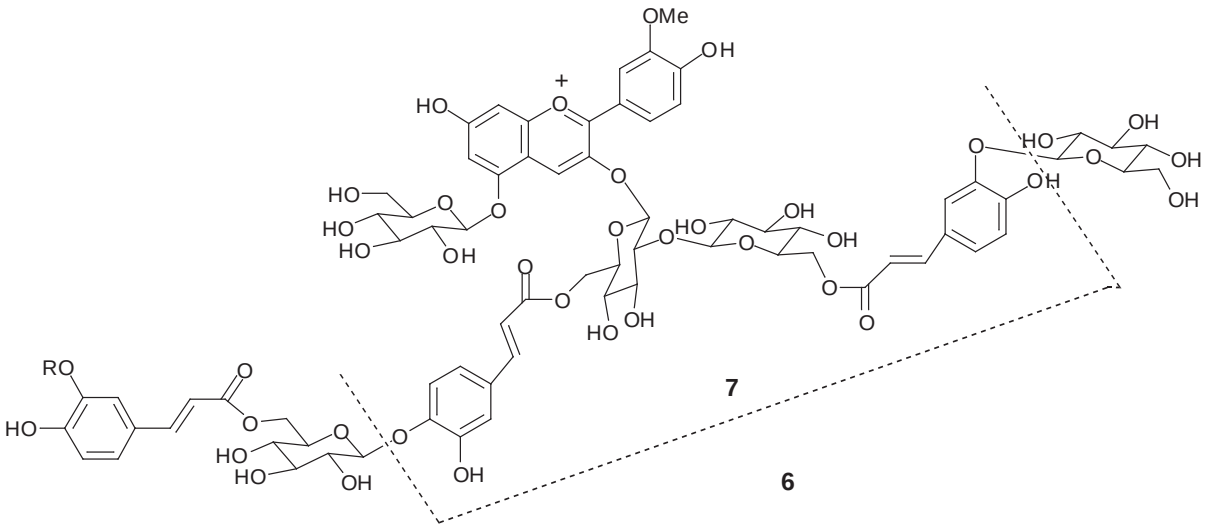
5

The similar type of substitution pattern was observed in the structure of Triteria anthocyanin; cyanidin 3-*O*-[6-*O*-(4-*O*-(6-*O*-(*p*-coumaroyl)-β-D-glucosyl)-*p*-coumaroyl)-β-D-glucoside]-5-*O*-[6-*O*-malonyl-β-D-glucoside] (**5**), which was isolated from the blue-violet flowers of *Triteria bridgesii* (Liliaceae) as a minor pigment.[40] In this pigment, *p*-coumaroyl group was attached in stead of caffeoyl group as its side chain. Moreover, the glucose residue at the 5-position was acylated with malonic acid, as similar to Evolvulus anthocyanin 2.

1-B-2) Sophorose as the sugar residue at the 3-position.

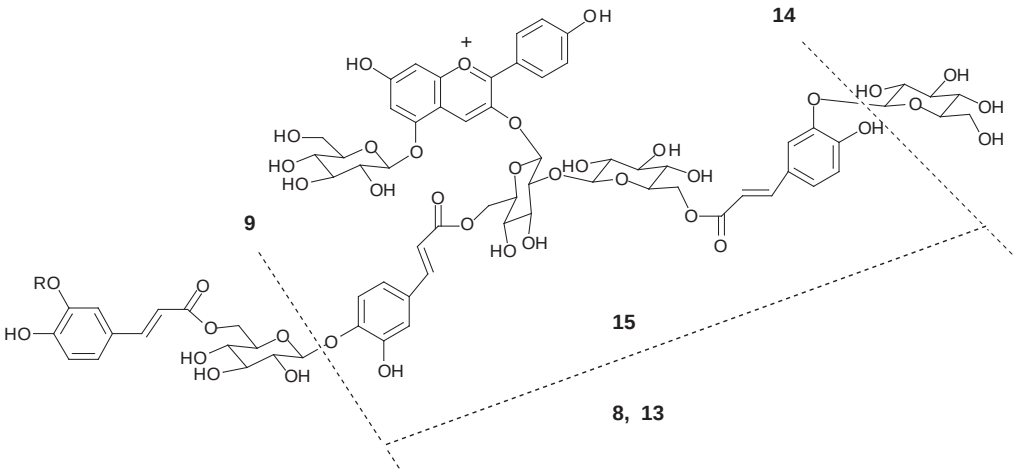
This type of pigments were mainly observed in the flowers of Convolvulaceae, such as *Pharbitis*[41,42,45] and *Ipomoea* genus,[43,44] and also found to be present in the leaves, stems and roots of *Brassica* [46,47] and *Raphanus*[4] (Cruciferae).

In the Convolvulaceae, a polyacylated anthocyanin, Heavenly Blue anthocyanin having a very complicated structure, was isolated from the blue flowers of *Ipomoea tricolor* by Ishikura and Shimizu,[10] and Asen *et al.*[12] The structure of this pigment was determined to be peonidin 3-*O*-[2-*O*-(6-*O*-(3-*O*-(β-D-glucosyl)-caffeoyl)-β-D-glucosyl)-6-*O*-(4-*O*-(6-*O*-(3-*O*-(β-D-glucosyl)caffeoyl)-β-D-glucosyl)caffeoyl)-β-D-glucoside]-5-*O*-β-D-glucoside (**6**) with its molecular weight of 1759 by Kondo *et al*. in 1987.[17]



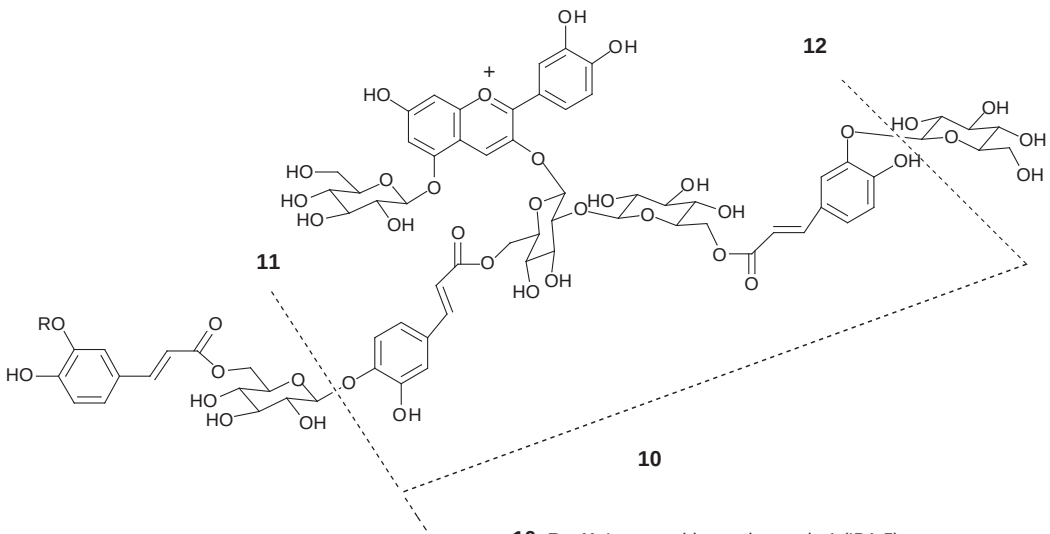
6: R = Glucose; Heavenly Blue Anthocyanin
7: Pharbitis blue Anthocyanin 3

We have also demonstrated that this pigment is distributed widely in the blue flowers of *Pharbitis nil* in 1992.[41] Interestingly, three more acylated pigments, peonidin 3-*O*-[2-*O*-(caffeoyl-β-D-glucosyl)-6-*O*-caffeoyl-β-D-glucoside]-5-*O*-β-D-glucoside (Pharbitis Blue anthocyanin-3) (**7**) bearing two molecules of caffeic acid, peonidin 3-*O*-[2-*O*-(β-D-glucosyl)-6-*O*-caffeoyl-β-D-glucoside]-5-*O*-β-D-glucoside (Pharbitis Blue anthocyanin-1), and peonidin 3-*O*-[2-*O*-(β-D-glucosyl)-6-*O*-(β-D-glucosylcaffeoyl)-β-D-glucoside]-5-*O*-β-D-glucoside (PBA-2), were also found in the blue flowers of *Pharbitis nil*. On the other hand, two polyacylated pelargonidin glycosides (Pharbitis red anthocyanins) were isolated from the red flowers of *Pharbitis nil*.[42] One of them has three molecules of caffeic acid and the other contains two molecules of caffeic acid. Based on the careful spectral analyses, the structure of this pigment having three molecules of caffeic acid was determined as pelargonidin 3-*O*-[2-*O*-(6-*O*-(3-*O*-(β-D-glucosyl)caffeoyl)-β-D-glucosyl)-6-*O*-(4-*O*-(6-*O*-(3-*O*-(β-D-glucosyl)caffeoyl)-β-D-glucosyl)caffeoyl)-β-D-glucoside]-5-*O*-β-D-glucoside (Pharbitis red anthocyanin-5) (**8**). The difference between Heavenly-Blue anthocyanin and this pigment is observed only in the aglycone parts; the former has peonidin and the later pelargonidin. The structure of the second pigment is pelargonidin 3-*O*-[2-*O*-(6-*O*-(3-*O*-(β-D-glucosyl)caffeoyl)-β-D-glucosyl)-6-*O*-caffeoyl-β-D-glucoside]-5-*O*-β-D-glucoside (PRA-3) (**9**).



8: **R = Glucose**; Pharbitis red anthocyanin 5 (PRA 5)
9: PRA 3
13: **R = H**; Ipomoea red anthocyanin 1 (IRA 1)
14: **R = H**; IRA 2
15: **R = H**; IRA 4

The structurally very similar pigments were also present in the flowers of *Ipomoea purpurea*.[43,44] In general, the blue flowers of this plant contain cyanidin pigments and red flowers contain pelargonidin pigments as their major anthocyanins, respectively. We named these blue pigments from *Ipomoea purpurea* as Ipomoea blue anthocyanins (IBA), in which the pigment (IBA-1) having the largest molecular weight contained three molecules of caffeic acid, and the characteristic feature of IBA-1 regarding the side chains can be seen with a loss of one glucose compared with those of HBA, as depicted as **6**. The structure of IBA-1 was determined to be cyanidin 3-*O*-[2-*O*-(6-*O*-(3-*O*-(β-D-glucosyl)caffeoyl)-β-D-glucosyl)-6-*O*-(4-*O*-(6-*O*-caffeoyl-β-D-glucosyl)caffeoyl)-β-D-glucoside]-5-*O*-β-D-glucoside (**10**), which contains three molecules of caffeic acid. Furthermore, the structures of IBA-2 and -3 were confirmed to be the pigments of cyanidin type, and they contain two molecules of caffeic acid in their structures. Finally, the structures of IBA-2 and IBA-3 were determined to be cyanidin 3-*O*-[2-*O*-(6-*O*-(3-*O*-(β-D-glucosyl)caffeoyl)-β-D-glucosyl)-6-*O*-caffeoyl-β-D-glucoside]-5-*O*-β-D-glucoside (**11**) and cyanidin 3-*O*-[2-*O*-(6-*O*-caffeoyl-β-D-glucosyl)-6-*O*-caffeoyl-β-D-glucoside]-5-*O*-β-D-glucoside (**12**), respectively.[43]



10: **R = H**; Ipomoea blue anthocyanin 1 (IBA 5)
11: IBA 2
12: IBA 3

In the red flowers of *Ipomoea purpurea*, four kinds of pigments having more than two molecules of caffeic acid are found. One of those pigments is identified as the same pigment with PRA-3 (**9**), which had already been isolated from *Pharbitis nil*.[42] Therefore, only three of Ipomoea red anthocyanins are recognized as new pigments, which are called as IRA-1 (**13**), -2 (**14**), and –4 (**15**). Based on the careful analysis of its

spectroscopy, IRA-1 was determined to be pelargonidin 3-*O*-[2-*O*-(6-*O*-(3-*O*-(β-D-glucosyl)caffeoyl)-β-D-glucosyl)-6-*O*-(4-*O*-(6-*O*-caffeoyl-β-D-glucosyl)caffeoyl)-β-D-glucoside]-5-*O*-β-D-glucoside (**13**).[44] This pigment has exactly the same side chain as that of IBA-1. The observed difference between IRA-1 and IBA-1 was aglycone part, where pelarginidin was found in the former pigment and cyanidin in the later pigment. The structure of IRA-2 was also elucidated as pelargonidin 3-*O*-[2-*O*-(6-*O*-caffeoyl-β-D-glucosyl)-6-*O*-(4-*O*-(6-*O*-caffeoyl-β-D-glucosyl)caffeoyl)-β-D-glucoside]-5-*O*-β-D-glucoside (**14**), which again involved three molecules of caffeic acid. The substitution pattern of the side chain of IRA-4 (**15**) was the same as that of IBA-3 (**12**), and the aglycone was again pelargonidin.

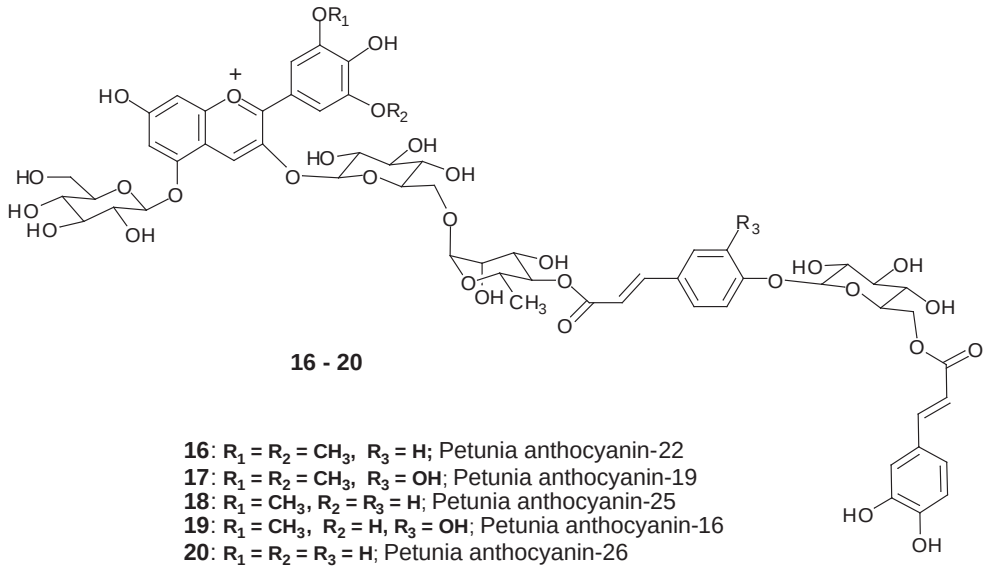
As discussed so far, the structures of the largest pigments of these three species, *Pharbitis nil, Ipomoea purpurea,* and *Ipomoea tricolor,* are closely related with each other. The main pigments (**10, 13**) in *Ipomoea purpurea* have only one glucose less than those (**6, 8**) in *Pharbitis nil* and *Ipomoea tricolor* on their side chains. Usually the presence of cyanidin as an aglycone makes the blue color stronger than that of peonidin. However, the blue color of the flowers of *Pharbitis nil* and *Ipomoea tricolor* containing peonidin as an aglycone, is much stronger than the flower color of *Ipomoea purpurea* containing cyanidin. This fact is very hard to explain the color variation by the presence or absence of methylation in anthocyanidin, and even by the difference of molecular number of glucose. The color of *Ipomoea purpurea* (aglycone: cyanidin) showed rather violet-blue. These phenomena must be explained by the other factor, such as pH degree in cell sap or co-pigmentation with phenolic compounds. On the other hand, regarding the red color of these flower pigments (IRA), none of such problematic phenomena was observed, since almost all of the same red flowers of these plants, *Pharbitis nil, Ipomoea purpurea, and Ipomoea tricolor,* have the similar side chains as seen in Heavenly Blue anthocyanin (**6**), and also have the same aglycone, pelargonidin.

The other example in this class is Red cabbage anthocyanins (RCA), isolated from the leaves of red cabbage *Brassica oleracea.*[46,47] From this red cabbage, three kinds of diacylanthocyanins having sophorose at the 3-position of the aglycone are isolated and named as Red cabbage anthocyanin-1, -2, and -3. The side chains of these pigments are a little shorter than those of *Pharbitis nil*. The structures of these three pigments are determined as cyanidin 3-*O*-[2-*O*-(2-*O*-sinapoyl-β-D-glucosyl)-6-*O*-*p*-coumaroyl-β-D-glucoside]-5-*O*-β-D-glucoside (RCA-1), cyanidin 3-*O*-[2-*O*-(2-*O*-sinapoyl-β-D-glucosyl)-6-*O*-feruloyl-β-D-glucoside]-5-*O*-β-D-

glucoside (RCA-2), and cyanidin 3-*O*-[2-*O*-(2-*O*-sinapoyl-β-D-glucosyl)-6-*O*-sinapoyl-β-D-glucoside]-5-*O*-β-D-glucoside (RCA-3), respectively.

1-B-3) Rutinoside as the acylated sugar residue at the 3-position.

Recently five acylated pigments (Petunia anthocyanins; PA) bearing two molecules of hydroxycinnamic acid were isolated from the flowers of *Petunia* group.[48-51] In these pigments, the first aromatic acid group is bonded with the 4-OH position of rhamnose residue as the glucosylcaffeic acid or glucosyl-*p*-coumaric acid unit as can be seen in the structures (**16-20**). In addition to this first aromatic acid group, another caffeic acid is esterified with the terminal glucose at the 6-OH position. In 1997, two pigments were isolated from the red-violet flowers of *Petunia guarapuavensis*.[48] These structures were determined as malvidin 3-*O*-[6-*O*-(4-*O*-(4-*O*-(6-*O*-caffeoyl-β-D-glucosyl)-*p*-coumaroyl)- α-L-rhamnosyl)-β-D-glucoside]-5-*O*-β-D-glucoside (PA-1) (**16**) and malvidin 3-*O*-[6-*O*-(4-*O*-(4-*O*-(6-*O*-caffeoyl-β-D-glucosyl)caffeoyl)- α-L-rhamnosyl)-β-D-glucoside]-5-*O*-β-D-glucoside (PA-19) (**17**).



16 - 20

16: **R_1 = R_2 = CH_3, R_3 = H;** Petunia anthocyanin-22
17: **R_1 = R_2 = CH_3, R_3 = OH**; Petunia anthocyanin-19
18: **R_1 = CH_3, R_2 = R_3 = H**; Petunia anthocyanin-25
19: **R_1 = CH_3, R_2 = H, R_3 = OH**; Petunia anthocyanin-16
20: **R_1 = R_2 = R_3 = H**; Petunia anthocyanin-26

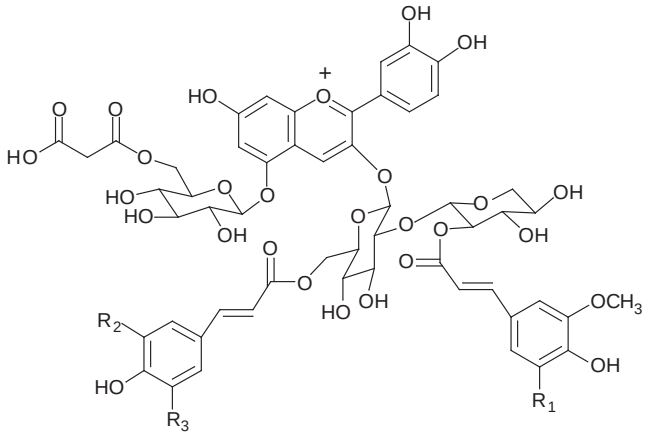
These pigments are also recognized to be present in the flowers of cultivated *Petunia hybrida* by Fukui *et al.*[49] and Ando *et al.*[50] More recently, they published the isolation of three novel pigments, having the same

substitution pattern of the side chains with different aglycone, such as petunidin [PA-25 (**18**) and PA-16 (**19**)][50] and delphinidin (PA-26) (**20**),[51] from the further investigation of *Petunia* group.

1-B-4) Sambubiose as the sugar residue at the 3-position.

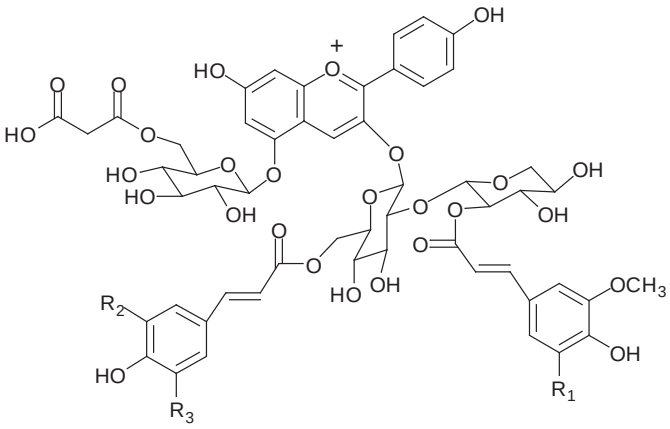
In the Cruciferae, some plants such as *Matthiola incana*[52,53] and *Sinapis alba*[54] contain pelargonidin and cyanidin series of pigments, which have sambubioside as the sugar residue at the 3-position. However, some pigments in this family have sophorose as their sugar residue at the 3-position such as the plants of Raphanus *sativus*[4,55,56] and *Brassica. oleracea*[46,47] The cyanidin pigments (four pigments) in *Matthiola* red-violet flowers (*Matthiola incana*) are called as Matthiola violet anthocyanins, MVA,[52] and pelargonidin pigments (five pigments) in *Matthiola* red flowers are also called as Matthiola red anthocyanins, MRA.[53]

The above pigments, MVA and MRA, have the same side chain, and two kind of aromatic acids are found to attach to sambubioside at the 3-position of anthocyanins. Whereas, the structures of Matthiola violet anthocyanins, MVA 1-3, isolated from the red-violet flowers of *Matthiola incana*, are also determined to be acylated cyanidin glycosides, in addition to MVA-4, a demalonyl derivative of MVA-1. On the basis of their spectral data and chemical degradation procedures, the structures of MVA-1, -2, and -3 were confirmed to be cyanidin 3-*O*-[2-*O*-(2-*O*-sinapoyl-β-D-xylosyl)-6-*O*-feruloyl-β-D-glucoside]-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**21**), cyanidin 3-*O*-[2-*O*-(2-*O*-sinapoyl-β-D-xylosyl)-6-*O*-*p*-coumaloyl-β-D-glucoside]-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**22**), and cyanidin 3-*O*-[2-*O*-(2-*O*-sinapoyl-β-D-xylosyl)-6-*O*-caffeoyl-β-D-glucoside]-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**23**), respectively.



