

A PROPOSED STRUCTURE OF COMMELININ, A SKY-BLUE ANTHOCYANIN COMPLEX
OBTAINED FROM THE FLOWER PETALS OF COMMELINA[†]

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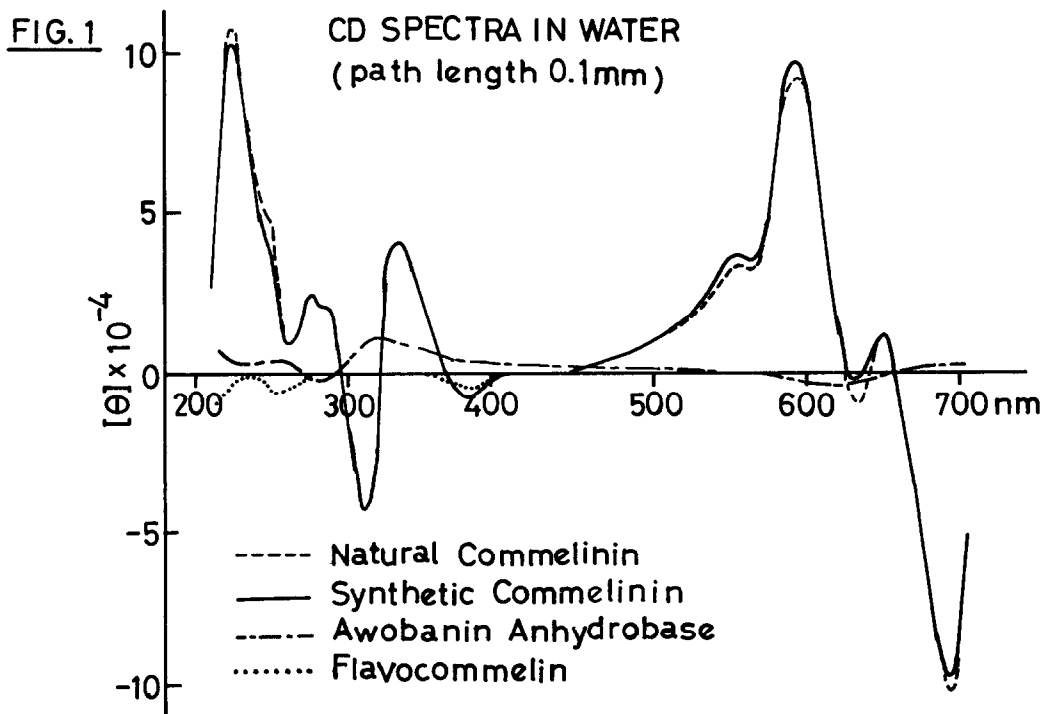
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Extensive studies on the correlation between structure of anthocyanin and flower color have been carried out, but some important problems still remain unsolved.¹ One of them is nature of blue color of flowers such as commelinin. Origin of such blue color cannot be explained simply in terms of pH change of the color of anthocyanins, because not only their long-wave absorption but also their stability in neutral solutions.¹ Shibata in 1918 attempted to explain this stability and color in terms of complex formation of anthocyanin with some metal ions.² Indeed, some anthocyanidins are well known to form deep colored complex with ferric or aluminum ion. Later Robinson³ suggested that flavones and tannins have stabilization and bluing effects on anthocyanins by co-pigmentation effect. Robinson³ further suggested that polypeptides and polysaccharides also combine with anthocyanins to produce change of stability and color.

Commelinin, which is obtainable from petals of Commelina (Japanese name: tsuyu-kusa), is one of the typical sky-blue flower pigments.^{4,5} It contains no high molecular components such as peptides or polysaccharides. Hayashi et al.⁶ assumed that it consists of two molecules each of an anthocyanin, awobanin (A),⁷ and a flavone, flavocommelin (F),⁸ and one atom each of Mg and K. They⁶ explained its color and stability in terms of co-ordinated complex of Mg and four molecules of the flavonoids; K being not an essential component. Bayer¹ opposed this explanation, however because in general Mg²⁺ does not form stable chelates with anthocyanins; he suggested the presence of Fe³⁺ or Al³⁺ in this pigment. No such trivalent metal ions, however, are involved as is evident from the analysis of Hayashi et al.⁶ and ours.⁹ Natural commelinin does contain Mg as reported by Hayashi et al.,⁶ but no evidence has been given whether Mg is essential for the formation of the complex. To clarify this point, we have examined reproduction of the pigment from A and F under the complete absence of Mg²⁺.

