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THE COMPOSITION OF COMMELININ, A HIGHLY ASSOCIATED METALLOANTHOCYANIN PRESENT IN THE BLUE FLOWER PETALS OF COMMELINA COMMUNIS

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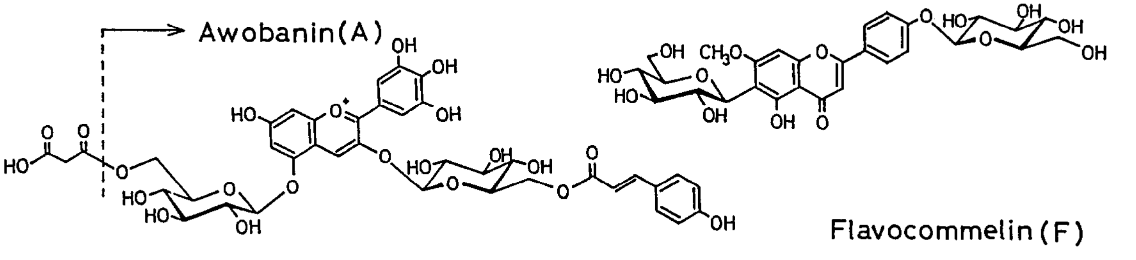
Commelinin was determined to be a highly associated complex molecule composed of 6 molecules each of malonylawobanin (M) and flavocommelin (F), and 2 atoms of magnesium in the form of $[M_6F_6Mg_2]^{6-}$; K^+ is possibly the counter cation in natural commelinin. Its molecular weight is about 9,100.

Hayashi[1,2] isolated a blue-colored anthocyanin, commelinin, in a crystalline form from deep blue flower petals of Commelina communis (Japanese name: tsuyukusa) and reported that it consists of two molecules each of an anthocyanin, awobanin (A), and a flavone, flavocommelin (F), and one atom each of magnesium and potassium.[3,4] This pigment is so stable that its blue color is not changed by changing pH from 7 to 2. He explained its color and stability in terms of co-ordinated complex of magnesium and four molecules of the flavonoids; potassium being not an essential component.[3,4] He named such a metal complex of anthocyanin metalloanthocyanin.[5] Bayer[6] opposed this explanation, however, because in general magnesium ion does not form stable chelates with anthocyanins.



Awobanin(A)

Flavocommelin (F)

Malonylawobanin (M)

Commelinin shows two surprising properties that are distinctly different from other anthocyanin pigments;[2] it is not dialyzable and migrates to the anode on electrophoresis at pH 4-6,[7] indicating that it is a high molecular pigment bearing negative charge(s) on it. It has long been mysterious, since neither awobanin nor flavocommelin has a negative charge at the pH, nor does magnesium ion. However, this problem was solved by finding a malonic acid

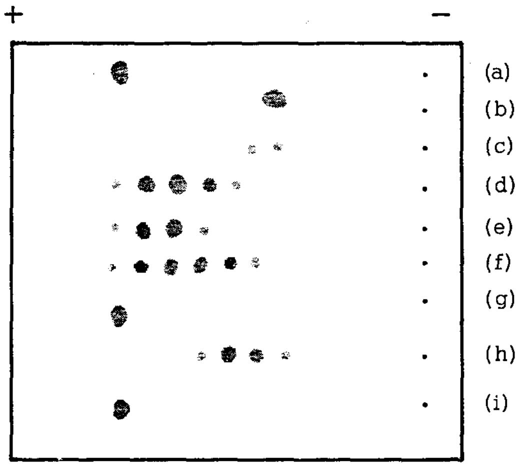


Fig. 1. Electrophoresis of natural and synthetic commelinin and commelinin-like pigments.
(a) M + F + Mg^{++}; (b) A + F + Mg^{++}; (c), (d), (e), and (f) M + A + F + Mg^{++}; (g) M + F + Mg^{++}; (h) natural C (aged: at room temp. for 1 year); (i) natural C (fresh); condition: cellulose acetate sheet, 0.02M acetate buffer, pH 5.7, 1 hour.