21: $R_1 = R_2 = OCH_3$, $R_3 = H$; Matthiola violet anthocyanin 1 (MVA-1)
22: $R_1 = OCH_3$, $R_2 = R_3 = H$; MVA-2
23: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$; MVA-3

As mentioned above, MRA-1 and -2 are determined as both acylated pelargonidin glycoside pigments of the red flowers. The characteristic structural difference between these pigments was found in the acyl groups. MRA-1 has feruloyl group and MRA-2 has sinapoyl group in their molecules, respectively. Accordingly, the structures of MRA-1 and -2 were determined as pelargonidin 3-*O*-[2-*O*-(2-*O*-feruloyl-β-D-xylosyl)-6-*O*-feruloyl-β-D-glucoside]-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**24**) and pelargonidin 3-*O*-[2-*O*-(2-*O*-sinapoyl-β-D-xylosyl)-6-*O*-feruloyl-β-D-glucoside]-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**25**), respectively. Moreover, MRA-3 in the red flowers was determined to be pelargonidin 3-*O*-[2-*O*-(2-*O*-sinapoyl-β-D-xylosyl)-6-*O*-*p*-coumaloyl-β-D-glucoside]-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**26**). Two demalonylated pigments, MRA-4 and -7, were also isolated together with the above pigments from the same flowers, and the former was identified as demalonyl MRA-2 (**25**) and the later as demalonyl MRA-3 (**26**).[53]



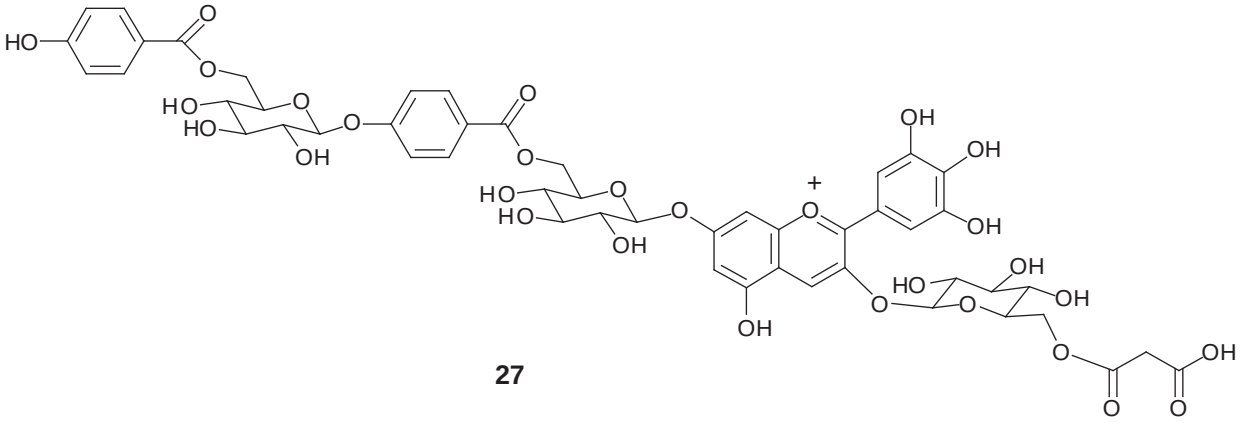
24: $R_1 = R_2 = H$, $R_3 = OCH_3$; Matthiola red anthocyanin 1 (MRA-1)
25: $R_1 = R_2 = OCH_3$, $R_3 = H$; MRA-2
26: $R_1 = OCH_3$, $R_2 = R_3 = H$; MRA-3

Type 2. 7-Polyacylglycoside group.

The second type of polyacylated anthocyanins includes polyacyl functions only in the sugar residue at the 7-position of the aglycone. Isolation of thirteen pigments belonging to this group was so far reported. In the plants of the Ranunculaceae as major pigment sources, the aromatic acid group presented in this group is commonly *p*-hydroxybenzoic acid instead of cinnamic acid. However, the presence of caffeic acid was also reported in the flowers of Senecio (Compositae).[59] An exceptional intermediate was observed in the plants of Campanulaceae, where caffeic acid was involved in the flowers of *Platycodon grandiflorum*,[5,60] and *p*-

hydroxybenzoic acid was observed in *Campanula medium*.[61,62] The pigments of this group are further classified into the following four types of sub-groups by their substitution pattern of the sugar residues of deacylanthocyanin. The first sub-group is 3-glucoside-7-glucoside (3,7-diglucoside), the second is 3-rutinoside-7-glucoside, the third is 3-rutinoside-7-sophoroside, and the last one is 3-rutinoside-7-laminaritrioside.

2-A) In the first sub-group pigments, their deacylanthocyanin have 3,7-diglucoside unit. On the occurrence of this sub-group, the two types of pigments are so far reported. The first type is represented by the pigment isolated from the violet-blue flowers of *Consolida armeniaca* (Ranunculaceae).[57] Its structure was determined as delphinidin 3-*O*-[6-*O*-malonyl-β-D-glucoside]-7-*O*-[6-*O*-(4-*O*-(6-*O*-*p*-hydroxybenzoyl-β-D-glucosyl)-*p*-hydroxybenzoyl)-β-D-glucoside] (Armeniaca anthocyanin-1) (**27**).



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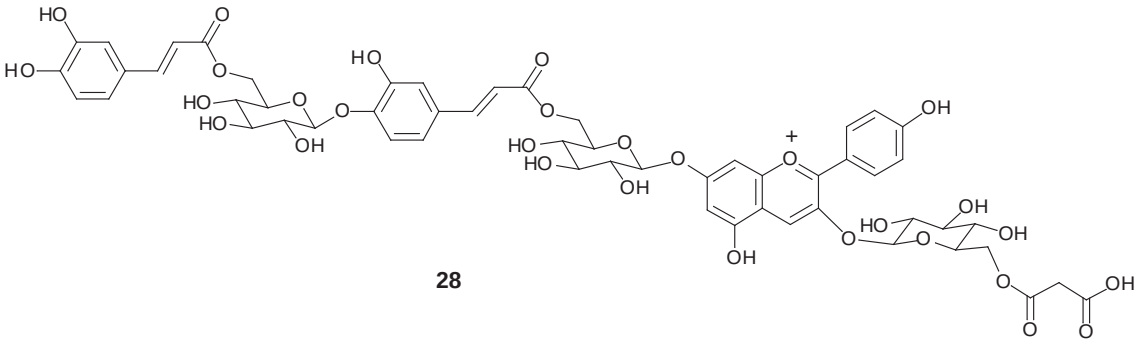
This pigment is a minor component in the flowers of this plant, and its deacylanthocyanin is delphinidin 3,7-diglucoside. On the other hand, the major pigment components of this plant exhibit the structures having sophorose instead of glucose at the 7-position, which will be mentioned later on as the pigments of the fourth sub-group.

Another example of the anthocyanin with 3,7-diglucoside in the plant of Ranunculaceae is a pigment from the red flowers of *Delphinium hybridum* 'Princes Caroline', which contained only one molecule of *p*-hydroxybenzoic acid.[58] The structure of this pigment was identified as pelargonidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-7-*O*-{[6-*O*-(4-*O*-(β-D-glucosyl)-*p*-hydroxybenzoyl)-β-D-glucoside].

The last pigment of this sub-group was found in the pink flowers of *Senecio cruentus* (Compositae) as Senecio pink anthocyanin-1.[59] Its structure was determined as pelargonidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-7-*O*-[6-*O*-(4-*O*-(6-*O*-caffeoyl-β-D-glucosyl)caffeoyl)-β-D-glucoside] (**28**) and two molecules of caffeic acid are contained in this pigment.



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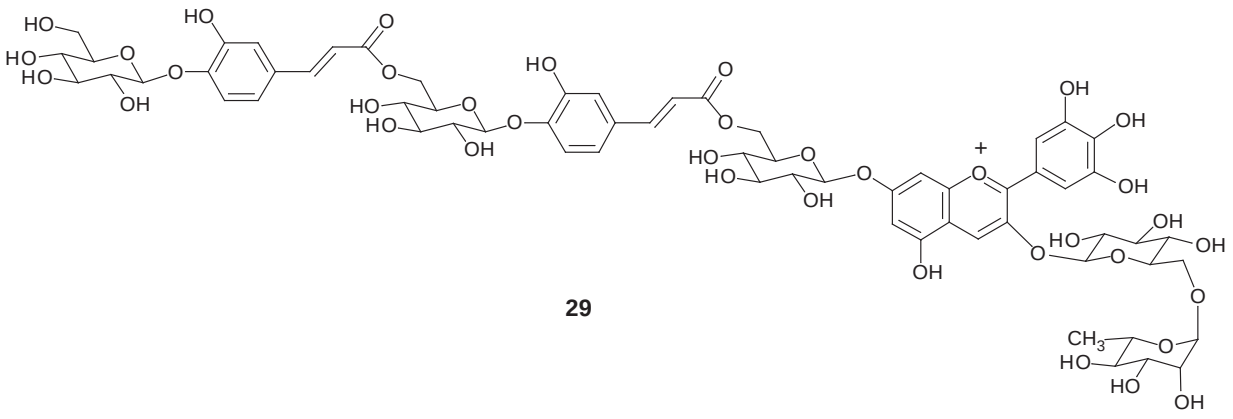
2-B) The pigments of the second sub-group contain a deacylanthocyanin having 3-rutinoside-7-glucoside unit. It is well recognized that both *p*-hydroxybenzoic acid and caffeic acid are involved as aromatic acid components in the pigments of this sub-group, and their acyl-substitution pattern is the same as the pigments belonging to the first sub-group. The typical example was the pigment, platyconin, which was isolated from the violet-blue flowers of *Platycodon grandiflorium* by Saito *et al*.[5,6] and its structure was determined by Goto *et al*. as delphinidin 3-*O*-rutinoside-7-*O*-[6-*O*-(4-*O*-(6-*O*-(4-*O*-(β-D-glucosyl)caffeoyl)-β-D-glucosyl)caffeoyl)-β-D-glucoside] (**29**).[60] This pigment is relatively stable in neutral aqueous solution and is the first example to prove making the intramolecular co-pigmentation for stabilizing the flower color.[6,60,66]



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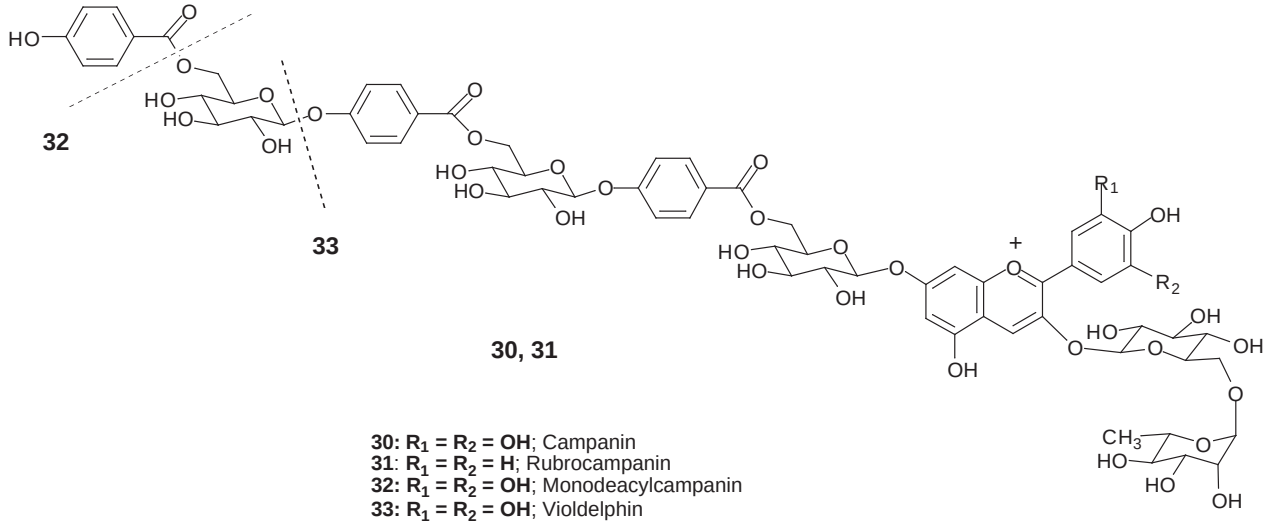
As structurally similar pigments to platyconin, two more pigments were isolated from *Campanula medium*.[61] One was named as campanin which was isolated from the blue-violet flowers of *Campanula medium*, and the structure of this pigment was confirmed to have delphinidin as an aglycone. The other pigment, rubrocampanin, was isolated from the pink flowers of this plant and had pelargonidin as an aglycone. Both pigments contained three molecules of *p*-hydroxybenzoic acid instead of caffeic acid as observed in platyconin, and the detailed structures were determined to be pelargonidin and delphinidin 3-*O*-rutinoside-7-*O*-[6-*O*-(4-*O*-(6-*O*-(4-*O*-(6-*O*-*p*-hydroxybenzoyl-β-D-glucosyl)-*p*-hydroxybenzoyl)-β-D-glucosyl)-*p*-hydroxybenzoyl)-β-D-glucoside] (**30** and **31**), respectively.

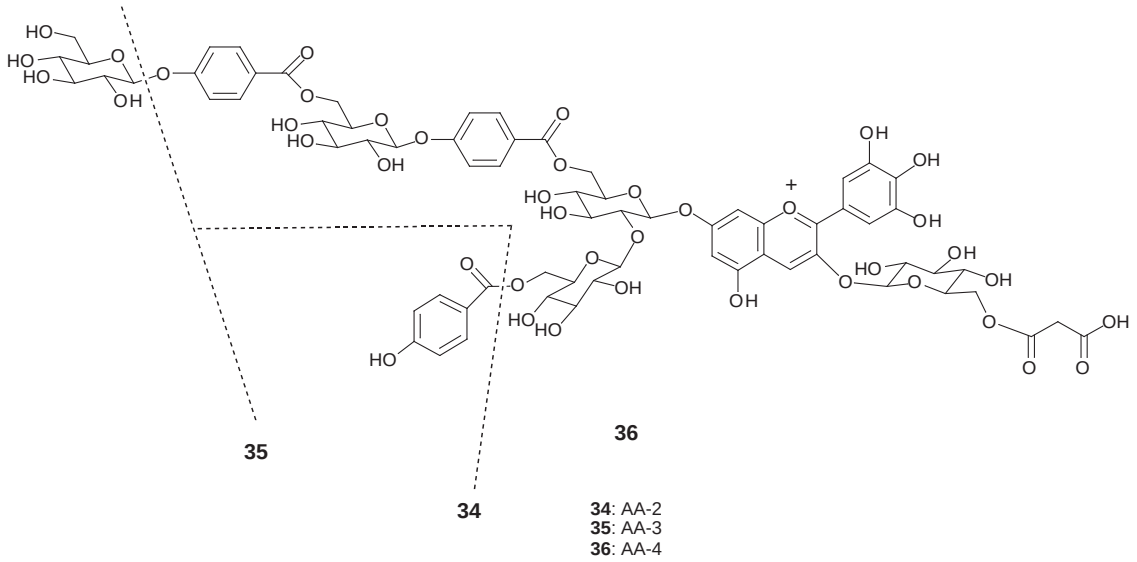
In *Campanula isophylla, C. carpatica*, and *C. poskarschyana* which belong to the same genus *Campanula* plant, campanin (**30**) and mono-deacylcampanin (**32**) having two molecules of *p*-hydroxybenzoic acid were isolated.[62] Moreover, violdelphine (**33**) with the loss of each one molecule of glucose and *p*-hydroxybenzoic acid, was further isolated from these plants. Violdelphine was also isolated from two plants of the Ranunculaceae, *Delphinium hybridum*[63] and *Aconitum chinense*.[64] This pigment has *p*-hydroxybenzoyl-β-D-glucosyl-*p*-hydroxybenzoic acid at the 7-glucoside as the acyl residue.



In *Delphinium hybridum*, the pigment of pelargonidin type with only one molecule of an aromatic acid at the 7-glucoside was also isolated from the red flowers of Princes Caroline.[58]

2-C) This sub-group pigments contain deacylanthocyanins with 3-rutinoside-7-sophoroside unit.

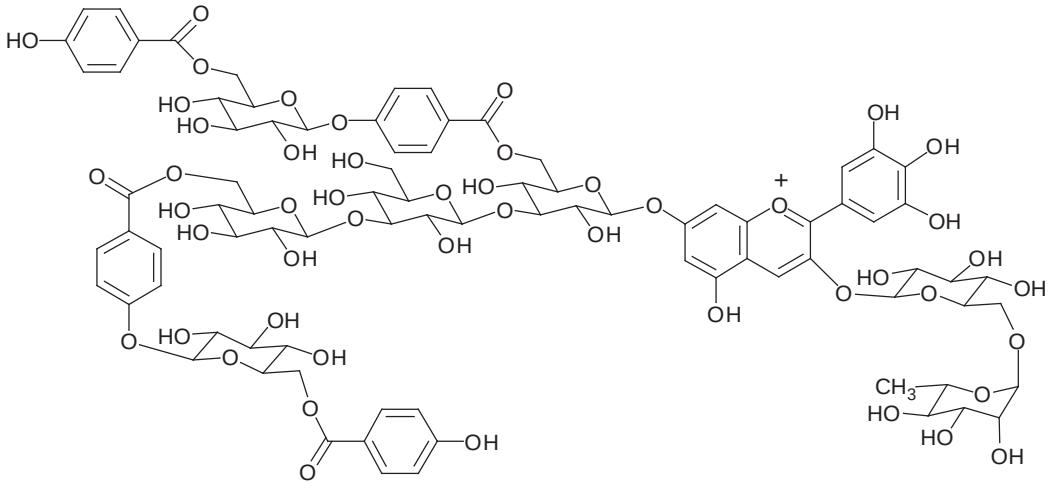
As described previously, we have isolated four acylated delphinidin glycosides from the violet-blue flowers of *Consolida armeniaca*.[50] These pigments are called as Armeniaca anthocyanins (AA). One of them, AA-1 belongs to the group 2-A as already mentioned, and the other three pigments belong to this sub-group.[57]



The structure of AA-2 was determined as delphinidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-7-*O*-[2-*O*-(β-D-glucosyl)-6-*O*-(4-*O*-(6-*O*-*p*-hydroxybenzoyl-β-D-glucosyl)-*p*-hydroxybenzoyl)-β-D-glucoside] (**34**), and AA-3 was determined to be delphinidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-7-*O*-[2-*O*-(6-*O*-*p*-hydroxybenzoyl-β-D-glucosyl)-6-*O*-(4-*O*-(6-*O*-*p*-hydroxybenzoyl-β-D-glucosyl)-*p*-hydroxybenzoyl)-β-D-glucoside] (**35**), which had one more molecule of *p*-hydroxybenzoic acid than AA-2. Finally, AA-4 (**36**) was elucidated to be the pigment having one more molecule of glucose than AA-3. The major anthocyanins of this flower were AA-2 and AA-3.

2-D) The pigment of the group 2-D contains a deacylanthocyanin having 3-rutinoside-7-laminaritrioside unit. Only one pigment belonging to this sub-group was reported so far, which was isolated from the blue flowers of *Delphinium hybridum*.[65] This pigment contains four molecules of *p*-hydroxybenzoic acid. Although its structure was proposed as delphinidin 3-*O*-rutinoside-7-*O*-[3-*O*-(3-*O*-(6-*O*-(4-*O*-(6-*O*-*p*-*h*ydroxybenzoyl-β-D-glucosyl)-*p*-hydroxybenzoyl)-β-D-glucosyl)-β-D-glucosyl)-6-*O*-(4-*O*-(6-*O*-*p*-

hydroxybenzoyl-β-D-glucosyl)-*p*-hydroxybenzoyl)-β-D-glucoside] (**37**), the accurate linkage form regarding the sugar region has not been determined yet.



37: Cyanodelphin

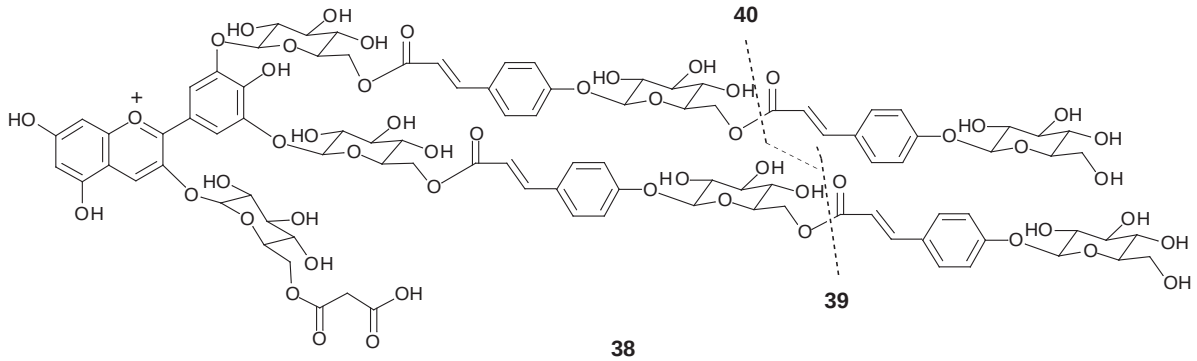
Type 3. 3'-Polyacylglycoside or 3',5'-di-polyacylglycoside group.

Third type group of polyacylated anthocyanins includes polyacyl functions at 3'- or 3',5'-positions of the aglycone.

The occurrence of the type 3 pigments is rather restricted, and these pigments are only present in the blue-violet flowers of *Clitoria ternatea* (Leguminosae).[66-74] So far the structures of thirteen pigments in this group are determined. The deacylanthocyanin of these pigments has a common structure which is delphinidin 3,3',5'-triglucoside. Usually malonic acid is attached at the 3-position with the 6-hydroxy group of glucose residue through the ester bond, and these pigments are named as Ternatins.[66] However, malonic acid is sometimes absent in the pigments at the early stage of blooming. Therefore, these demalonylated pigments might be thought to be derived before Ternatins are synthesized in the flowers, and named as preternatins.[74] The aromatic acid observed in this flower pigment is only *p*-coumaric acid which is attached with the 6-hydroxy group of the glucose residues at the 3'- and/or 5'-position of delphinidin. This group is also divided into the following five sub-groups (A - E) based on the difference of the linkage patterns of *p*-coumaric acid and glycosyl *p*-coumaric acid.

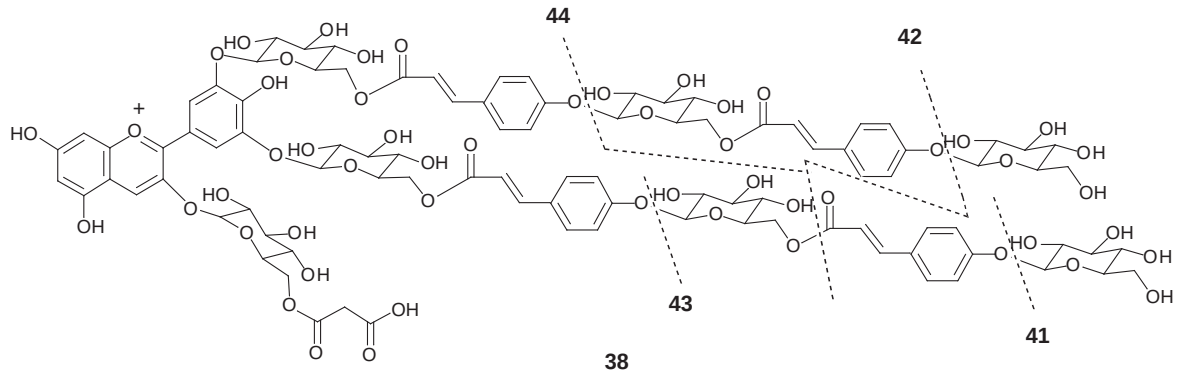
3-A) In the pigments of the first sub-group, the terminal substituents at the 3' and 5'-positions are both glucose. This type (3-A) is classified as the group ternatin A, and the isolation of three pigments, ternatin

A1 (TA-1) (**38**), A2 (TA-2) (**39**) and A3 (TA-3) (**40**) was reported.[68-71,73] The structure of TA-1 was assigned as delphinidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-3',5'-di-*O*-[6-*O*-(4-*O*-(6-*O*-(4-*O*-β-D-glucosyl-*p*-coumaroyl)-β-D-glucosyl)-*p*-coumaroyl)-β-D-glucoside] (**38**).[70] The molecular weight of this pigment is 2107, which is the largest molecular weight so far found as anthocyanin pigments in plants. The structures of TA-2 and TA-3 pigments were determined as depicted as **39** and **40**. Moreover, the structure of demalonyl TA-3 was also elucidated and named as preternatin A3.[74]



38: Ternatin A1 (TA1)
39: Ternatin A2 (TA2)
40: Ternatin A3 (TA3)

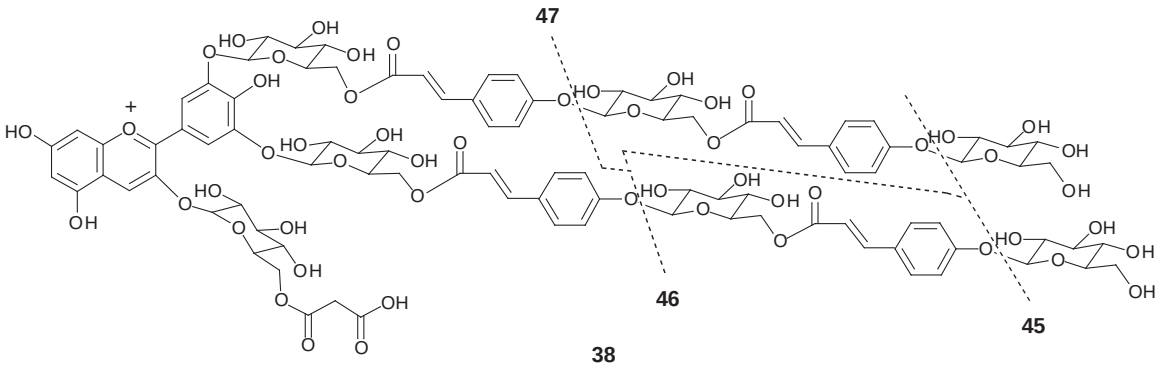
3-B) In the pigments of the second sub-group, the terminal substituents at the 3' and 5'-positions are glucose and *p*-coumaric acid.[68,69,72,73]



41: Ternatin B1 (TB1)
42: Ternatin B2 (TB2)
43: Ternatin B3 (TB3)
44: Ternatin B4 (TB4)

The pigments in this sub-group were classified as the group ternatin B, and four pigments were isolated as ternatin B1 (TB-1) (**41**), B2 (TB-2) (**42**), B3 (TB-3) (**43**) and B4 (TB-4) (**44**) from the blue-violet flowers. The most complicated pigment in this sub-group is TB-1, whose structure was determined as delphinidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-3'-*O*-[6-*O*-(4-*O*-(6-*O*-(4-*O*-β-D-glucosyl-*p*-coumaroyl)-β-D-glucosyl)-*p*-coumaroyl)-β-D-glucoside]-5'-*O*-[6-*O*-(4-*O*-(6-*O*-*p*-coumaroyl-β-D-glucosyl)-*p*-coumaroyl)-β-D-glucoside] (**41**).[72]

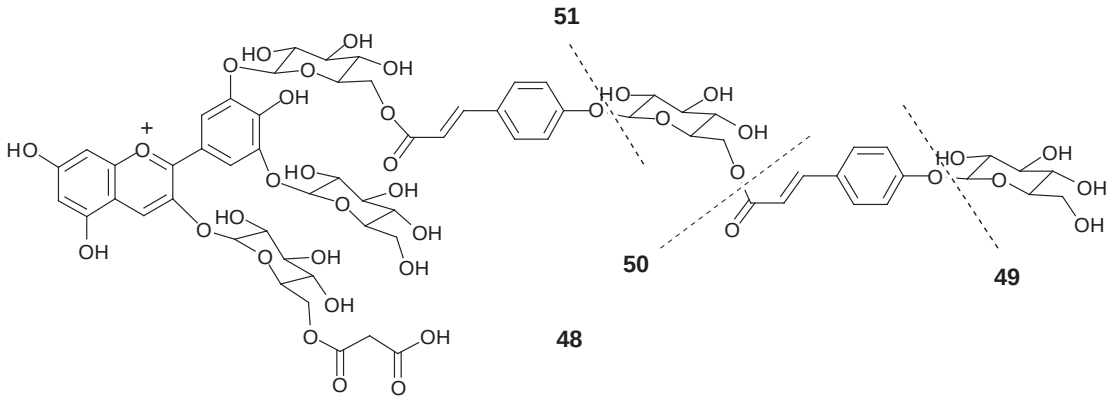
3-C) In the pigments of the third sub-group, the terminal substituents at the 3' and 5'-positions are both *p*-coumaric acid. This sub-group pigments are again classified as the group ternatin D, and the isolation of three pigments, ternatin D1 (TD-1) (**45**), D2 (TD-2) (**46**) and D3 (TD-3) (**47**) was reported.[67,69,73] The pigment having the largest molecular weight in this sub-group was TD-1, whose structure was determined as delphinidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-3',5'-di-*O*-[6-*O*-(4-*O*-(6-O-*p*-coumaroyl-β-D-glucosyl)-*p*-coumaroyl)-β-D-glucoside] (**45**).[67]



45: Ternatin D1 (TD1)
46: Ternatin D2 (TD2)
47: Ternatin D3 (TD3)

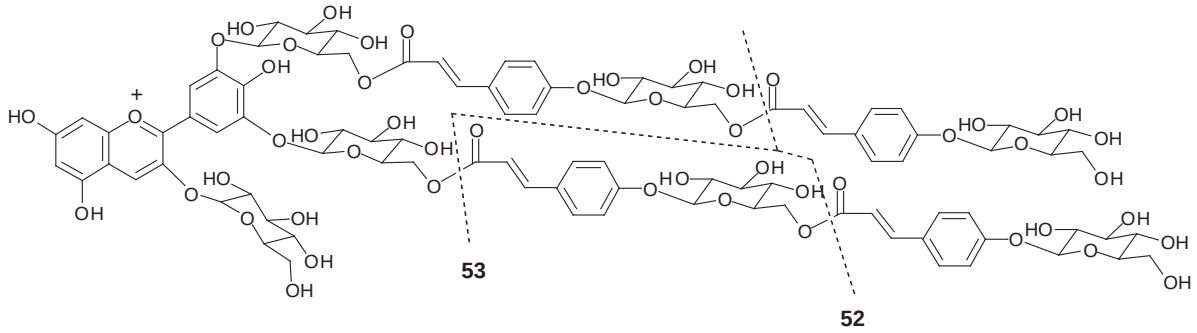
3-D) In the pigments of the fourth sub-group, the terminal substituent at the 5'-position is simply glucose and the substituent at the 3'-position is acylated with aromatic acid. This type of sub-group is classified as the group ternatin C, and isolation of four pigments, ternatin C1 (TC-1) (**49**), C2 (TC-2) (**48**), C3 (TC-3) (**51**) and C4 (TC-4) (**50**) was reported.[74] The pigment having the largest molecular weight in this sub-group was TC-2, whose structure was determined as delphinidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-3'-*O*-

[6-*O*-(4-*O*-(6-*O*-(4-*O*-β-D-glucosyl-*p*-coumaroyl)-β-D-glucosyl)-*p*-coumaroyl)-β-D-glucoside]-5'-*O*-β-D-glucoside (**48**).



48: Ternatin C2 (TC2)
49: Ternatin C1 (TC1)
50: Ternatin C4 (TC4)
51: Ternatin C3 (TC3)

3-E) The pigments without malonic acid moiety at the 3-position of glucose were mainly isolated from the flowers at the early blooming stage of *Clitoria ternatea* as minor pigments, and were called as preternatin.[74]



52: Preternatin A3
53: Preternatin A4

Only two pigments belonging to this sub-group are known as preternatin A3 and preternatin C4 at the present time. The structures of these pigments were confirmed as delphinidin 3-*O*-β-D-glucoside-3',5'-di-*O*-[6-*O*-(4-*O*-β-D-glucosyl)-*p*-coumaroyl]-β-D-glucoside] (**52**) for A3, and delphinidin 3,3'-di-*O*-β-D-glucoside-5'-*O*-[6-*O*-(4-*O*-β-D-glucosyl-*p*-coumaroyl)-β-D-glucoside] (**53**) for C4, respectively.

Type 4. 7,3'-Polyacylglycoside group.

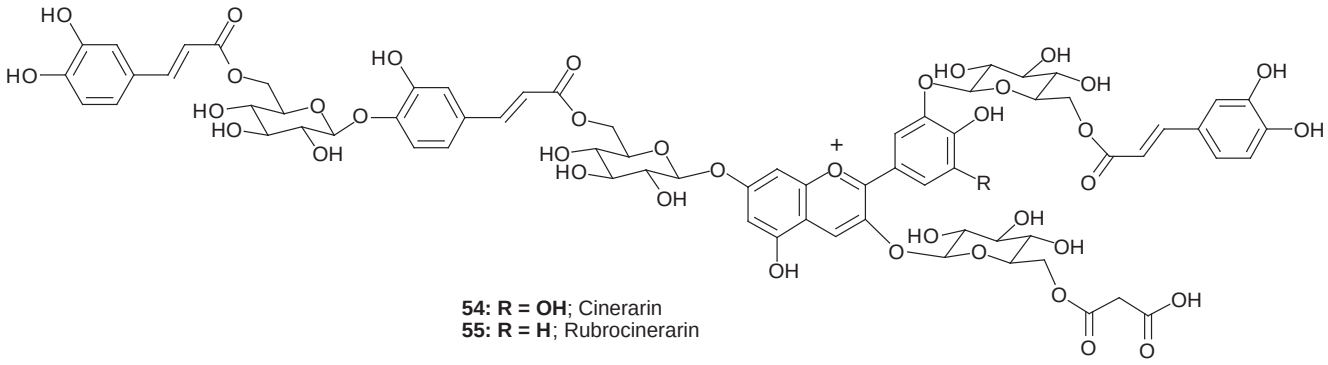
The pigments in the fourth group of polyacylated anthocyanins include polyacyl functions at 7- and 3'-positions of aglycone in the sugar residues. The presence of twenty-three pigments belonging to this group are so far known, and these pigments are usually found in the more advanced plants, such as the plants of the Compositae and Orchidaceae. Based on the characteristics of both uncommon glycosylation and acylation at the 7- and 3'-OH groups, the pigments in this group are considered to be one of the most advanced anthocyanin pigments from the chemotaxonomical point of view. The common aromatic acid moiety in this group is hydroxycinnamic acid, such as *p*-coumaric, caffeic and sinapic acids, except one report[77] for the presence of *p*-hydroxybenzoic acid. The pigments of this group are also divided into the following two sub-groups by their glycoside types of deacylanthocyanin, such as 3,7,3'-triglucosides (A) and 3-rutinoside-7,3'-diglucosides (B), where the aglycones are cyanidin or delphinidin. This first sub-group (4-A) is further classified into three sub-classes (4-A-1 to 4-A-3) by their linkage pattern in their acylated side chains. The pigments in the 4-A-1 class have one molecule of aromatic acid at the 3'-position and more than two molecules of aromatic acids at the 7'-position. Whereas, the pigments in the 4-A-2 class have opposite substitution pattern to the 4-A-1 class, indicating that the pigments in the 4-B-1 class have more than two molecules of aromatic acids at the 3'-position. The pigments in the 4-A-3 class have each one molecule of aromatic acid at the 3'- and 7'-positions, respectively.

4-A) The pigments in this type contain the deacylanthocyanin having 3,7,3'-triglucoside.

4-A-1) The pigments of the 4-A-1 class are 7-diacyl-3'-monoacyl glycoside type, and have more than two molecules of caffeic acid on their sugar residues at the 7-position. The aromatic acid in this class is limited only to caffeic acid and two kinds of polyacylated pigments are found in the plants of the Compositae.[9,16,75,76] Cinerarin was firstly isolated from the blue flowers of *Senecio cruentus*,[9] and thereafter rubrocinerarin was also isolated from its red flowers.[75] Their structures were delphinidin 3-*O*-[6-*O*-malonyl-β-D-glucoside]-7-*O*-[6-*O*-(4-*O*-(6-*O*-caffeoyl-β-D-glucosyl)caffeoyl)-β-D-glucoside]-3'-*O*-[6-*O*-caffeoyl-β-D-glucoside] (**54**) as a blue pigment (cinerarin),[16] and cyanidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-7-*O*-[6-*O*-(4-*O*-(6-*O*-caffeoyl-β-D-glucosyl)caffeoyl)-β-D-glucoside]-3'-*O*-[6-*O*-caffeoyl-β-D-glucoside] (**55**) as a red pigment (rubrocinerarin),[76] respectively. In these pigments, two molecules of

caffeic acid and one molecule of caffeic acid were linearly bonded with the sugars at the 7-position and at the 3'-position, respectively, in addition to malonylation with glucose residue at the 3-position. Rubrocinerarin was also found to be present in red leaves and red stalks (stems) of *Gynura aurantiaca* 'Purple Passion'.[76]

Generally, these polyacylated pigments in the Compositae have longer side chains at the 7-position than the 3'-position in comparison with those of the Orchidaceae, which are described in the next section.

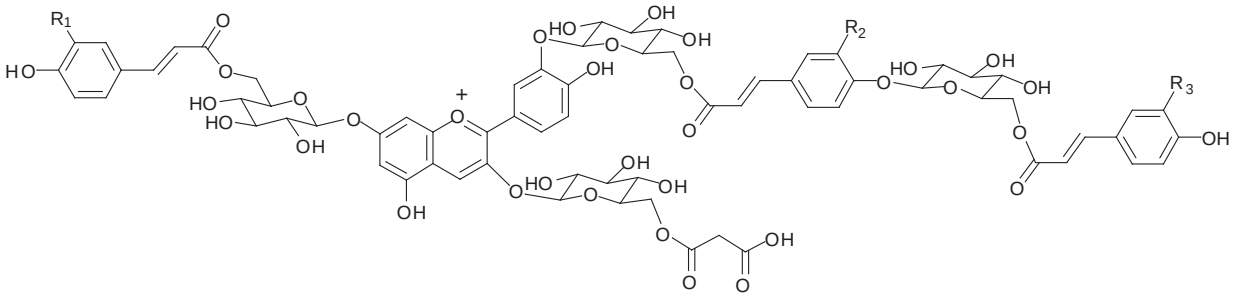


54: R = OH; Cinerarin
55: R = H; Rubrocinerarin

4-A-2) The pigments belonging to this class are so far observed in the plants of the Orchidaceae.[77-80] This class of pigments have one molecule of aromatic acid at the 7-position and more than two molecules of aromatic acids at the 3'-position, and were thought to be the 7-monoacyl-3'-diacylglycoside type. In 1994, we have isolated one pigment, Laeliocattleya anthocyanin-1 (LA-1) having this substitution pattern, from the red-violet flowers of *xLaeliocattleya* cv 'Mini Purple', and determined its structure as cyanidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-7-*O*-(6-*O*-*p*-coumaroyl-β-D-glucoside)-3'-*O*-[6-*O*-(4-*O*-(6-*O*-*p*-coumaroyl-β-D-glucosyl)-*p*-coumaroyl)-β-D-glucoside] (**56**).[78] In practice, the presence of more than sixteen pigments was observed in this flower by HPLC analysis, and their chemical properties were found to be closely related to each other. Therefore, the difficulties were sometimes encountered for the isolation of these pigments as pure forms. In 1995, we could succeed in the isolation of ten pigments from this flower, and figured out that three of these pigments were the same structures with the pigments isolated from the flowers of *Bletilla stricta* (Bletilla anthocyanin, BA).[79] Among those ten pigments isolated, we have determined six LAs, such as LA-1 (**56**), LA-2 (**57**), LA-3 (**58**), LA-6 (**59**), LA-7 (**60**), and LA-10 (**63**), accurately.[80] The structures of these pigments (LA's) were basically very similar to each other. The observed difference was

only their set of aromatic acid residues. For example, LA-1 had *p*-coumaric acid as the sole aromatic acid residue, and in LA-2, two molecules of aromatic acid units were ferulic acid. On the other hand, LA-3 had one molecule of caffeic acid and LA-6 had two molecules of caffeic acid in their three aromatic acids. The aromatic acids in LA-7 (**60**) were all caffeic acid. Finally LA-10 (=BA-3) (**63**) had one more molecule of glucose than LA-7, which located at the 3'-position of caffeic acid.

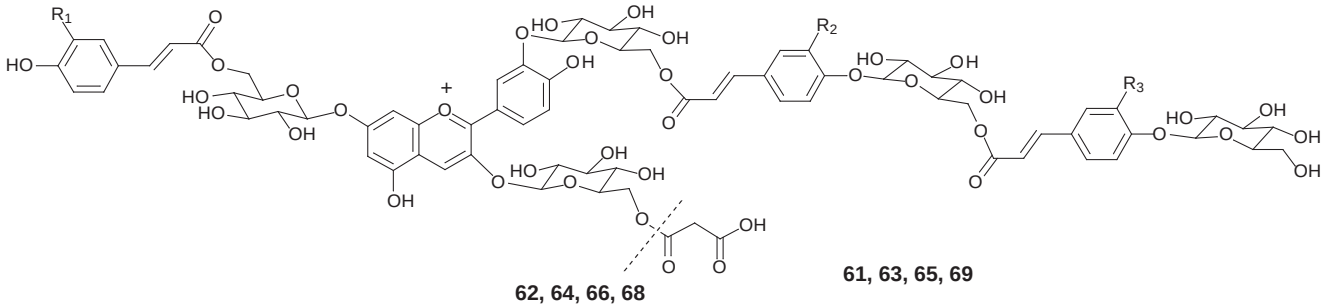
As mentioned before, the plants of the Orchidaceae have generally the longer acyl side chain at the 3'-position than that of 7-position.