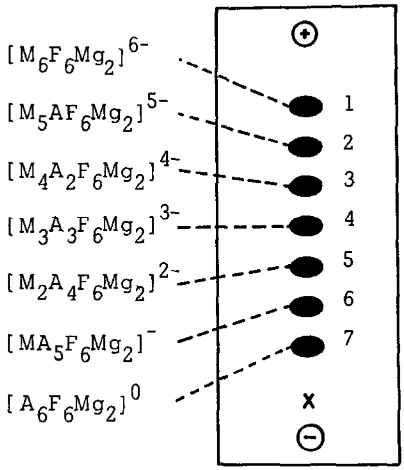


Fig. 2. Schematic representation of electrophoresis of commelinin and commelinin-like pigments.

(C: Commelinin; A: Awobanin; M: Malonylawobanin; F: Flavocommelin)

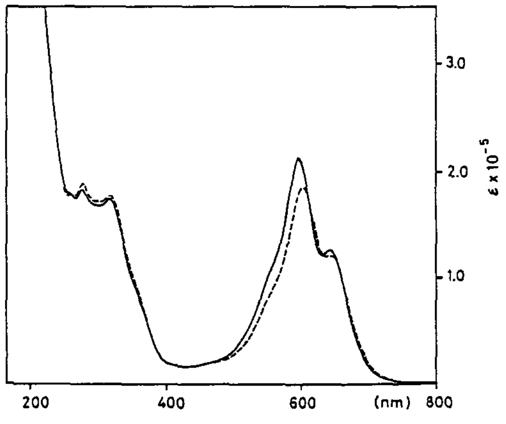


Fig. 3. UV-VIS spectra of commelinin and commelinin-like pigment.[13]

Solid line: natural and synthetic commelinin; dotted line: commelinin-like pigment prepared from awobanin (A), F , and Mg^{++}; condition: 5×10^{-5} M in 0.1M phosphate buffer at pH 7.0

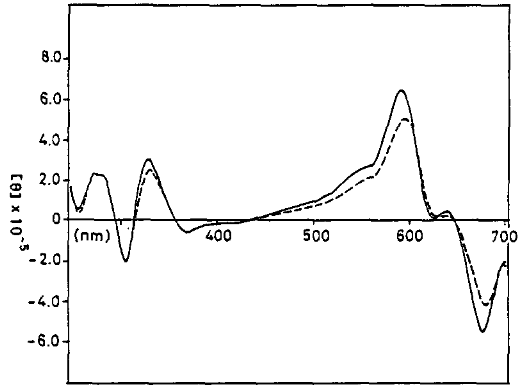


Fig. 4. CD spectra of commelinin and commelinin-like pigment.[13]

Solid line: natural and synthetic commelinin; dotted line: commelinin-like pigment prepared from awobanin (A), F, and Mg^{++}; condition: 5×10^{-5} M in 0.1M phosphate buffer at pH 7.0

moiety on the anthocyanin. Thus, we have found[8] that the true anthocyanin present in the flower is not awobanin (A), but a new anthocyanin containing a malonic acid molecule. We named it malonylawobanin (M). At pH 4-6 malonylawobanin exists in the carboxylate anion form of anhydrobase. Another mysterious finding has been that natural commelinin gave several blue spots on electrophoresis[7] (Fig. 1). We have found that natural commelinin freshly prepared from the live flower petals gave only one blue spot on electrophoresis, whereas several spots were found in the case of the commelinin stored at room temperature for several months or heated at 80°C for a few hours. We synthesized commelinin from the components, malonylawobanin (M), flavocommelin (F), and magnesium ions[9,10] (see below). It showed only one spot, which coincided with the spot that moved fastest. On the other hand, commelinin-like pigments synthesized from awobanin (A), flavocommelin (F), and magnesium ion gave a spot with the least movement. We have mixed malonylawobanin and awobanin and used this mixture for synthesis of commelinin-like pigments. In this case we obtained seven spots each separated by an equal distance from the adjacent spot (Fig. 1). The spot (spot 1) that moved fastest was identical with the spot prepared from malonylawobanin, and the spot (spot 7) that moved least was identical with the spot prepared from awobanin. We extracted each spot and analyzed the contents of malonylawobanin (M) and awobanin (A). Spot 1 gave only M and spot 7 only A. Other spots contained M and A[11] in the ratios of 5.0:1.0 (spot 2), 4.0:1.9 (spot 3), 3.0:2.9 (spot 4), 2.1:3.8 (spot 5), and 1.3:4.7 (spot 6). Now it is evident from this result that commelinin (spot 1) must have six molecules each of malonylawobanin (M) in its carboxylated anion form and flavocommelin (F) in its molecule (Fig. 2).