Laeliocattleya anthocyanins

	R_1	R_2	R_3	
56	H	H	H	(LA-1)
57	OH or OCH_3	OH or OCH_3	OH or OCH_3	(LA-2)
58	H	OH	H	(LA-3)
59	H	OH	OH	(LA-6)
60	OH	OH	OH	(LA-7)

As another source of these pigments, we have also isolated eight pigments from the violet-red flowers of *Bletilla stricta,* and these pigments are called as Bletilla anthocycnins (BA).[79] Three of these eight pigments were proved to be the same as xLaeliocattleya anthocyanins.



62, 64, 66, 68

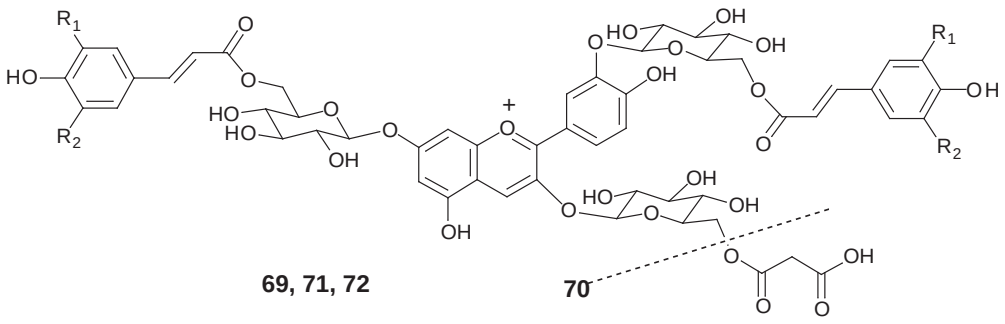
61, 63, 65, 69

Bletilla anthocyanins

	R_1	R_2	R_3	
61:	H	H	H	(BA-1)
62:	H	H	H	(BA-2)
63:	OH	OH	OH	(BA-3)
64:	OH	OH	OH	(BA-4)
65:	H or OH	H or OH	H or OH	(BA-5)
66:	H or OH	H or OH	H or OH	(BA-6)
67:	H or OH	H or OH	H or OH	(BA-7)
68:	H or OH	H or OH	H or OH	(BA-8)

Compared the structures of BA with LA, it was figured out that BA has only one more glucose than LA locating as the terminal substituent at the 3'-position. The structure of BA-1 was determined as cyanidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-7-*O*-(6-*O*-*p*-coumaroyl-β-D-glucoside)-3'-*O*-[6-*O*-(4-*O*-(6-*O*-(4-*O*-β-D-glucosyl-*p*-coumaroyl)-β-D-glucosyl)-*p*-coumaroyl)-β-D-glucoside] (**61**). The aromatic acids in this pigment were all *p*-coumaric acid. BA-2 was determined to be demalonylated BA-1 (**62**). In the structure of BA-3 (**63**), three molecules of aromatic acids were found to be all caffeic acid, which was also found in the flowers of x*Laeliocattleya* cv 'Mini Purple' as LA-10 (**63**) and both pigments are of the same structure, as mentioned before. BA-4 (**64**) was found to be demalonylated BA-3. The substitution pattern of aromatic acid in BA-5 (**65**) was similar to that of LA-8, and BA-5 contained two molecules of *p*-coumaric acid and one molecule of caffeic acid. BA-6 (**66**) was again demalonylated BA-5. BA-7 (**67**) was composed with two molecules of caffeic acid and one molecule of *p*-coumaric acid, and this pattern was also similar to that of LA-9. BA-8 (**68**) is demalonylated BA-7.

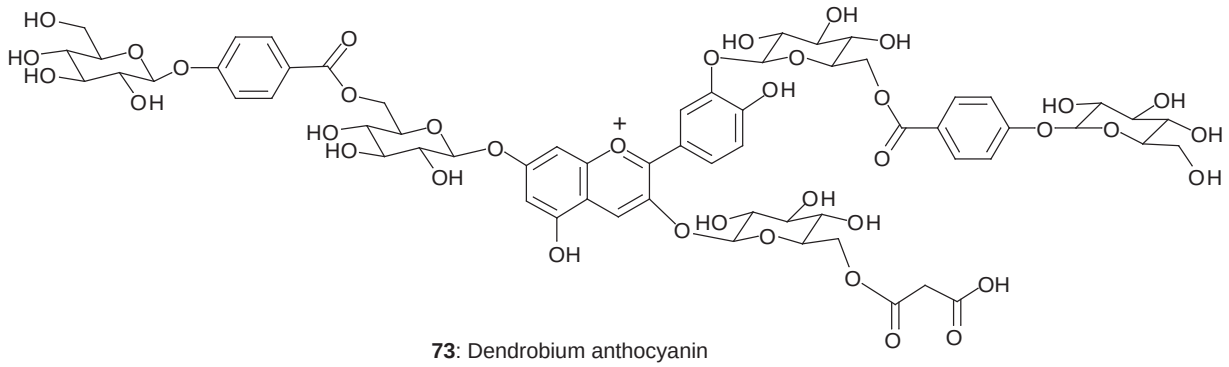
4-A-3) The pigments having each one molecule of aromatic acid in the sugar residue at the 7- and 3'-positions (7,3'-diacylglycoside type). This type of pigments are observed in the red-violet flowers of five kinds of Phalaenopsis species and also in *P.* hybrid cultivars (Orchidales).[81] We have isolated four pigments from this flower and these pigments are called as *Phalaenopsis* anthocyanin 1-4 (PA-1 to PA-4) which contained two molecules of hydroxycinnamic acid. The structure of PA-3 was confirmed as cyanidin 3-*O*-(6-*O*-malonyl-β-D-glucoside)-7-*O*-[6-*O*-sinapoyl-β-D-glucoside]-3'-*O*-[6-*O*-sinapoyl-β-D-glucoside] (**69**), and PA-1 was demalonylated PA-3 (**70**).



69, 71, 72 **70**

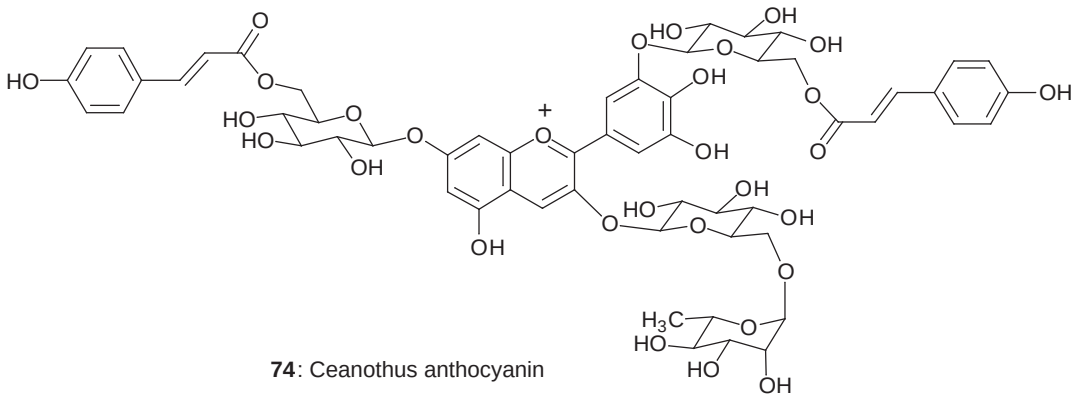
69: **$R_1 = R_2 = OCH_3$**; Phalaenopsis anthocyanin-3 (PA-3)
70: **$R_1 = R_2 = OCH_3$**; Phalaenopsis anthocyanin-1 (PA-1)
71: **R_1 = H or OCH_3, $R_2 = OCH_3$ or H**; Phalaenopsis anthocyanin-4 (PA-4)
72: **$R_1 = R_2 = OCH_3$ and R_1 = H, $R_2 = OCH_3$**; Phalaenopsis anthocyanin-2 (PA-2)

In PA-4, both aromatic acids were ferulic acid (**71**). On the other hand, PA-2 had each one molecule of sinapic acid and ferulic acid (**72**). Moreover, the similar pigment having two molecules of aromatic acids was isolated from the red-violet flowers of *Dendrobium* 'Pramat' (*Phalaenopsis* type cv.) (Orchidaceae) and its structure was determined as cyanidin 3-*O*-[6-*O*-malonyl-β-D-glucoside]-7,3'-di-*O*-{[6-*O*-(4-*O*-β-D-glucosyl-*p*-hydroxybenzoyl)-β-D-glucoside] (*Dendrobium* anthocyanin) (**73**).[77] In this pigment, the aromatic acid was *p*-hydroxybenzoic acid instead of caffeic acid.



73: Dendrobium anthocyanin

4-B) The pigment in this class belongs to 7,3'-di-monoacylated glycoside type, and its deacylanthocyanin contains 3-rutinoside-7,3'-diglucoside unit. Only one pigment classified into this group was so far reported. This pigment was isolated from the blue-violet flowers of *Ceanothus papillosus* (Rhamnaceae), and its structure was determined as delphinidin 3-*O*-rutinoside-7,3'-di-*O*-(6-*O*-*p*-coumaroyl-β-D-glucoside) (**74**).[82]



74: Ceanothus anthocyanin

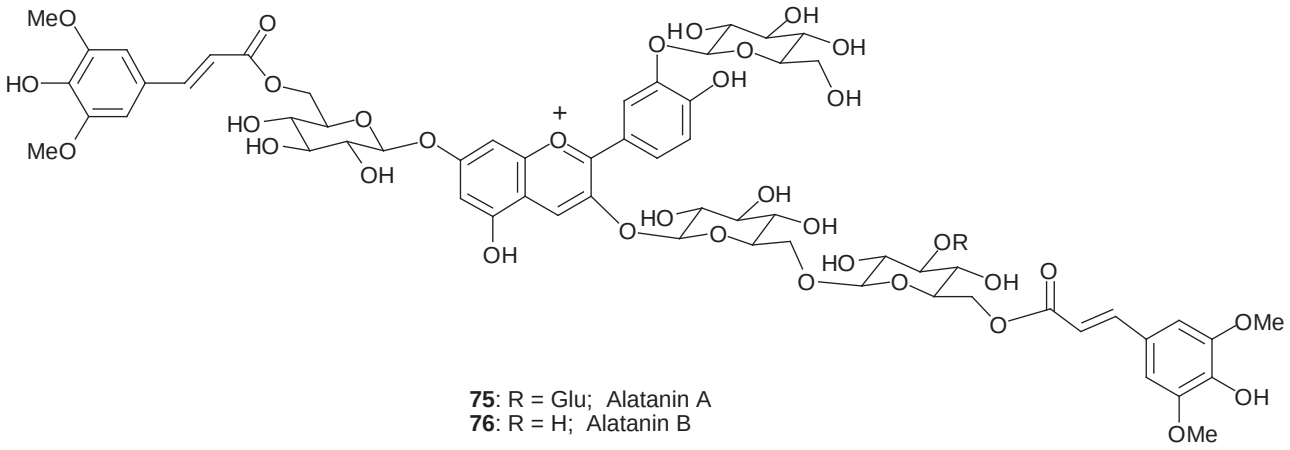
Type 5. 3,7-, 3,3'- and 3,7,3'-Polyacylglycoside groups.

The fifth group pigments are the polyacylated anthocyanins which could not be classified into the above four groups (type 1 to type 4), and these pigments certainly have one molecule of aromatic acid at 3-position and 7- and/or 3'-positions of the aglycone. These pigments are conventionally divided into the following four sub-groups according to the position of their second or other aromatic acid residues.

5-A) The pigments having aromatic acids at the 3- and 7-positions are classified as the group 5-A.

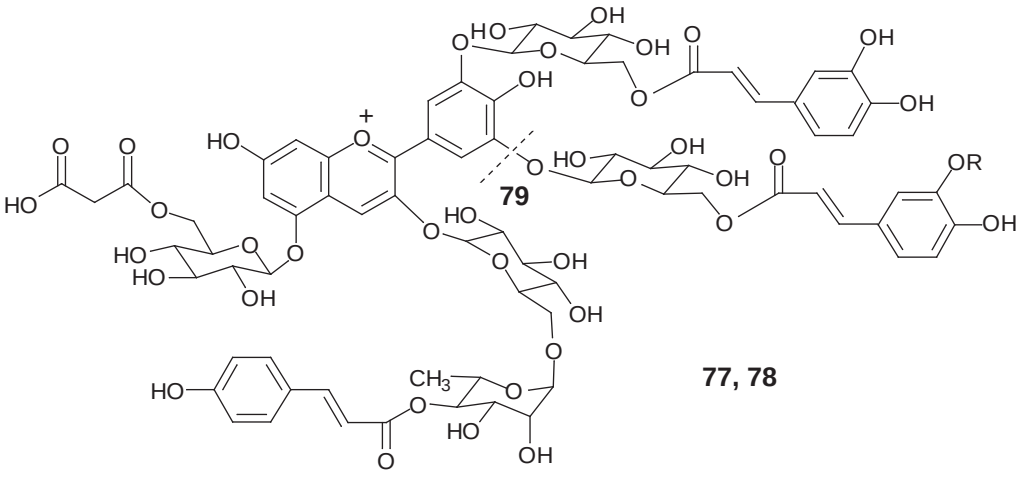
From the violet tuber of *Dioscorea alata* (Dioscoreaceae), two pigments with two molecules of sinapic acid were isolated.[83] These pigments were named as Alatanins A and B. The structures of Alatanins A and B were determined as cyanidin 3-*O*-[6-*O*-(3-*O*-(β-D-glucosyl)-6-*O*-sinapoyl-β-D-glucosyl)-β-D-glucoside]-7-*O*-(6-*O*-sinapoyl-β-D-glucoside)-3'-*O*-β-D-glucoside (**75**), and cyanidin 3-*O*-[6-*O*-(6-*O*-sinapoyl-β-D-glucosyl)-β-D-glucoside]-7-*O*-(6-*O*-sinapoyl-β-D-glucoside)-3'-*O*-β-D-glucoside (**76**), respectively.

These pigments keep relatively stable color in the solution and the violet tuber is used as edible colorant in the tropical area. Hence, these pigments are sometimes utilized as coloring agents with some kinds of foods.



75: R = Glu; Alatanin A
76: R = H; Alatanin B

5-B) The pigments having aromatic acid functions at the 3,3'- or at the 3,3',5'-positions: Three pigments of this group were isolated from the flowers of *Lobelia erinus* (Lobeliaceae).[84-85] From the blue-violet flowers, crude Loberinin was firstly isolated by Yoshitama.[84]



79

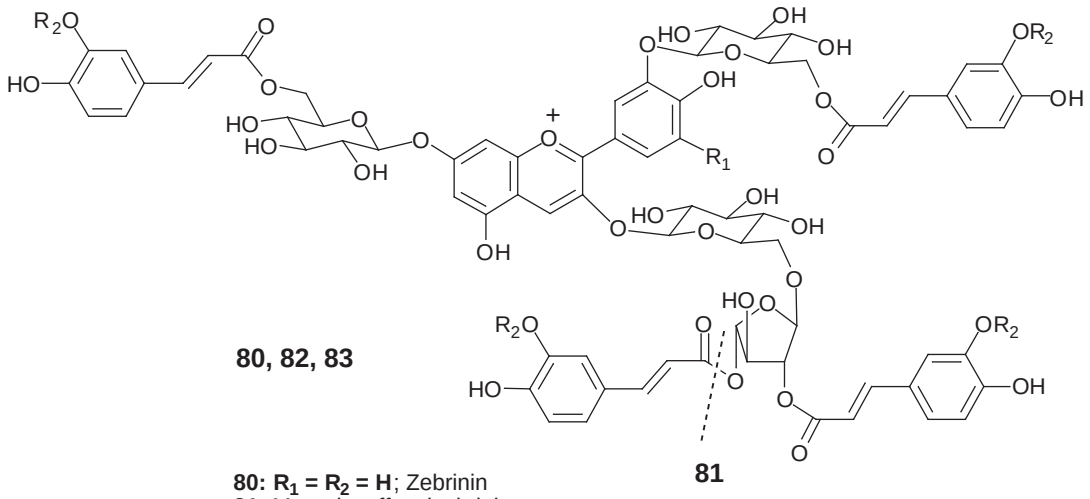
77, 78

77: R = H; Lobelinin A
78: R = CH_3; Lobelinin B
79: Lobelia red anthocyanin

Thereafter, Loberinins A and B were isolated from the same flowers, and their structures were also determined by Kondo *et al.*[85] Lobelinin A had three molecules of aromatic acids and its structure was proposed to be delphinidin 3-*O*-[6-*O*-(4-*O*-*p*-coumaroyl-α-L-rhamnosyl)-β-D-glucoside]-5-*O*-(6-*O*-malonyl-β-D-glucoside)-3',5'-di-*O*-(6-*O*-caffeoyl-β-D-glucoside) (**77**). Lobelinin B (**78**) had basically the same structure as Lobelinin A, and the difference was only the aromatic acid at the 3'-position, in which Lobelinin B contained ferulic acid instead of caffeic acid in Lobelinin A. From the red flowers of this plant, Lobelia red anthocyanin was isolated and determined as cyanidin 3-*O*-[6-*O*-(4-*O*-*p*-coumaroyl- α-L-rhamnosyl)-β-D-glucoside]-5-*O*-(6-*O*-malonyl-β-D-glucoside)-3'-*O*-(6-*O*-caffeoyl-β-D-glucoside) (**79**).[86]

5-C) The pigments having aromatic acids at the 3,7,3'-positions: The pigments having the 3,7,3'-acyl substitution pattern were found in *Zebrina pendula*,[87] *Tradescantia pallida*,[88,89] and *Tradescantia reflux*[90] (Commelinaceae). Deacylanthocyanin in this group of pigments is 3-arabinosylglucoside-7,3'-diglucoside, and the aglycones are delphinidin and cyanidin. Zebrinin was isolated from the violet leaves of *Zebrina pendula*[91] and the structure for this pigment was determined as cyanidin 3-*O*-[6-*O*-(2-*O*-caffeoyl-4-*O*-caffeoyl-α-L-arabinosyl)-β-D-glucoside]-7,3'-di-*O*-(6-*O*-caffeoyl-β-D-glucoside) (**80**), and also another pigment was determined to be monodecaffeoylzebrinin (**81**).[87] From the violet-red leaves and stems of *Tradescantia pallida*, two structurally similar pigments, Tradescantias A (**82**) and B (**83**) with the same acyl

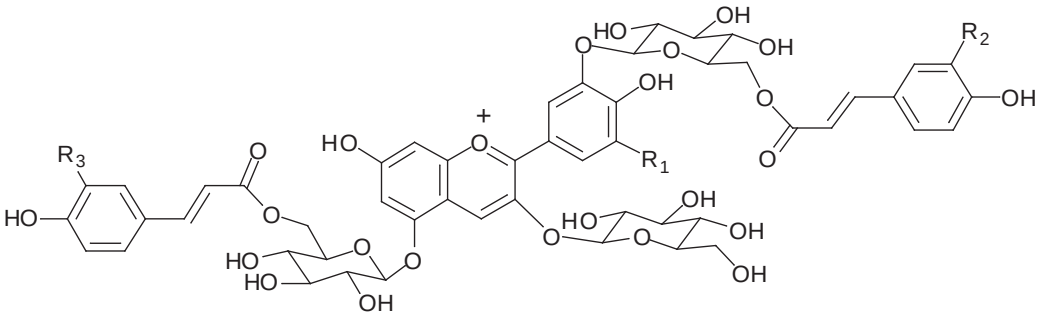
side chains, were also isolated, in which the aromatic acid was ferulic acid instead of caffeic acid in Zebrinin.[88,89] Furthermore, two pigments composed from delphinidin and cyanidin with caffeic acid and ferulic acid were isolated from *Tradescantia reflex* as major pigments.[90]



80: $R_1 = R_2 = H$; Zebrinin
81: Monodecaffeoylzebrinin
82: $R_1 = H$, $R_2 = CH_3$; Tradescantia A
83: $R_1 = OH$, $R_2 = CH_3$; Tradescantia B

Type 6. 5- and 3'-Polyacylglycosides.

Four pigments having aromatic acid at the 5- and 3'-positions were found in the flowers of *Gentiana makinoi* (Gentianaceae). From the blue-violet flowers of this plant, gentiodelphin was firstly isolated by Goto *et al.*[92] and its structure was determined to be delphinidin 3-*O*-β-D-glucoside-5-*O*-(6-*O*-caffeoyl-β-D-glucoside)-3'-*O*-(6-*O*-caffeoyl-β-D-glucoside) (**84**). In the red-violet flowers of this plant, gentiocyanin B was also observed, and its structure was determined as cyanidin 3-*O*-β-D-glucoside-5,3'-di-*O*-(6-*O*-caffeoyl-β-D-glucoside) (**85**).[93] Moreover, Albireodelphins D and E were isolated from the cultivated flowers[94] and their structures were proposed to be delphinidin 3-*O*-β-D-glucoside-5-*O*-(6-*O*-*p*-coumaroyl-β-D-glucoside)-3'-*O*-(6-*O*-caffeoyl-β-D-glucoside) (**86**), and delphinidin 3-*O*-β-D-glucoside-5-*O*-(6-*O*-caffeoyl-β-D-glucoside)-3'-*O*-(6-*O*-*p*-coumaroyl-β-D-glucoside) (**87**), respectively. Again these pigments have each one molecule of aromatic acid at the 5- and 3'-positions.

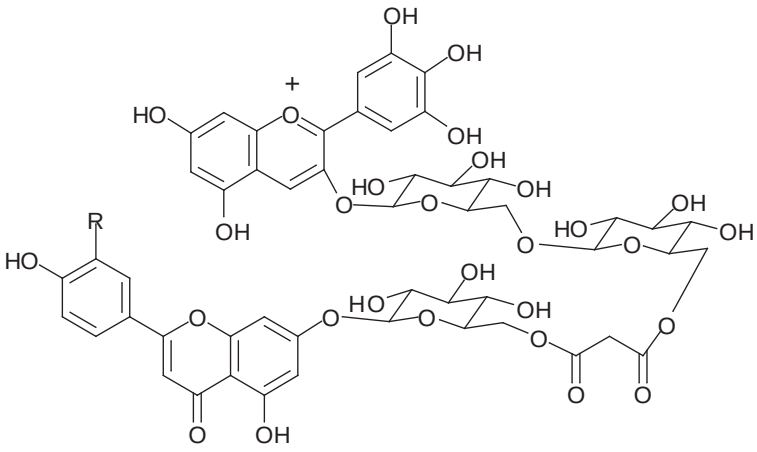


84: $R_1 = R_2 = R_3 = OH$; Gentiodelphin;
85: $R_1 = H$, $R_2 = R_3 = OH$; Gentiocyanin B
86: $R_1 = OH$, $R_2 = H$, $R_3 = OH$; Albireodelphin D
87: $R_1 = R_2 = OH$, $R_3 = H$; Albireodelphin E

Type 7. Bis-acylated malonyl and succinyl anthocyanin.

As a new acylated pigment type, four bis-acyalted malonyl- or succinylanthocyanins were recently isolated by Toki *et al.*[95,97,98] and Bloor and Falshaw, [96] independently. The characteristic feature of these pigments is the presence of bis-acylated aliphatic acid functions in their molecules.

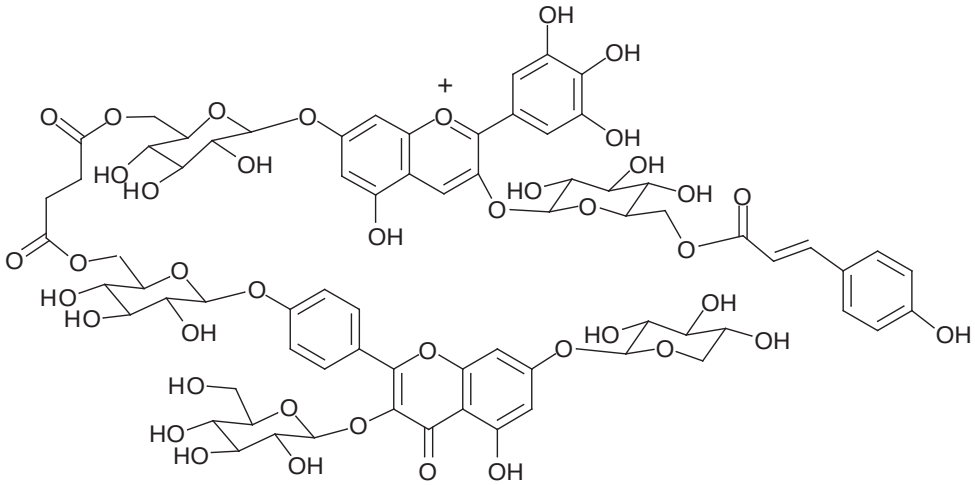
7-A) Eichhornia anthocyanins: Two pigments of this type were observed in the blue-violet flowers of *Eichhornia crassipes* (Pontederiaceae).[95] In these pigments, malonic acid was bonded with both flavone and anthocyanin through the ester bond. These pigments were supposed to make a folding structure between anthocyanin and flavone as an anthocyanin-co-pigment attached covalently.



88: R = H; Eichhornia anthocyanin A
89: R = OH; Eichhornia anthocyanin B

Eichhornia anthocyanin A was determined as {6’’’-*O*-[delphinidin 3-*O*-(6”-*O*-(β-D-glucosyl)-(β-D-glucosyl))]}-[6”-*O*-(apigenin 7-*O*-β-D-glucosyl)]malonate (**88**),[95] and Eichhornia anthocyanin B was determined as {6’’’-*O*-[delphinidin 3-*O*-(6”-*O*-(β-D-glucosyl)-(β-D-glucosyl))]}-[6”-*O*-(luteolin 7-*O*-β-D-glucosyl)]malonate (**89**).[97] These pigments exhibited relatively strong co-pigmentation effect.

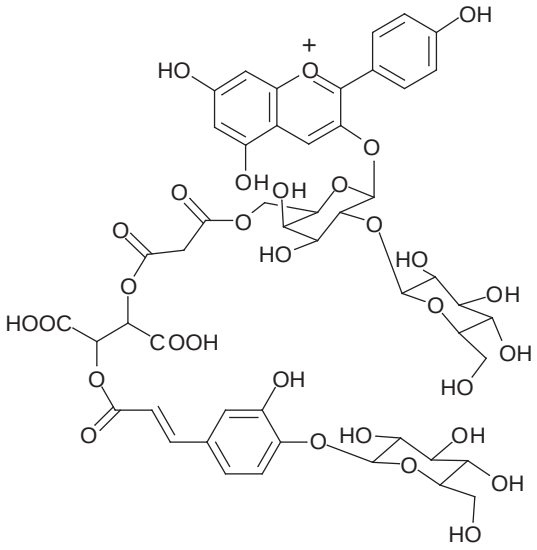
7-B) Very recently, two *Agapanthus* anthocyanins of novel bis-acylated anthocycnin type were isolated from *Agapanthus praecox* sp. *orientalis* (Liliaceae).[96] In these structures, bis-acylated succinates were involved, and Agapanthus anthocyanin 1 (AA-1) was determined to be [6’’-*O*-(delphinidin 3-*O*-(6’’-*O*-*p*-coumaloyl-β-D-glucoside)-7-*O*-β-D-glucosyl)]-[6’’-*O*-(kaempferol 3-*O*-β-D-glucoside-7-*O*-β-D-xyloside-4’-*O*-β-D-glucosyl)]succinate (**90**), and AA-2 was the same structure with AA-1 having 7-*O*-glucose residue instead of 7-*O*-xylose residue.



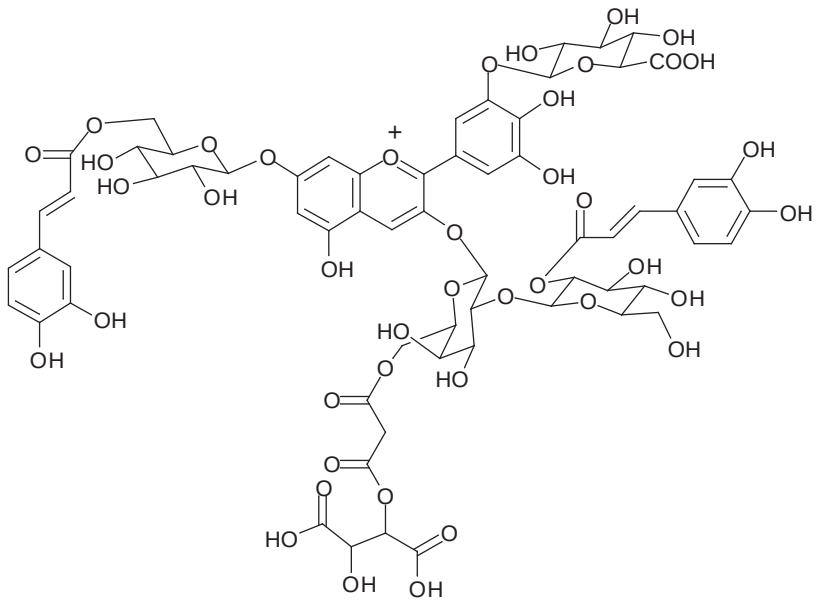
90

These compounds represent unique examples of anthocyanin pigments in which co-pigmentation of anthocyanidin with both an aromatic acyl group and a flavonoid was exhibited.

7-C) Anemone anthocyanins: Two bis-acylted pigments were also isolated from the flowers of *Anemone coronaria.*[98] In Anemone red anthocyanin 4, only one aromatic acid was involved, however, as aliphatic acids, such as malonic acid and tartaric acid, were also involved in this pigment.



91: Anemone red anthocyanin



92: Anemone blue-violet anthocyanins

Especially, interesting feature of this pigment was the presence of a bis-acylated malonic acid with galactose and tartaric acid, this substitution pattern was very rare example in the anthocyanin chemistry. Anemone red anthocyanin 4, isolated from the scarlet flowers of *Anemone coronaria* was determined as {6''-*O*-[pelargonidin 3-*O*-(2"-*O*-(β-D-xylosyl)-β-D-glucoside)]}-[2-*O*-(4-*O*-(β-D-glucosylcaffeoyl)tartaryl)]-malonate (**9 1**) and the presence of a bis-acylted malonic acis was confirmed.[98]

Another structurally similar pigment was also isolated from the blue-violet flowers of *Anemone coronaria* and determined to be {6''-O-[delphinidin 3-*O*-(2-*O*-(2-*O*-caffeoyl)-β-D-xylosyl)-β-D-glucosyl]-[7-*O*-(6-*O*-caffeoyl-β-D-glucoside)-3'-*O*-glucuronide]}-[1-tartryl]malonate (**9 2**), as the major pigment.[97]

Table 1 shows the summary of new polyacylated anthocyanin distribution with their species, family, deacylanthocyanin and the species of aromatic acids. As can be seen in Table 1, the polyacylated anthocyanins having more than two molecules of aromatic acids, are commonly present in much more advanced plants. For example, the highly advanced monocotyledons, such as some plants of the Orchidaceae and Liliaceae contain structurally complicated polyacyl anthocycanins. In the dicotyledons, some plants of the Compositae contain structurally more complexed polyacyl anthocyanins. However, an exception is observed in the plants of Ranunculaceae, which are thought to be a rather primitive plant family

of evolutionary or phylogenetic status, and these plants still contain a number of polyacylated anthocyanins. These facts will be an interesting subject to be discussed in future.

II. The UV and VIS spectral properties in HCl-MeOH of polyacylated anthocyanins.

Polyacylated anthocyanins of the above seven groups are dissolved in 0.1% HCl-MeOH solution and the UV and VIS spectra of their flavylium cations were measured at the wavelength of 210-700 nm. Tables 2a - f show the λmax values for each groups of pigments. As mentioned by Harborne,[4] absorption maxima (λmax) of anthocyanins in the UV and VIS regions are changed and shifted by the spectral facts relating to anthocyanidin structures, such as hydroxylation, glycosilation, methylation, acylation and so on. As can be seen in Table 2-1 to 2-3, increasing of the number of aromatic acid in each group of pigments causes shifting of their λmax in VIS spectra into longer-wavelength region (bathochromic shift). This phenomenon was already observed and mentioned in the reports.[22,41,42] Table 3 indicated the largest λmax values of the each group of pigments with the number of aromatic acids included in their structures. In order to make the comparison easier, the standard λmax values of corresponding deacylanthocyanins are also inserted in Table 2. In the group 1, remarkable bathochromic shifts of VIS-λmax in the pigments having polyacyl groups at the 3-position, could not be observed in comparison with those of other groups. These pigments having the largest λmax exhibit their λmax at 515 nm for pelargonidin 3,5-glycoside type, 533 nm for cyanidin 3,5-glycoside type, and 546 nm for delphinidin 3,5-glycoside type (Table 2-1). These different values due to bathochromic shift ranged 7–8 nm to compare with the λmax of deacylanthocyanins. On the other hand, polyacylated pigments of the groups 3 and 4 exhibited more remarkable bathochromic shifts in their spectra with 17–28 nm long wavelength shift at the λmaxs of both groups. By consideration of the values of bathochromic shift depending on the type of aglycones in all polyacylated anthocyanins, polyacylated pigments of pelargonidin type showed their λmax at between 512 to 517 nm. Whereas, nonacylated pelargonidin diglycosides exhibited their λmax at 503–512 nm (Table 3).

Table 2-1. Absorption spectral data of polyacylated anthocyanins in 0.1% HCl-MeOH

Acyl Type (Group)	Name of Anthocyanin	λmax in 0.1% HCl-MeOH (nm)			Structure †	Compd. No.
	Ipomoea brownish-red anthocyanin 4 (IBRA 4)	523	314	284	Cy 3-(2-(glu•caff•glu•caff•glu)-glu)	**1**
	IBRA 5	527	316	286	Cy 3-(tri-(glu•caff)-soph)	
	IBRA 6	521	318	282	Cy 3-(di-(caff)-soph)	
1- A	Cyanidin 3-sophoroside	515				
	Petunia du sky violet anthocyanin 1 (PDVA 1)	545	324	287	Mal 3-(6-(4-(caff•glu•*p*-coum)rham)-glu)	**2**
	PDVA 2	542	320	283	Mal 3-(6-(*p*-coum•rham)-glu)	
	Malvidin 3-rutinoside	539		282		
	Evolvulus anthocyanin 1 (EA 1)	546	320	282	Del 3-(glu•caff•glu•caff•glu)-5-(mal•glu)	**3**
	EA 2	546	320	284	Del 3-(glu•caff•glu•caff•glu)-5-glu	**4**
1- B-1	Delphinidin 3,5-di-glucoside	538				
	Triteleia anthocyanin	528	313	282	Cy 3-(p-coum•glu•*p*-coum•glu)-5-(mal•glu)	**5**
	Cyanidin 3,5-di-glucoside	526				
	Heavenly Blue anthocyanin	530	318	294	Peon 3-(2-(glu•caff•glu)-6-(glu•caff•glu•caff•glu)-glu)-5-glu	**6**
	Pharbitis blue anthocyanin 3 (PBA 3)	528	331	296	Peon 3-(2-(caff•glu)-6-(caff)-glu)-5-glu	**7**
	PBA 2	525	324	280	Peon 3-(2-glu-6-(glu•caff)-glu-5-glu	
	Ipomoea blue-violet anthocyanin 1 (IBA 1)	532	320	295	Cy 3-(2-(glu•caff•glu)-6-(caff•glu•caff)-glu)-5-glu	**10**
	IBA 2	530	323	295	Cy 3-(2-(glu•caff•glu)-6-(caff)-glu)-5-glu	**11**
	IBA 3	530	329	296	Cy 3-(2-(caff•glu)-6-(caff)-glu)-5-glu	**12**
	IBA 6	533	312	297	*p*-coum•caff•glu•caff-Cy 3-soph-5-glu	
1 - B-2	IBA 5	532	316	298	*p*-coum•caff-Cy 3-soph-5-glu	
	Pharbitis red anthocyanin 5 (PRA 5)	515	318	288	Pel 3-(2-(glu•caff•glu)-6-(glu•caff•glu•caff)-glu)-5-glu	**8**
	Ipomoea red anthocyanin 1 (IRA 1)	512	319	287	Pel 3-(2-(glu•caff•glu)-6-(caff•glu•caff)-glu)-5-glu	**13**
	IRA 2	511	323	287	Pel 3-(2-(caff•glu)-6-(caff•glu•caff)-glu)-5-glu	**14**
	IRA 3	510	326	288	Pel 3-(2-(glu•caff•glu)-6-(caff)-glu)-5-glu	**9**
	IRA 4	510	329	289	Pel 3-(2-(caff•glu)-6-(caff)-glu)-5-glu	**15**
	PRA 2	509	331	288	Pel 3-(2-glu-6-(caff)-glu)-5-glu	
	Pelargonidin 3-sophoroside-5-glucoside	507				
	Petunia anthocyanin 25 (PA 25)	543	325	284	Pet 3-(caff•gul•*p*-coum•rut)-5-glu	**18**
	PA 26	542	305	280	Del 3-(caff•gul•*p*-coum•rut)-5-glu	**20**
	PA 16	542	320	285	Pet 3-(caff•gul•caff•rut)-5-glu	**19**
1 - B-3	PA 19	543	321	283	Mal 3-(caff•gul•caff•rut)-5-glu	**17**
	PA 22	543	325	284	Mal 3-(caff•gul•*p*-coum•rut)-5-glu	**16**
	Delphinidin 3-rutinoside-5-glucoside	540				
	Petunidin 3-rutinoside-5-glucoside	538				
	Malvidin 3-rutinoside-5-glucoside	537				

Table 2-2. Absorption spectral data of polyacylated anthocyanins in 0.1% HCl-MeOH

Acyl Type (Group)	Name of Anthocyanin	λmax in 0.1% HCl-MeOH (nm)			Structure †	Comd. No.
	Matthiola violet anthocyanin 4 (MVA 4)	531	325	281	Cy 3-(2-sm•xyl-6-fer-glu)-5-glu	
	MVA 3	531	321	281	Cy 3-(2-(sm•xyl)-6-caff-glu)-5-(mal•glu)	**23**
	MVA 2	529	317	281	Cy 3-(2-(sm•xyl)-6-*p*-coum-glu)-5-(mal•glu)	**22**
I - B-4	MVA 1	528	326	281	Cy 3-(2-(sm•xyl)-6-fer-glu)-5-(mal•glu)	**21**
	Cyanidin 3-sumbubioside-5-glucoside	527				
	Matthiola red anthocyanin 1 (MRA 1)	512	327	289	Pel 3-(2-(fer•xyl)-6-(fer)-glu)-5-(mal•glu)	**24**
	MRA 2	510	329	289	Pel 3-(2-(sm•xyl)-6-fer-glu)-5-(mal•glu)	**25**
	MRA 3	511	320	289	Pel 3-(2-(sm•xyl)-6-*p*-coum-glu)-5-(mal•glu)	**26**
	MRA 6	512	325	289	Pel 3-(2-(sm•xyl)-6-fer-glu)-5-glu	
	MRA 7	512	320	289	Pel 3-(2-(sm•xyl)-6-*p*-coum-glu)-5-glu	
	Pelargonidin 3-sumbubioside-5-glucoside	507				
	AA 1	548		250	Del 3-(mal•glu)-7-(HB•glu•HB•glu)	**27**
	Senecio pink anthocyanin 1 (SPA 1)	513	324	284	Pel 3-(mal•glu)-7-(caff•glu•caff•glu)	**28**
2-A	SPA 2	508	331	282	Pel 3-(mal•glu)-7-(caff•glu)	
	Delphinium red anthocyanin 3	508		252	Pel 3-rut-7-(glu•HB•glu)	**26**
	Pelargonidin 3-rutinoside-7-glucoside	504				
	Rubrocampanin	517		251	Pel 3-rut-7-(HB•glu•HB•glu•HB•glu)	**31**
	Campanin	550		242	Del 3-rut-7-(HB•glu•HB•glu•HB•glu)	**35**
2-B	Monodeacylcampanin	550		242	Del 3-rut-7-(glu•HB•glu•HB•glu)	**32**
	Violdelphin	530			Del 3-rut-7-(HB•glu•HB•glu)	**33**
	Platyconin	549	323	286	Del 3-rut-7-(caff•glu•caff•glu)	**29**
	Delphinidin 3-glucoside-7-sophoroside	537				
	Armeniaca anthocyanin 4 (AA 4)	550		250	Del 3-(mal•glu)-7-[2-(glu•HB•glu)-6-(HB•glu•HB)]-glu	**36**
2-C	AA 3	546		249	Del 3-(mal•glu)-7-[2-(HB•glu)-6-(HB•glu•HB)]-glu	**35**
	AA 2	548		250	Del 3-(mal•glu)-7-(HB•glu•HB•soph)	**34**
2-D	Cyanodelphin	548		250	Del 3-rut-7-[3-(HB•glu•HB•glu•glu)-6-(HB•glu•HB)]-glu	**37**
	Ternatin A1	550		287	Del 3-(mal•glu)-3',5'-di-(glu•*p*-coum•glu•*p*-coum•glu)	**38**
	Ternatin B1	548		288	Del 3-(mal•glu)-3'-(glu•*p*-coum•glu•*p*-coum•glu)-5'-(*p*-coum•glu•*p*-coum•glu)	**41**
	Ternatin D1	550		290	Del 3-(mal•glu)-3',5'-di-(*p*-coum•glu•*p*-coum•glu)	**45**
	Ternatin A2	547		286	Del 3-(mal•glu)-3'-(glu•*p*-coum•glu•*p*-coum•glu)-5'-(glu•*p*-coum•glu)	**39**
	Ternatin B2	548		289	Del 3-(mal•glu)-3'-(*p*-coum•glu•*p*-coum•glu)-5'-(glu•*p*-coum•glu)	**42**
	Ternatin B3	548		290	Del 3-(mal•glu)-3'-(glu•*p*-coum•glu•*p*-coum•glu)-5'-(*p*-coum•glu)	**43**
	Ternatin D2	547		290	Del 3-(mal•glu)-3'-(*p*-coum•glu•*p*-coum•glu)-5'-(*p*-coum•glu)	**46**
3	Ternatin A3	545		286	Del 3-(mal•glu)-3',5'-di-(glu•*p*-coum•glu)	**40**
	Ternatin B4	543		287	Del 3-(mal•glu)-3'-(glu•*p*-coum•glu)-5'-(*p*-coum•glu)	**44**
	Ternatin D3	544		299	Del 3-(mal•glu)-3',5'-di-(*p*-coum•glu)	**47**
	Ternatin C1	537		288	Del 3-(mal•glu)-3'-(*p*-coum•glu•*p*-coum•glu)-5'-glu	**49**
	Ternatin C2	538		285	Del 3-(mal•glu)-3'-(glu•*p*-coum•glu•*p*-coum•glu)-5'-glu	**48**
	Ternatin C3	535		282	Del 3-(mal•glu)-3'-(*p*-coum•glu)-5'-glu	**51**
	Ternatin C4	534		281	Del 3-(mal•glu)-3'-(glu•*p*-coum•glu)-5'-glu	**50**
	Delphinidin 3,3',5'-triglucoside	522				