If we assume that commelinin consists of 6 molecules of malonylawobanin, 6 molecules of flavocommelin, and metal ions, the calculated molecular weight is about 9,100. The observed value by analytical ultracentrifugation (30,000 rpm, 14 hours at 10°C) in 0.1M NaCl was around 9,300. The content of magnesium in commelinin analyzed by atomic absorption spectroscopy was 0.47%, corresponding to 2 atoms of Mg (calcd. value 0.54%). Thus, commelinin molecule must have a composition of $[M_6F_6Mg]^{6-}$; K^+ is possibly the counter cation in natural commelinin.[12]

SYNTHESIS OF COMMELININ[13]

Pure malonylawobanin trifluoroacetate[8] was treated with 0.5N ammonia and then evaporated to produce M anhydrobase. To a solution of the anhydrobase in water was added crystalline flavocommelin,[14] magnesium acetate, and potassium acetate (M:F:Mg:K = 1:1:1.9:1). The mixture was stirred at room temp. for 30 min to a deep blue solution. The resulting mixture was passed through a column of Sephadex G-10. To the blue eluate was added ethanol to give the blue pigment as precipitates in 60% yield. Synthetic commelinin was completely identical with the freshly prepared natural one in UV-VIS (Fig. 3), CD (Fig. 4), and ^{1}H-NMR (not shown) spectra, and mobilities in electrophoresis (Fig. 1) and the retention time of HPLC using MCI gel (Mitsubishikasei Co.). Composition of M and F in natural and synthetic commelinin was analyzed by ODS HPLC to be 1:1 (found: nat. F/M = 0.94; synth. F/M = 0.91).[15]

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11. Contents of M and A in the spot eluted from the cellulose acetate sheet were analyzed by ODS HPLC detected at 520 nm (column: Deverosil ODS, 5μ, 250 x 4.6 mm; flow rate 1ml/min, solvent: $H_3PO_4:CH_3CN:AcOH:H_2O$ = 1.5:12.5:10:76). The following ε values were used for calculation: M, 27,000; A, 26,000. Analysis of spot 5 and 6 are not very accurate due to scarcity of samples.
12. Magnesium ion possibly makes bonds with 3'-hydroxy and 4'-quinonoidal carbonyl groups of each of three M molecules to form a hexacoordinated complex. The coordinated magnesium might have a negative charge, which is not considered in this paper, however.
13. We previously assumed[10] that Mg ion would not be assential in commelinin, because a pigment could be produced from A and F without Mg ion. However, now a careful experiments without Mg ions have given only a trace amount of the blue pigment, which was very similar to natural commelinin but contained other metals such as Zn and Fe than Mg. UV and CD spectra of the pigment are almost identical with those of the commelinin-like pigment [see K. Takeda, Proc. Japan Acad., 53, 257 (1977)]. Thus, metal ions such as Mg^{2+}, Zn^{2+}, Cd^{2+}, etc., are indeed necessary to form the commelinin-like pigments.
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15. Contents of M and F in the pigments were analyzed by HPLC detected at 280 nm (column: Deverosil ODS, 5μ, 250 x 4.6 mm; flow rate 1 ml/min, solvent: $H_3PO_4:CH_3CN:AcOH:H_2O$ = 1.5:12.5:10:76). The following ε values were used for calculation: M, 20,300; F, 18,200.

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