Table 2-3. Absorption spectral data of polyacylated anthocyanins in 0.1% HCl-MeOH

Acyl Type (Group)	Name of Anthocyanin	λmax in 0.1% HCl-MeOH (nm)			Structure †	Comd. No.
4-A-1	Cinerarin	550	328	290	Del 3-(mal•glu)-7-(caff•glu•caff•glu)-3'-(caff•glu)	54
	Rubrocinerarin	533	324	286	Cy 3-(mal•glu)-7-(caff•gul•caff•glu)-3'-(caff•glu)	55
4-A-2	Bletilla anthocyanin 1 (BA 1)	537	306	290	Cy 3-(mal•glu)-7-(*p*-coum•glu)-3'-(glu•*p*-coum•glu•*p*-coum•glu)	61
	BA 2	536	306	289	Cy 3-(glu)-7-(*p*-coum•glu)-3'-(glu•*p*-coum•glu•*p*-coum•glu)	62
	BA 3	537	320	288	Cy 3-(mal•glu)-7-(caff•glu)-3'-(glu•caff•glu•caff•glu)	63
	BA 4	533	320	287	Cy 3-(glu)-7-(caff•glu)-3'-(glu•caff•glu•caff•glu)	64
	BA 5	538	307	294	caff•*p*-coum•*p*-coum•glu•glu-Cy 3-(mal•glu)-7,3'-diglu	65
	BA 6	535	307	287	caff•*p*-coum•*p*-coum•glu•glu-Cy 3,7,3'-triglu	66
	BA 7	538	309	288	caff•caff•*p*-coum•glu•glu-Cy 3-(mal•glu)-7,3'-diglu	67
	BA 8	538	309	286	caff•caff•*p*-coum•glu•glu-Cy 3,7,3'-triglu	68
	Laeliocattleya anthocyanin 1 (LA 1)	535	305	290	Cy 3-(mal•glu)-7-(*p*-coum•glu)-3'-(*p*-coum•glu•*p*-coum•glu)	56
	LA 2	537	320	290	Cy 3-(mal•glu)-7-(caff•glu)-3'-(fer•glu•fer•glu)	57
	LA 3	538	310	290	Cy 3-(mal•glu)-7-(caff•glu)-3'-(*p*-coum•glu•caff•glu)	58
	LA 6	538	320	290	Cy 3-(mal•glu)-7-(*p*-coum•glu)-3'-(caff•glu•caff•glu)	59
	LA 7	536	325	293	Cy 3-(mal•glu)-7-(caff•glu)-3'-(caff•glu•caff•glu)	60
	LA 4	536	310	290	*p*-coum•caff•caff•glu-Cy 3-(mal•glu)-7,3'-diglu	
	LA 5	532	310	285	caff•caff• fer•glu-Cy 3-(mal•glu)-7,3'-diglu	
4-A-3	Phalaenopsis anthocyanin 3 (PA 3)	535	331	295	Cy 3-(mal•glu)-7-(sm•glu)-3'-(sm•glu)	69
	PA 1	534	330	295	Cy 3-glu-7-(sm•glu)-3'-(sm•glu)	70
	PA 4	533	330	295	Cy 3-glu-7-(fer•glu)-3'-(fer•glu)	71
	PA 2	533	330	295	sm•fer-Cy 3-(mal•glu)-7,3'-diglu	72
	Dendrobium anthocyanin (DA)	533		248	Cy 3-(mal•glu)-7,3'-di(glu•HB•glu)	73
	Demalonyl DA 3	533		249	Cy 3-glu-7,3'-di(glu•HB•glu)	
	Cyanidin 3,7,3'-triglucoside	513				
4-B	Ceanothus blue anthocyanin	542	305	290	Del 3-rut-7-(*p*-coum•glu)-3'-(*p*-coum•glu)	74
5-A	Alatanin A	534	334	297	Cy 3-[6-(3-glu-6-snp-glu))glu]-7-(snp•glu)-3'-glu	75
	Alatanin B	533	344	296	Cy 3-[6-(6-snp-glu)glu]-7-(snp•glu)-3'-glu	76
5-B	Lobelinin A	544	320	302	Del 3-(*p*-coum•rut)-5-(mal•glu)-3',5'-di(caff•glu)	77
	Lobelia red anthocyanin	528	318	295	Cy 3-(*p* -coum•rut)-5-(mal•glu)-3'-(caff•glu)	79
5-C	Zebrinin	532	329	292	Cy 3-(di-caff•ara•glu)-7-(caff•glu)-3'-(caff•glu)	80
	Tradescantia anthocyanin	532	329	292	Cy 3-(di-fer•ara•glu)-7-(fer•glu)-3'-(fer•glu)	82
6	Gentiodelphin	538	328	295	Del 3-glu-5-(caff•glu)-3'-(caff•glu)	84
7-A	Water-hyacinth anthocyanin	548	342	272	[Del 3-(gent•glu)][apig-7-glu]mal	88
7-B	Agapanthus pigment	541	313	283 274	[Del 3-(*p*-coum•glu)-7-glu][kaem-3-glu-7-xyl-4'-glu]succin	90
7-C	Anemone red anthocyanin	512	433	324 288	[Pel 3-(xyl-glu)][1-(glu•caff)-tart]mal	91
	Anemone blue-violet anthocyanin	537	332	285	[Del 3-(caff•xyl•glu)-7-(caff•glu)-6-gluc]-[tart]mal	92

Table 3. The observed largest λmax (nm) of polyacylated anthocyanins in 0.1% HCl-MeOH and the number of aromatic acid in each anthocyanins

Acyl Type (Group)	Deacylanthocyanin Type †	Pelargonidin	Cyanidin	Delphinidin
Nonacyl-antho-cyanins	3-glu*	512	528	540
	3,5-diglu*	507	526	538
	3,7-diglu*	503	524	537
	3,5,3'-triglu*		518	528
	3,7,3'-triglu*		513	525
	3,3',5'-triglu*			522
1-A	3-glu		527 (3) **	545 (2) **
1-B-1	3,5-diglu		528 (2)	546 (2)
1-B-2	3-soph-5-glu	515 (3)	533 (3)	
1-B-3	3-rut-5-glu			543 (2)
1-B-4	3-sm-5-glu	512 (2)	531 (2)	
2-A	3-glu-7-glu	513 (2)		550 (3)
2-B	3-rut-7-glu	517 (3)		550 (3)
3	3,3,5-triglu			550 (4)
4-A-1	3,7,3'-triglu		533 (2)	550 (2)
4-A-2			538 (3)	
4-A-3			535 (2)	
4-B	3-rut-7,3'-diglu			542 (2)
5-A	3-glu-7,3'-diglu		534 (2)	
5-B	3-rut-3',5'-triglu		528 (2)	544 (3)
5-C	3,7,3'-triglu		532 (4)	548 (3)
6	3,5,3'-triglu		526 (2)	538 (3)
7	3-gen(flavone)			548

* λmax of deacylanthocyanin; ** number of aromatic acid in parenthesis

† Pel: pelargonidin; Cy: cyanidin; Peon: peonidin; Del: delphinidin; Pet: petunidin; Mal: malvidin; *p*-coum: *p*-coumaric acid; caff: caffeic acid; fer: ferulic acid; sm: sinapic acid; HB: *p*-hydroxybenzoic acid; mal: malonic acid; tart: tartaric acid; glu: glucose; xyl: xylose; ara: arabinose; rham: rhamnose; rut: rutinose; soph: sophorose; sm: sumbubiose; gen: gentiobiose.

Particularly, rubrocampanin in the group 2-B (Table 2-2) has the largest λmax at 517 nm, and its λmax value (517 nm) in the VIS spectrum was shifted bathochromically into 13 nm at the longer wavelength region compared to its deacylanthocyanin (504 nm). Polyacylated pigments of cyanidin type usually showed their λmax values at between 526 to 538 nm (Table 3), in which Bletilla and Laeliocattleya anthocyanins belonging to the group 4 exhibited the largest λmax value at 538 nm (Table 2-3). Again this value showed a bathochromic shift of Δ 25 nm from that of their deacylanthocyanin (513 nm).

Furthermore, the λmax values of polyacylated delphinidin pigments were observed at 538-550 nm, and the largest shift (λmax value = 550 nm) could be found in the groups 2, 3, and 4, respectively (Table 3). Among these groups, ternatin D1 of the group 3 showed the largest value (Δ 28 nm) of bathochromic shift at 550 nm compared to its deacylanthocyanin (522 nm) (Table 2-2). Based on the consideration of these results, we thought that these spectral variations have the close relation to the stereochemical structures between anthocyanidin nucleus (chromophore) and aromatic acid residue(s) in these acylated pigments by making the stacking conformation. Actually, by increasing the molecular numbers of aromatic acids in pigments, the larger bathochromic shift, bluing the flower color, and also making the higher stability of the flower color were observed.[18,22,29,42,66] These phenomena were also supposed to be attributed to the attaching position of the aromatic acids, strongly. This relationship can clearly be explained by ternatins of the group 3 (3',5'-polyacylated glycoside type) and Bletilla anthocyanins of the group 4 (7,3'-polyacylated glycoside type), in which, the remarkable bathochromic shifts are observed by depending on increasing the number of aromatic acid (Tables 2-2 and 2-3). As can be seen clearly in Table 2, the characteristic bathochromic shifts are confirmed to be mainly responsible for the molecular number of aromatic acids in these polyacylated anthocyanins. Furthermore, the proportion of the bathochromic shift is closely related to the anthocyanidin structures. Usually, pelargonidin type shows a little bathochromic shift, which probably stems from the formation of a weak co-pigmentation. This results support that the pigments of pelargonidin type may form a folding structure (half-sandwich form) or loose the sandwich form between the aglycone (chromophore) and the aromatic acid at 3- or 7-position (Figure 1). Whereas, the pigments folded with at least two aromatic acids at the 3'- and 7-positions make the rigid co-pigmentation from both up- and down-sides of the aglycone, such as the pigments of cyanidin and delphinidin types. These facts clearly indicate that the aromatic acids in the polyacylated anthocyanins play an important role to make more bluing of the flower color than that of the flower color produced from unacylated pigments. These conclusions will be consistent with the results obtained from the NMR study.

III. The UV and VIS spectral properties of polyacylated anthocyanins under neutral or weakly acidic condition

We have already mentioned the spectral characteristics of anthocyanins under acidic condition where the pigments were present as the flavilium cation structure. Here the spectral characteristics of polyacylated

pigments under neutral or weakly acidic conditions will be discussed, since anthocyanin pigments are usually present in the cell saps and their pH circumstances are considered to be pH 3-8 in flower tissues. As means to investigate the spectral properties of anthocyanins under the similar conditions as in flower tissues, a variety of absorption spectra of fresh petals or anthocyanin-containing cells have been appeared to date, and these spectra have also been classified into several groups according to their main features, such as the number of peak maxima and the position of maxima in the visible range by many groups.[28,99-102]

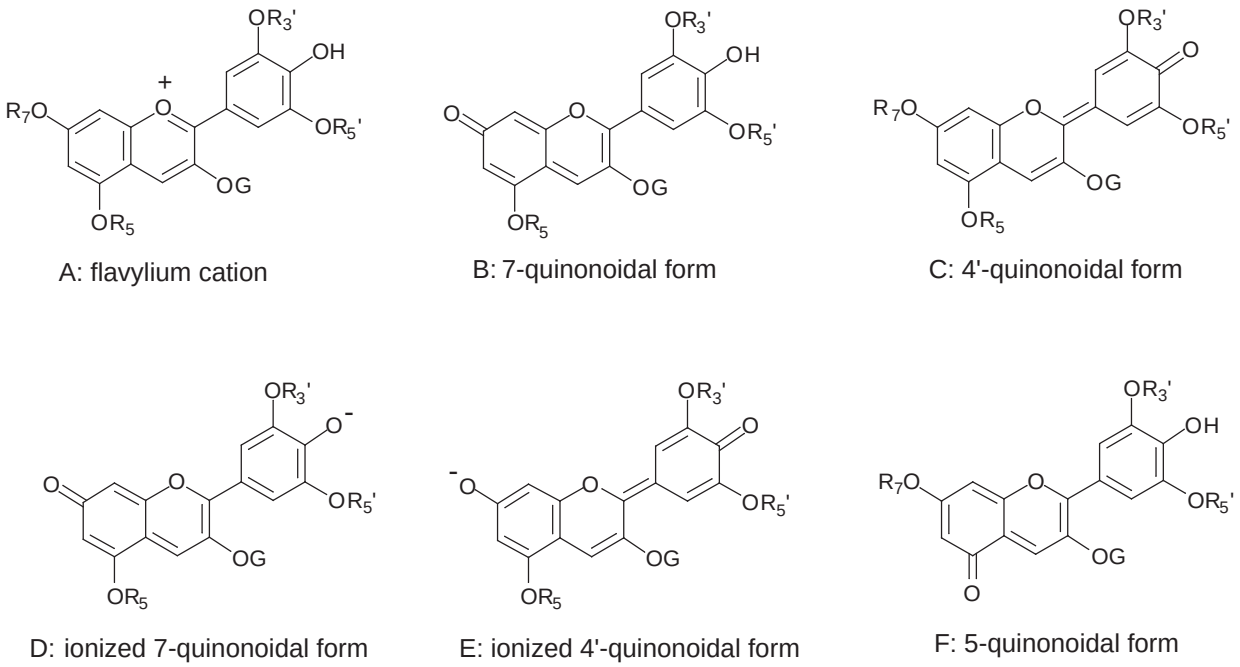


Figure 2. Main prototropic tautomerism-isomers

Since anthocyanidins have the hydroxy groups at the 3, 5, 7, 3', 4' and 5'-positions, many isomeric structures will be considered to be present in the aqueous solutions at pH 3-8.[29,103] The formation of these isomers arises from the difference of pH-conditions as well as their glycosylation patterns as shown in Figure 2, and these isomers exhibit various colors in their solutions depending on their aromatic ring π bond energy levels.[28,29,103] The important isomeric forms for anthocyanidin are depicted in Figure 2. Since polyacylated anthocyanins are very stable in the neutral or weakly acidic solutions, the spectral measurements of quinonoidal and ionized quinonoidal bases [B(7), C(4'), D(7), E(4')] are easily able to carry out for the solutions at pH 3-8. Therefore, the spectral data for quinonoidal and flavylium cation

structures are mainly obtained at the pH 4.01 aqueous solution, and those of ionized and neutral quinonoidal structures are taken for the solutions at pH 6.86, in this paper (Table 4).

Table 4. The observed VIS λmax (nm) in buffer solution at pH 4.01 or at pH 6.86 of typical polyacylated anthocyanins in each group

Acyl Type (Group)		Name of Anthocyanin	λ max pH 4.01	λ max pH 6.86	Deacyl anthocyanin	Compd. No.
1	A	IBRA-4	(460) 525	555 - 575	Cy 3-glu-glu	**1**
	B	Heavenly Blue anthocyanin	536	560 - 595	Peon 3-soph-5-glu	**6**
	B	IBA-1	537 (570)	560 - 585	Cy 3-soph-5-glu	**10**
	B	MVA-1	543 (575)	555 - 595	Cy 3-sam-5-glu	**21**
	B	IRA-1	520	532 - 570	Pel 3-soph-5-glu	**13**
	B	MRA-2	536 (560)	535 - 575	Pel 3-sam-5-glu	**25**
	B	EA-1	537 - 565 (610)	(545) 570 613	Del 3,5-diglu	**3**
	B	PA-22	(540) - 567 (610)	530 - 560 (610)	Mal 3-rut-5-glu	**16**
2	A	AA-1	(510) 540 (620)	(510) 543 (620)	Del 3,7-diglu	**27**
	B	Platyconin	532 568 617	(533) 572 618	Del 3-rut-7-glu	**29**
	B	Violdelphin*	534 569 620	(534) 569 620	Del 3-rut-7-glu	**33**
	B	Senecio pink anthocyanin	(450) 515 (558)	455 (500) 530 564	Pel 3,7-diglu	**28**
	B	Rubrocampanin	(468) (500) 523 (560)	(465) 500 530 564	Pel 3-rut-7-glu	**31**
3	A	Ternatin A1	(533) 572 617	(580) 623	Del 3,3',5'-triglu	**38**
	D	Ternatin C1	(530) 565 608	(530) (580) 612	Del 3,3',5'-triglu	**49**
4	A	Bletilla anthocyanin 1	(483) 510 545 587	(480) 510 546 588	Cy 3,7,3'-triglu	**61**
	A	Rubrocinerarin	(480) 512 545 587	(480) 512 546 588	Cy 3,7,3'-triglu	**55**
	A	Cinerarin	(505) 537 574 620	(505) 540 576 622	Del 3,7,3'-triglu	**54**
5	A	Alatanin A	(480) (506) 539 578	506 539 579	Cy 3-glu-glu -7,3'-diglu	**75**
	C	Zebrinin**	508 545 585		Cy 3-glu-glu -7,3'-diglu	**80**
	C	Tradescantia anthocyanin	510 542 583	509 543 584	Cy 3-glu-glu -7,3'-diglu	**82**
6	A	Gentiodelphin	(535) 566 (608)	(585) 618	Del 3,5,3'-triglu	**84**

* see in Ref. 64. pH 4.01: Phthalate buffer solution
** see in Ref. 87. pH 6.86: Phosphate buffer solution
The peaks observed as shoulders are in parentheses.

In the groups 2 and 4, where the 7-position is blocked with the sugar substituents, the major contribution of the structural isomers will be the forms A, C, and E. On the other hand, for the pigments without substituents at the 7-position, the interesting results are already reported by Abe *et al.*[104] They mentioned by the spectral study of the synthetic anthocyanidins that pelargonidin and cyanidin anthocyanins, without substituents at the 7-position, take 7-quinonoidal form as the major contributed form, whereas, 4'-quinonoidal form will be the major isomeric form in delphinidin anthocyanins under neutral or weakly acidic conditions. As the typical example of the group 1, we first measured the light absorption spectra of

Ipomoea red anthocyanin 1 as a typical pelargonidin pigment in the pH buffer solutions (Figure 3). This pelargonidin pigment showed its λmax at 520 nm under pH 4.01, and at 532 - 570 nm under pH 6.86. On the basis of these experimental results and their substitution types of pigments, it will be realized that the former absorption will mainly be due to the isomeric forms A and B of pelargonidin 3,5-glycoside, and the latter arise from the mixture of its ionized isomers D and B (Figure 3 and Table 4).

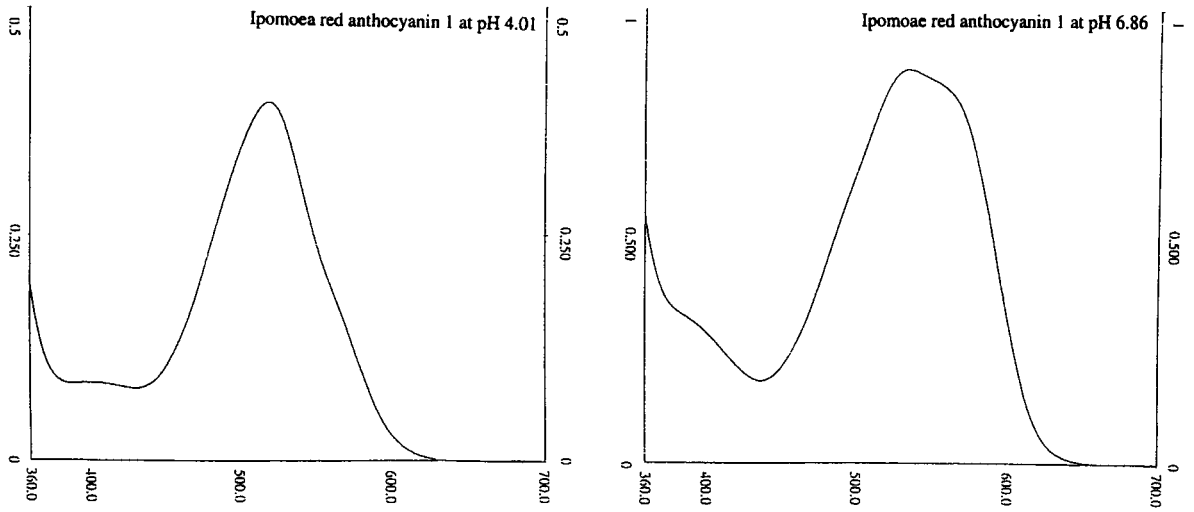


Figure 3

In the similar spectrum of Matthiola violet anthocyanin 1 (MVA-1) (a cyaniding 3,5-glycoside type) as the group 1, MVA-1 exhibited its λmax at 543 nm under pH 4.01, and λmax at 555 - 595 nm under 6.86 (Figure 4). These results will support that the former is mainly produced by the isomeric form A and B, and the latter is due to the presence of isomeric forms B and D, respectively.

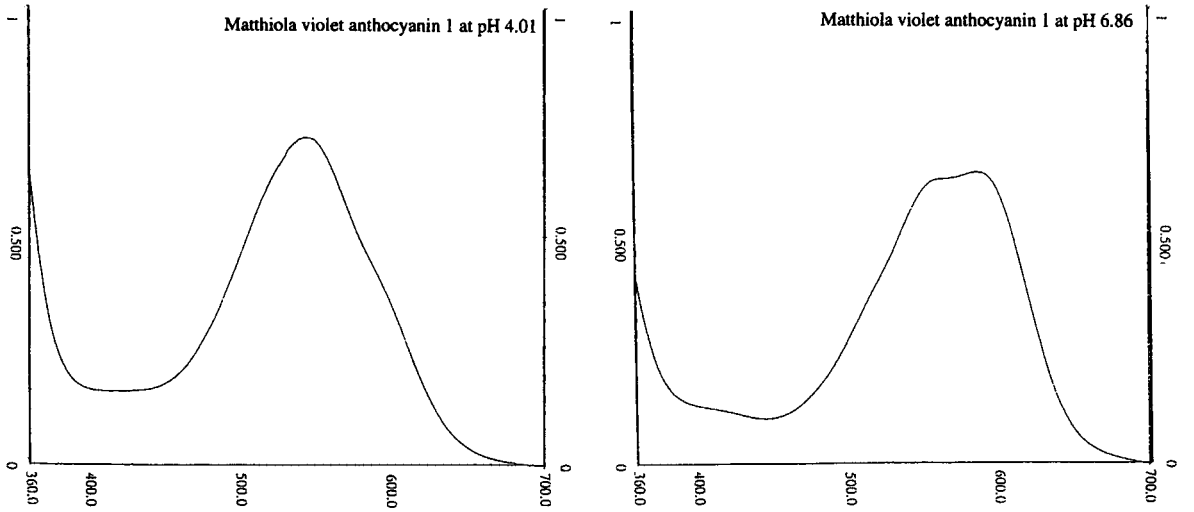


Figure 4

For the delphinidin pigment of the group 1, we have measured the spectra of Evolvulus anthocyanin 1, and obtained lmax at 537 – 565 and (610) nm under pH 4.01, in which the latter is appeared as shoulder, and also λmax at (545), 570 and 613 nm under pH 6.86 (Figure 5). The isomeric forms A, B, and C of delphinidin and a small amount of D or E will contribute to the former spectrum, and the latter will mainly be due to the mixture of isomeric forms B, C, D and E. Probably, the contribution of C and E will be dominant in this spectrum.

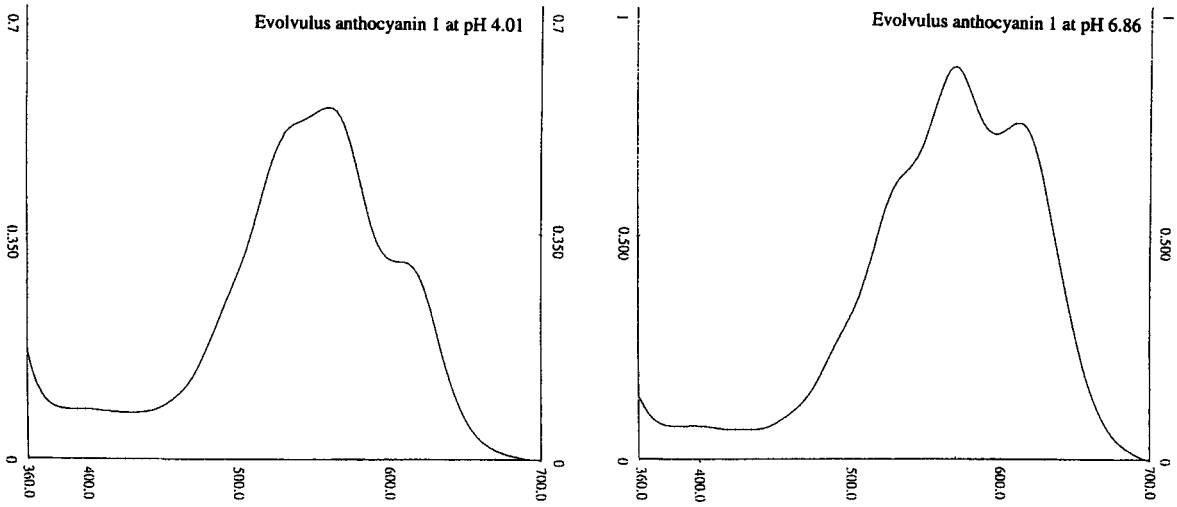


Figure 5

On the analysis of the spectra of pelargonidin pigments and also the absence of isomeric forms B and D due to the presence of the substituent at the 7-position in the group 2, the major isomeric forms at pH 6.86 is suggested to be C and E, whereas the isomers C and A will take the majority at pH 4.01, probably contribution of A will be dominant than C in this case. For example, Senecio pink anthocyanin exhibited the absorption bands at (450), 515, and (558) nm at pH 4.01 (Figure 6) corresponding mainly the isomeric form A (515 nm in Figure 6), and Rubrocampanin also showed its absorption bands at (468), (500), 523, and (560) nm at pH 4.01 (Table 4), which also mainly correspond to the isomeric form A in addition to the form C. However, the strong absorption band at 564 nm is observed at pH 6.86 in both pigments as well as 530 nm mainly correlated to form C. These data indicated that both pigments take E form (λmax 564 nm) as the major isomer under weakly acidic condition (Table 5).

As typical delphinidin type of pigments in this group, we measured the spectra of platyconin, in which λmax at 532, 568, and 617 nm were observed at pH 4.01 as main absorption (Figure 7).

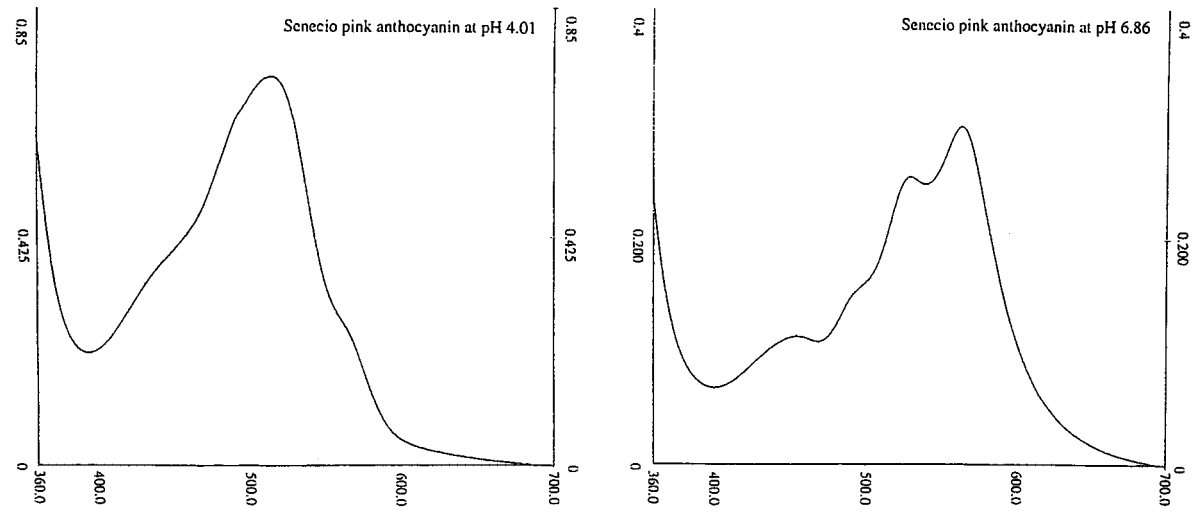


Figure 6

These absorption probably arose from the contribution of the structures of A and C. Whereas the absorption bands at 572 and 618 nm at pH 6.86 indicated the contribution of the isomers C and E to this spectrum.

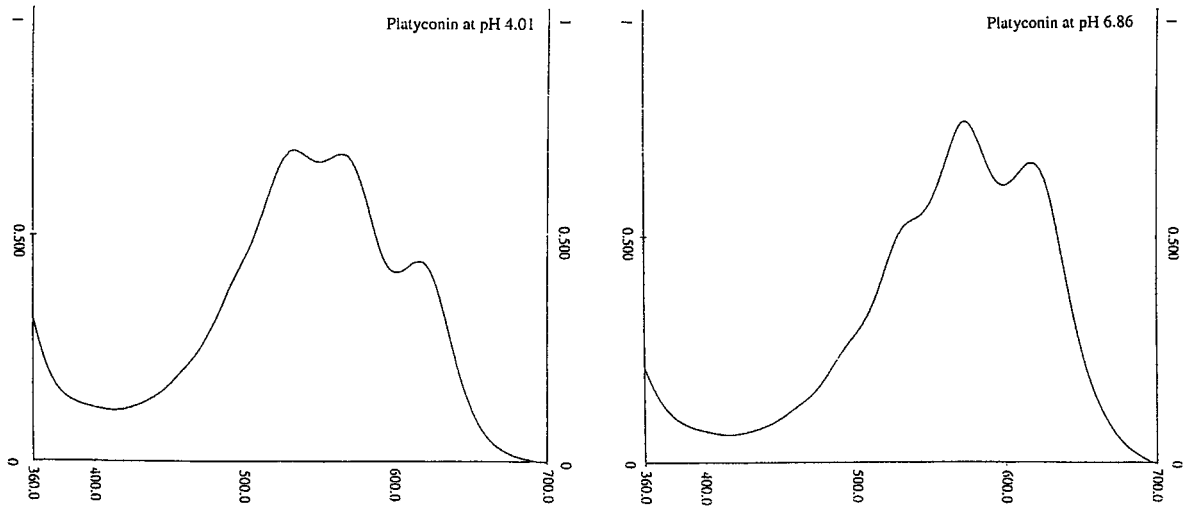


Figure 7

As the pigments of the group 3, the spectra of ternatin A1 were measured, and the absorption bands at (533), 572 and 617 nm were obtained for this pigment at pH 4.01 (Figure 8). These values might be considered as the contribution of the isomeric forms of C and E. The spectrum gave the band at 623 nm at pH 6.86, and these these absorption supported the contribution of E form under this condition with the presenceof a small amount of D form.

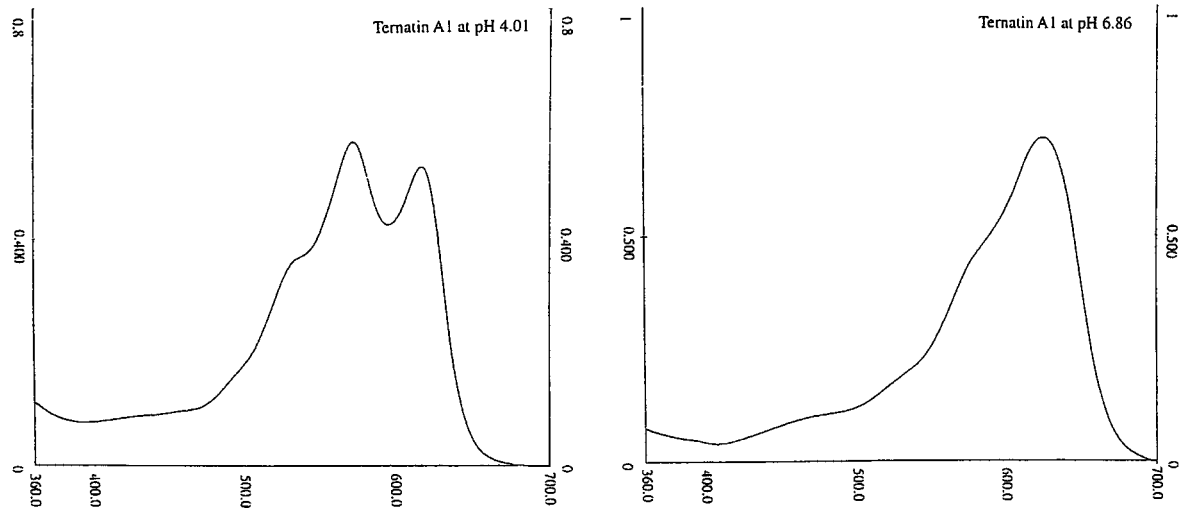


Figure 8

Since the pigments in the group 4 were substituted at 3,7,3'-OH of the anthocyanidins, the presence of the 7-quinonoidal form could be ignored, and the isomeric forms A, C, and E should be considered among the contribution of these isomeric mixtures. Actually, rubrocinerarin, a cyanidin type of pigments, showed its λmax at 512, 545, and 587 nm at pH 4.01 (Figure 9).

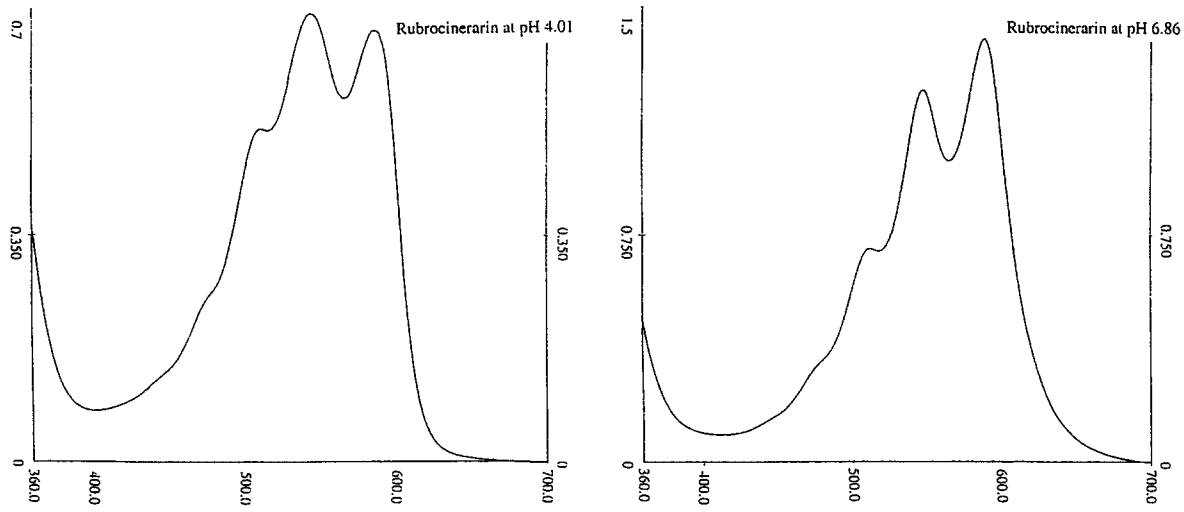


Figure 9

In the spectrum of Blatilla anthocyanin 1, three λmax at 510, 545, and 586 nm were observed. In these λmax values, the second and third λmax (545 and 586 nm) appeared bigger than that of the first λmax, which meant that the light absorption spectrum of Bletilla anthocyanin 1 was produced by the major contribution of the isomers C and E with a minor contribution of isomer A at pH 4.01 (Table 4). On the

other hand, the spectra of this pigment and also of rubrocinerarin gave two strong λmax at 588 and 546 nm at pH 6.86, in which the absorption λmax at 588 nm (isomer E) is observed as a remarkably strong peak. This fact supported that the isomer E contributed mainly to produce its absorption spectrum at pH 6.86 as well as the minor contribution of the isomer C.

The spectrum of Cinerarin, a delphinidin pigments in this group, afforded λmax at 505, 537, 574, and 620 nm at pH 4.01 (Figure 10). These three λmax are relatively strong peaks, but the strongest peak of them is at 574 nm. These peaks were considered to be produced by the mixture of the isomeric forms C, E and A, where the contribution of the isomer C will be dominant. Similarly, the isomeric form E (622 nm) of cinerarin is deminant in the solution at pH 6.86.

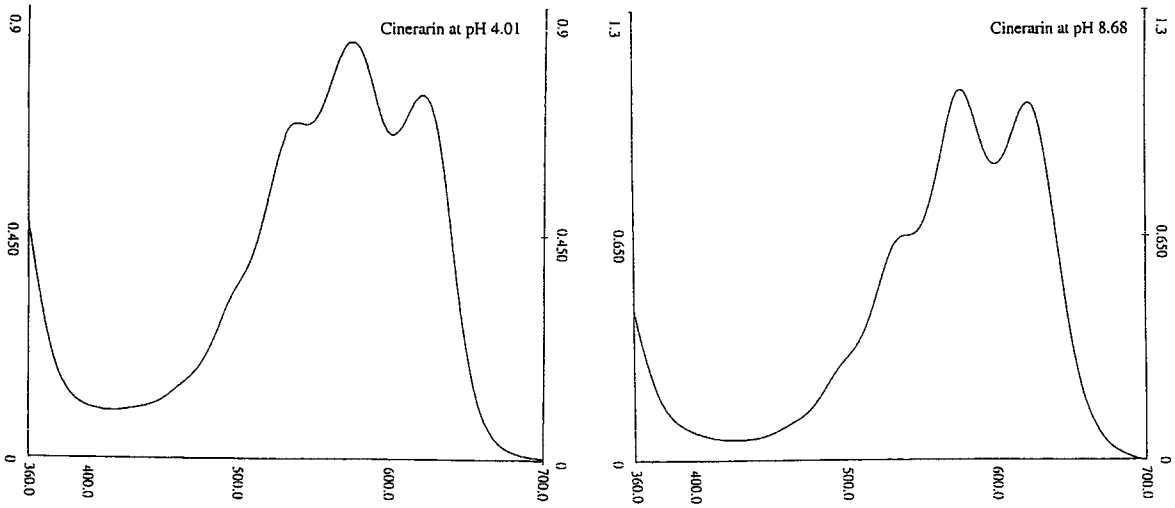


Figure 10

As the pigment in the group 5, Tradescantia anthocyanin and Alatanin A, cyanidin pigments, gave the similar spectra to those of the pigments in the group 4 under both pH 4.01 and 6.86 solutions (Table 4).

Gentiodelphin showed a large λmax at 566 nm with the relatively strong shoulder peak at 535 nm at pH 4.01, which indicated the presence of the major contribution of isomer B with rather minor of isomer A (Figure 11). Whereas the λmax at 618 nm with the relatively strong shoulder peak at 585 nm were obtained in its absorption spectrum at pH 6.86. In this case, the isomers E and D are thought to contribute the formation of its spectrum as the major isomers. Fortunately, due to the potential stability of those polyacylated anthocyanins, the presence of each possible tautomers could apparently be observed and their

expected λmaxs were obtained (see Table 5), although the presence of these isomers for the rather simple and non-acylated anthocyanins have long been proposed, however, direct observation of such isomers has not succeeded in to date, because of their instability in the neutral or acidic conditions.

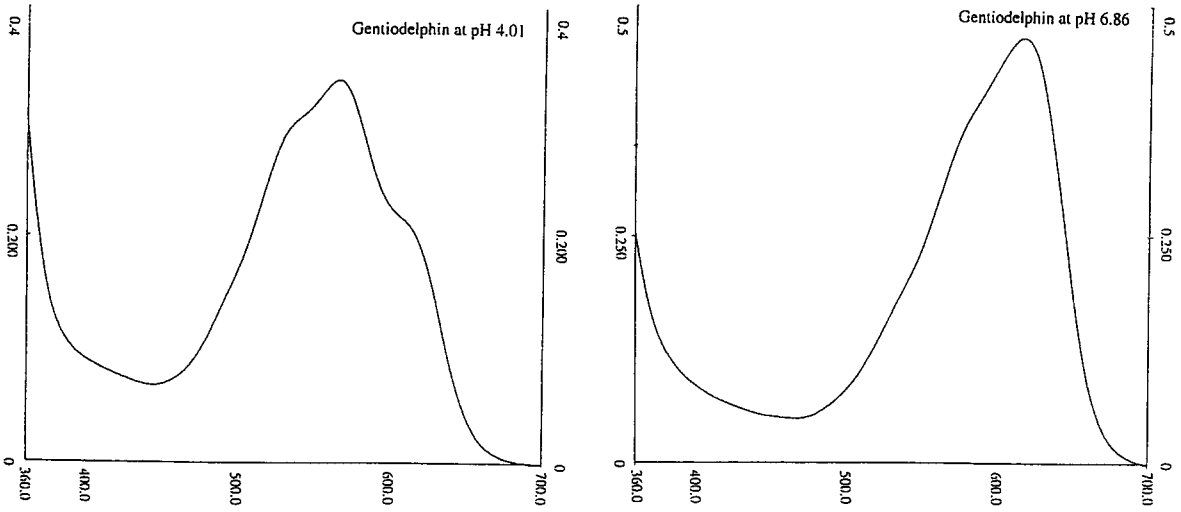


Figure 11

Table 5. Expected λmax (nm) for each isomers of anthocyanidin glycosides from the experimental.

Acyl Type (Group)	Substitutions R_3	R_5	R_7	$R_{3'}$	$R_{5'}$	Prototropic tautomerism - isomer A*	A	$B_{(7)}$	$C_{(4')}$	$D_{(7^-)}$	$E_{(4'^-)}$	F	Compd. No.
1-A	G	H	H	OH	H	523	525	555-575		555-575			**1**
3-A	G	H	H	OG	OG	523	533		572		623		**38**
1-B	G	G	H	H	H	515	520	533		563		—	**13**
1-B	G	G	H	OH	H	529	543	560		588		—	**21**
6-A	G	G	H	OG	OH	538	540	566		585	618	—	**84**
5-B**	G	G	H	OG	OG	544	530		568		612	—	**77**
1-B	G	G	H	OH	OH	546	540	(562)	570		610	—	**3**
2-B	G	H	G	H	H	513	515	—	530	—	564	460	**28**
2-B	G	H	G	OH	OH	549	533	—	570	—	618	510	**29**
2-A	G	H	G	OH	OH	550	540	—		—	625	514	**27**
4-A	G	H	G	OG	H	536	512	—	546	—	588	480	**55**
5-A	G	H	G	OG	H	534	506	—	539	—	578	480	**75**
4-A	G	H	G	OG	OH	550	537	—	575	—	622	505	**54**

* λmax in 0.1% HCl - MeOH; ** λmax in H_2O; G: glycoside.
Prototropic tautomerism - isomers: see in Figure 2.
The peaks observed as shoulders are in parentheses.

IV. The ^{1}H-NMR spectra of polyacylated anthocyanin

As we discussed above, the aromatic acids in the polyacylated anthocyanins play an important role as a factor of controlling cyanic colors of flowers in bluing (bathochromic shift effect) in addition to the stabilization of flower color. The stabilization of the flower color is well explained and rationalized by several factors, such as self-association, inter- and intramolecular co-pigmentation, and metal complexation, which prevent the attack of water to anthocyanins from both up and down sides of anthocyanidin nucleus.[18,28-31,103,105] Especially in the polyacylated anthocyanins, intramolecular co-pigmentation is well accepted to stabilize the flower color for many hours.[18,22,66] This co-pigmentation usually occurred between anthocyanidin and the substituted aromatic acid in the side chains by making the sandwich-like stacking model. We thought that this co-pigmentation will cause some changes for the chemical shifts of aromatic rings of anthocyanins. We, therefore, investigated the ^{1}H-NMR spectra of the polyacylated anthocyanins by the focusing our mind on the difference of substituion position and number of aromatic acid.

In Tables 6-1 to 6-3, the proton chemical shifts (ppm) of anthocyanidin nucleus and the number of aromatic acids are indicated for the main anthocyanins in each group 1 – 7. As can be seen in these Tables, particularly Tables 6-2 and -3, the pigments having more aromatic acids have inclination to shift δ values of anthocyanin protons to higher magnetic field. These phenomena will be caused mainly by the presence of ring current effects by the stacking structure between anthocyanidin and aromatic acids. The up-field shift of δ values of proton signals in their NMR spectra is remarkably observed for the protons at the 4-position. This fact means that the sandwich-like stacking state might take place the strongest π–π interaction on the hetero-ring (C-ring) of anthocyanins. These characteristic proton chemical shift movements are observed especially for the pigments in the groups 3 and 4, in which these pigments have the acylated substituents at the 7- and 3'-positions. In these groups, Bletilla anthocyanins and Laeliocattleya anthocyanins contained three molecules of aromatic acids in their structures, and exhibited the chemical shifts of H-4 at 8.36 ppm (cf. 9.04 ppm of cyanidin 3,3',7-triglycoside), which seems to be very high magnetic region usually expected for the proton signals at the 4-position of structurally simple anthocyanins. From these data, it may be assumed that the increase-rate of one molecule of aromatic acid generally shifts δ value of the 4-position to be appeared at *ca.* 0.2-0.3 ppm upper magnetic field. In fact, other large chemical shift changes

Table 6-1. ^{1}H NMR spectra of polyacylated anthocyanins in DMSO-d_6/CF_3CO_2D or DMSO-d_6/DCl

Acyl Type (Group)	Name of Anthocyanin (Pelargonidin type)	4	6	8	2',6'	3',5'	λmax in 0.1% HCl-MeOH (nm)	Number of aromatic acid	Compd. No.
1	Pharbitis red anthocyanin 5 (PRA 5)	8.91	6.99	7.07	8.58	7.10	515	3	**8**
	Ipomoea red anthocyanin 1 (IRA 1)	8.92	6.95	7.02	8.55	7.07	512	3	**13**
	IRA 2	8.92	6.98	7.00	8.56	7.08	511	3	**14**
	PRA 3*	8.90	6.95	7.03	8.58	7.09	511	2	**9**
	IRA 4	8.91	6.97	7.01	8.57	7.09	510	2	**15**
	PRA 1*	8.88	7.04	7.12	8.62	7.13	507	1	
	PRA 2	8.98	6.96	7.11	8.62	7.10	506	1	
	Pelargonidin 3-sophoroside-5-glucoside#	8.97	7.02	7.17	8.60	7.14	507	0	

Acyl Type (Group)	Name of Anthocyanin (Cyanidin type)	4	6	8	2'	5'	6'	λmax in 0.1% HCl-MeOH (nm)	Number of aromatic acid	Compd. No.
1	Ipomoea brownish-red anthocyanin	8.90	6.66	6.82	8.01	7.06	8.18	527	2	**1**
	Ipomoea blue-violet anthocyanin 1 (IBA 1)	8.81	6.96	6.97	8.05	7.10	8.25	532	3	**10**
	IBA 2	8.80	6.98	6.95	8.05	7.11	8.25	530	2	**11**
	IBA 3	8.81	6.92	6.95	8.05	7.11	8.26	530	2	**12**
	Triteleia anthocyanin	8.83	7.03	7.16	8.06	7.10	8.23	528	2	**5**
	PBA-4	8.74	6.97	7.03	8.02	7.07	8.23	528	1	
	Cyanidin 3-sophoroside-5-glucoside#	8.90	7.04	7.16	8.11	7.13	8.29	523	0	
	Name of Anthocyanin (Peonidin type)									
	Heavenly blue anthocyanin	8.89	6.96	7.08	7.94	6.77	8.32	530	3	**6**
	PBA-3	8.94	6.98	7.05	7.99	7.09	8.34	528	2	**7**
	PBA-1	9.04	7.01	7.17	8.02	7.15	8.44	525	1	
	Peonidin 3-sophoroside-5-glucoside#	9.01	7.00	7.32	8.07	7.12	9.43	522	0	

Acyl Type (Group)	Name of Anthocyanin (Delphinidin type)	4	6	8	2',6'	λmax in 0.1% HCl-MeOH (nm)	Number of aromatic acid	Compd. No.
1	Petunia dasky violet anthocyanin (Malvidin)	8.87	6.94	7.08	7.89	545	2	**2**
	Malvidin 3-sophoroside#	8.91	7.01	7.16	7.95	539	0	
	Petunia anthocyanin 16 (PA 16) (Petunidin)	8.95	7.15	7.28	7.90	542	2	**19**
	Petunidin 3-rutinoside-5-glucoside#	8.69	7.09	7.30	7.90	538	0	
	PA 19 (Malvidin)	9.09	7.11	7.40	7.99	543	2	**17**
	PA 22 (Malvidin)	9.07	7.16	7.39	7.98	543	2	**16**
	Malvidin 3-rutinoside-5-glucoside#	9.09	7.21	7.44	8.02	537	0	
	Evolvulus anthocyanin 1	8.77	6.18	7.09	7.75	546	2	**3**
	Delphinidin 3,5-di-glucoside #	8.87	6.99	7.18	7.80	538	0	

* measured in CD_3OD - DCl
# non-acyl anthocyanin

Table 6-2. ^{1}H NMR spectra of polyacylated anthocyanins in DMSO-d_6/CF_3CO_2D or DMSO-d_6/DCl

Acyl Type (Group)	Name of Anthocyanin (Pelargonidin type)	4	6	8	2',6'	3',5'	λmax in 0.1% HCl-MeOH (nm)	Number of aromatic acid	Compd. No.
2	Rubrocampanin	8.68	6.77	7.45	8.78	7.00	517	3	**31**
	Senecio pink anthocyanin 1 (SPA 1)	8.88	6.86	7.39	8.56	7.04	513	2	**28**
	SPA 2	8.91	6.92	7.42	8.62	7.07	508	1	
	Delphinium red anthocyanin 6 (DRA 6)	8.93	7.00	7.49	8.65	7.13	508	1	
	DRA 3	8.90	7.07	7.46	8.65	7.15	508	1	
	Pelargonidin 3-rutinoside-7-glucoside	8.93	6.99	7.44	8.65	7.15	504	0	
	Pelargonidin 3,7-diglucoside#	8.91	7.07	7.43	8.66	7.15	503	0	
	Name of Anthocyanin (Delphinidin type)								
	Campanin	8.60	6.90	7.27	7.85		550	3	**30**
	Aemeniaca anthocyanin 4 (AA 4)*	8.39	6.70	7.38	7.89		550	3	**36**
	AA 3	8.59	6.63	7.30	7.89		546	3	**35**
	AA 2	8.59	6.70	7.14	7.76		548	2	**34**
	AA 1	8.57	6.69	7.20	7.77		548	1	**27**
	Violdelphin*	8.65	6.68	7.14	7.80		525	1	**33**
	Delphinidin 3-glucoside-7-sophoroside#	8.93	6.88	7.01	7.92		537	0	

Acyl Type (Group)	Name of Anthocyanin (Delphinidin type)	4	6	8	2',6' or 2'	6'	λmax in 0.1% HCl-MeOH (nm)	Number of aromatic acid	Compd. No.
3	Ternatin A1	8.58	6.92	6.64	8.02		550	4	**38**
	Ternatin D1	8.59	6.93	6.65	8.04		550	4	**45**
	Ternatin B2	8.52	6.93	6.64	8.05		548	3	**42**
	Ternatin B3	8.47	6.88	6.68	8.03	7.98	548	3	**43**
	Ternatin D2	8.48	6.87	6.67	8.04	8.00	547	3	**46**
	Ternatin A3	8.44	6.93	6.63	8.05		545	2	**40**
	Ternatin B4	8.44	6.89	6.64	8.02	7.99	543	2	**44**
	Ternatin C1	8.75	7.00	6.72	8.12	8.09	537	2	**49**
	Ternatin C3	8.70	6.97	6.72	8.12	8.03	535	1	**51**
	Ternatin C4	8.71	7.00	6.69	8.12	8.08	534	1	**50**
	Delphinidin 3-malonylglucoside-3',5'-di-glucoside#	8.92	7.09	6.77	8.13		528	0	
	Delacylternatin*	9.01							

* measured in CD_3OD - DCl
\# non-acyl anthocyanin

Table 6-3. ^{1}H NMR spectra of polyacylated anthocyanins in DMSO-d_6/CF_3CO_2D or DMSO-d_6/DCl

Acyl Type (Group)	Name of Anthocyanin (Cyanidin type)	4	6	8	2'	5'	6'	λmax in 0.1% HCl-MeOH (nm)	Number of aromatic acid	Compd. No.
4	Bletilla anthocyanin 1 (BA 1)	8.36	6.68	6.91	7.84	7.14	8.56	537	3	**61**
	BA 2	8.47	6.67	6.91	7.88	7.10	8.57	536	3	**62**
	BA 3	8.51	6.67	6.90	7.83	7.12	8.60	537	3	**63**
	BA 4	8.61	6.66	6.91	7.87	7.13	8.60	533	3	**64**
	Laeliocattleya anthocyanin 1 (LA 1)	8.41	6.71	6.87	7.75	7.09	8.51	535	3	**56**
	LA 3	8.48	6.78	6.93	7.72	7.09	8.52	538	3	**58**
	LA 6	8.50	6.79	6.98	7.77	7.07	8.51	538	3	**59**
	Dendrobium anthocyanin	8.56	6.59	7.78	8.09	7.17	9.03	533	2	**73**
	demalonyl DA	8.66	6.60	7.69	8.16	7.15	8.95	533	2	
	Phalaenopsis anthocyanin 3 (PA 3)	8.62	6.69	6.98	7.97	7.02	8.49	533	2	**69**
	PA 1	8.69	6.69	7.03	7.99	7.07	8.51	535	2	**70**
	Sophronitis anthocyanin 3 (SA 3)	8.85	6.85	7.50	8.10	7.01	8.57	524	1	
	SA 1	8.89	6.83	7.50	8.18	7.07	8.63	521	1	
	Cyanidin 3,7,3'-triglucoside#	9.04	6.85	7.23	8.27	7.16	8.69	513	0	
	Cinerarin	8.41	6.76	6.76	8.05		7.32	549	3	**54**
	Rubrocinerarin	8.53	6.76	6.93	7.76	7.07	8.53	533	3	**55**

Acyl Type (Group)	Name of Anthocyanin	4	6	8	2'	5'	6'	λmax in 0.1% HCl-MeOH (nm)	Number of aromatic acid	Compd. No.
5	Alatanin A*	8.55	6.39	7.06	7.94	7.01	8.45	534	2	**75**
	Alatanin B*	8.56	6.82	7.06	7.91	7.01	8.44	533	2	**76**
	Alatanin C*	8.55	6.62	6.48	7.84	7.01	8.18	539	1	
	Zebrinin*	8.39	6.75	6.72	7.50	7.00	8.43	532	4	**80**
	Tradescantin A	8.47	6.84	6.70	7.76	7.02	8.34	532	4	**82**
6	Gentiocyanin B*	8.78	6.96	6.85	8.02	7.19	8.45		2	**85**
	Gentiodelphin*	8.80	6.90	7.10	7.80		7.89	538	2	**84**
	Albiodelphin D*	8.80	6.97	6.80	7.83		7.83		2	**86**
	Albiodelphin E*	8.78			7.79		7.88		2	**87**
		4	6	8	2',6'					
7	Eichhornia anthocyanin	8.63	7.21	6.82	7.80			548	0	**88**
	Agapanthus pigment 1*	8.80	6.63	7.15	7.82			541	1	**90**
	Anemone blue anthocyanin 1	8.91	7.07	7.25	7.79		8.13	536	2	**92**
	Anemone red anthocyanin	8.89	6.89	7.04	8.60	7.08		512	1	**91**

* measured in CD_3OD - DCl
\# non-acyl anthocyanin

for the 4-position were also observed at δ 8.39 – 8.60 ppm for ternatin pigments in the group 3 and in some others. Actually, the proton signal of deacylanthocyanin appears at 9.01 ppm in ternatin A3 (8.44 ppm),

hence the difference of the chemical shift between these two pigments is 0.57 ppm. Since ternatin A3 has two molecules of aromatic acids, the increase-rate of one aromatic acid for the chemical shift of the proton at the 4-position is *ca.* 0.29 (0.57/2) ppm in upper magnetic field. Interestingly, the similar changes of this increase-rate were observed in the pigments having two or three molecules of aromatic acids in their glycosidic residues. However, the pigments having four molecules of aromatic acids do not show such remarkable changes in their NMR spectra, and these pigments exhibit the chemical shifts as almost same as the case of three molecules of aromatic acids. It may be thought that the saturation of intramolecular co-pigmentation take place in these pigments. Such observed chemical shift changes will reflect on the formation of intramolecular co-pigmentation, where the presence of two or three molecules of aromatic acids will be required to make expected stacking structure between anthocyanidin and aromatic acid, and form the relatively stable co-pigmentation. On the other hand, the fourth aromatic acid may not usually participate for making such co-pigmentation. At present, it is assumed from these data that the polyacylated pigments of the groups 3 and 4 keep mainly the sandwich forms, however, those of the group 1 probably lose the important interaction between chromophore (anthocyanidin) and aromatic acid(s), and exhibit the mixtures of open, half-sandwich, and sandwich forms (Figure 1).

Again these results well support that the aromatic acids in polyacylated anthocyanins play important roles in both controlling the bluing and stabilization of the flower colors.

V. The stereostructures of polyacylated anthocyanins

It is well recognized that the stability of the flower color depends on the stereostructures of polyacylated anthocyanins as mentioned above, since the rigid sandwich-like structures of polyacylated anthocyanins prevent the attack of water to the 2-position of anthocyanidins. It is also understood that the intramolecular stacking state between anthocyanidin and aromatic acid controls the π-electron energy level of polyacylated anthocyanin with making "bluing effect" in the flower colors. We already discussed based on the spectral properties which types of anthocyanin make such intramolecular sandwich-like structure more rigid. Typical examples of proposed stereostructures so far published for making intramolecular co-pigmentation are indicated as Figures 12 - 15.

Figure 12 showed one of the proposed stereostructure of the anthocyanin pigment, isolated from *Lobelia erinus*. This pigment was supposed to take half-sandwich-like form, based on the NMR study including

2D-COSY, NOE, HMBC, HMQC and so on (observed NOE's are indicated by arrows). The proposed stereostructure indicated that the flower color of this pigment would be stabilized by the presence of π-stacking interaction between the aromatic ring of caffeic acid at the 3'-position and flavylium aromatic ring.[86,106]

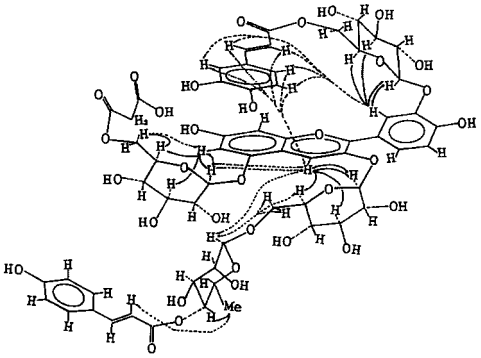


Figure 12

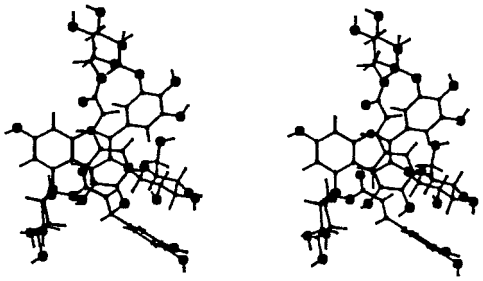


Figure 13

Another half-sandwich-like stereostructure for gentiodelphin was also reported by Yoshida *et al.*[20] as depicted in Figure 13, where the similar interaction of the aromatic ring at the 3'-position with anthocyanidin nucleus was proposed.

Typical example of the sandwich-like form for the anthocyanin was reported in the case of the pigment isolated from *Bletilla striata*, in which the interaction of both aromatic rings of the aromatic acid substituents on the side chains at the 3'- and 7-positions with anthocyanidin nucleus was observed to make the stable flower color as shown in Figure 14.[26]

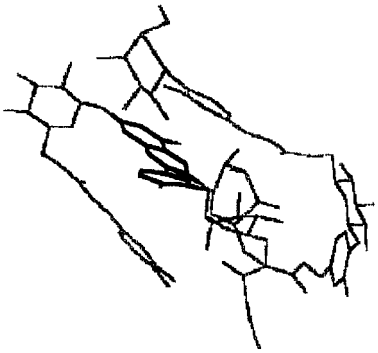


Figure 14

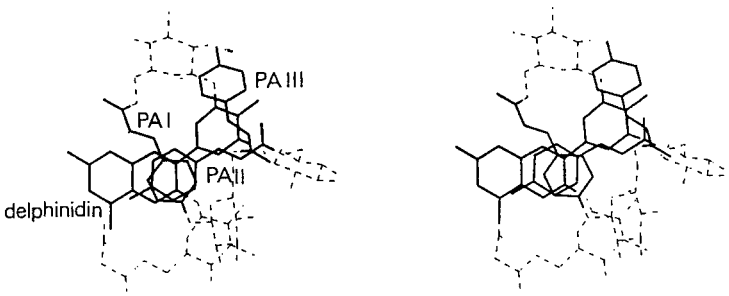


Figure 15

The similar stable intramolecular stacking conformation was also proposed for ternatin D2 based on the NMR study.[107]

VI. Conclusion

Since the isolation of platyconin from the blue-violet flower of *Platycodon grandiflorum* in 1971, about 100 kinds of polyacylated anthocyanins having more than two molecules of aromatic acids in their structures were newly isolated. In these polyacylated anthocyanins, the aromatic acids play important roles in both stabilization of flower color and also in bluing of the flower color. In these two important roles, polyacyl functions would be considered to contribute predominantly in bluing of the flower color at the early stage of the investigation of polyacylated anthocyanin chemistry. However, since the isolation of pelargonidin and cyanidin types of polyacylated anthocyanins, having glycosyl residues at 3-OH, 3,7-OH and 3,7,3'-OH, from the reddish flowers of *Ipomoea* species, *Cineraris* (*Senecio*) species, and some species of the Orchidaea, the stabilization of flower color is considered to be more important subject by making the intramolecular copigmentation between aglycone and the aromatic acids. In the earlier investigation of polyacylated anthocyanins, it is also suggested that the stabilization of the flower color will simply and strongly depend on the number of aromatic acid presented in their structures. However, based on our systematic investigation of the structures of about 100 kinds of polyacylated anthocyanins, the position of acyl group is also a very important factor for the stabilization of the flower color, probably more important than the number of aromatic acid as far as we are concerned. Based on our investigation, it would be suggested that the anthocyanins with the aromatic acids at 7- and 3'-positions are considered to make the most stable colors in the flowers. In other word, these anthocyanins make the relatively rigid stacking sandwich structures.

In the polyacylated anthocyanins, aromatic acids are obviously linked with the sugar residues through the ester bonds. Therefore, the polyacylated anthocyanins are classified into seven groups by their substitution pattern of acyl functions in order to understand their variable functionality systematically. Again, as the results of our investigation, it was figured out that the stabilization of the flower color depended on the substitution position of the acyl group on deacylanthocyanins due to formation of the stacking structures between aglycones and aromatic acids in the polyacyl groups. We would like to see further isolation of new polyacylated anthocyanins and their functionality for the flower color stabilization and bluing effect. We hope that this kind of classification of polyacylated anthocyanins, described here, will make great

contributions to investigate the role of aromatic acyl group, systematically.

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