Freshly prepared A chloride was treated with aq. ammonia to produce A anhydrobase. To a concd. solution of A anhydrobase was added crystalline F and the mixture was stirred at room temperature. Although the flavone is almost insoluble in water, it rapidly dissolved in the solution by formation of a complex. The resulting mixture was passed through a column of Sephadex G-10 to remove the starting materials, and the blue pigment was precipitated from the eluates by addition of ethanol. Commelinin thus prepared showed UV, IR (KBr disc), and CD spectra superimposable to those of natural commelinin. The characteristic CD spectrum

(Fig. 1) has a strong diagnostic value for the formation of the complex, since only weak CD is observed with the components, A and F. Mg content of this synthetic pigment was given by atomic absorption spectroscopic analysis to be 0.013%, which is far less than that expected from the theoretical value of 1 atom Mg in the complex (calcd. value 0.87%; Mg content in natural commelinin 0.5-0.7%). Thus, evidently Mg is not an essential component to produce the blue color of commelinin. Incidentally, commelinin has negative charge(s) and tends to include divalent cations such as Mg^{2+} in the molecule¹⁰ possibly by formation of a salt.¹¹



A proposed structure of the molecular complex of A and F is shown in Fig. 2; four glucose moieties on A and F are so ingeniously arranged that aromatic rings of A and F are faced each other and are surrounded by the four glucose moieties. In this arrangement phenyl groups of both of the flavonoid nuclei twist in a same direction, which may cause the large CD of commelinin. These AF units can be arranged side by side to form dimer $(AF)_2$, trimer $(AF)_3$, and so on.^{12,13} Driving force for the formation of this tight complex would be hydrophobic interactions between the aromatic rings of both components, and covering effect on the hydrophobic parts of aromatic rings with the hydrophilic glucose moieties. Indeed, commelinin dissociates in organic solvents such as 80% dimethyl. sulfoxide¹⁴ and loses its large CD, because hydrophobic interactions weaken in such aprotic solvents.

Commelinin ($2.56 \times 10^{-5}M$ as AF) gradually decomposed in dilute neutral aq. solution (85% after 6 hrs) to awobanin pseudobase and F. Addition of F ($3 \times 10^{-5}M$) strongly suppressed the decomposition (25% after 6 hrs), while Mg^{2+} ($4 \times 10^{-3}M$)^{13,15} and A pseudobase ($4 \times 10^{-5}M$)

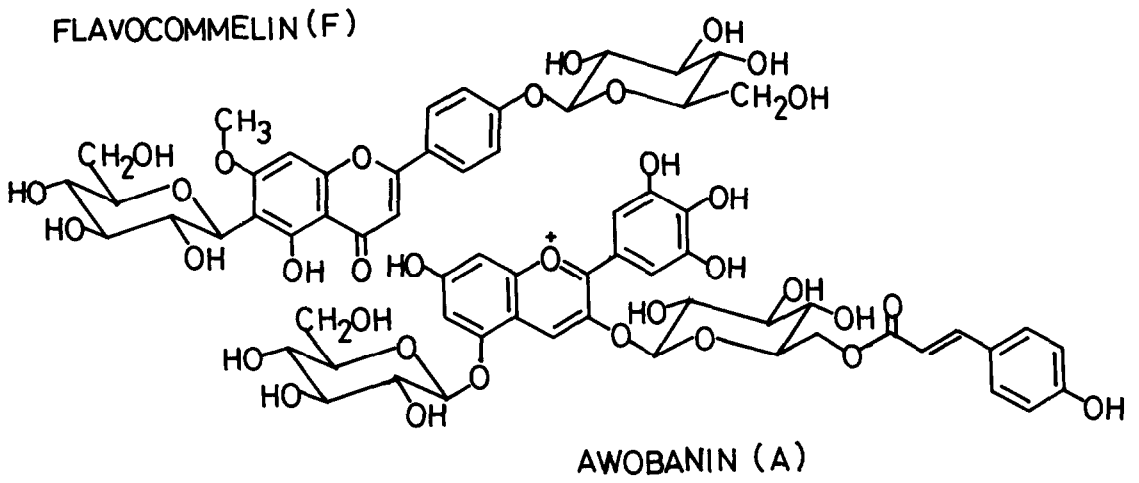
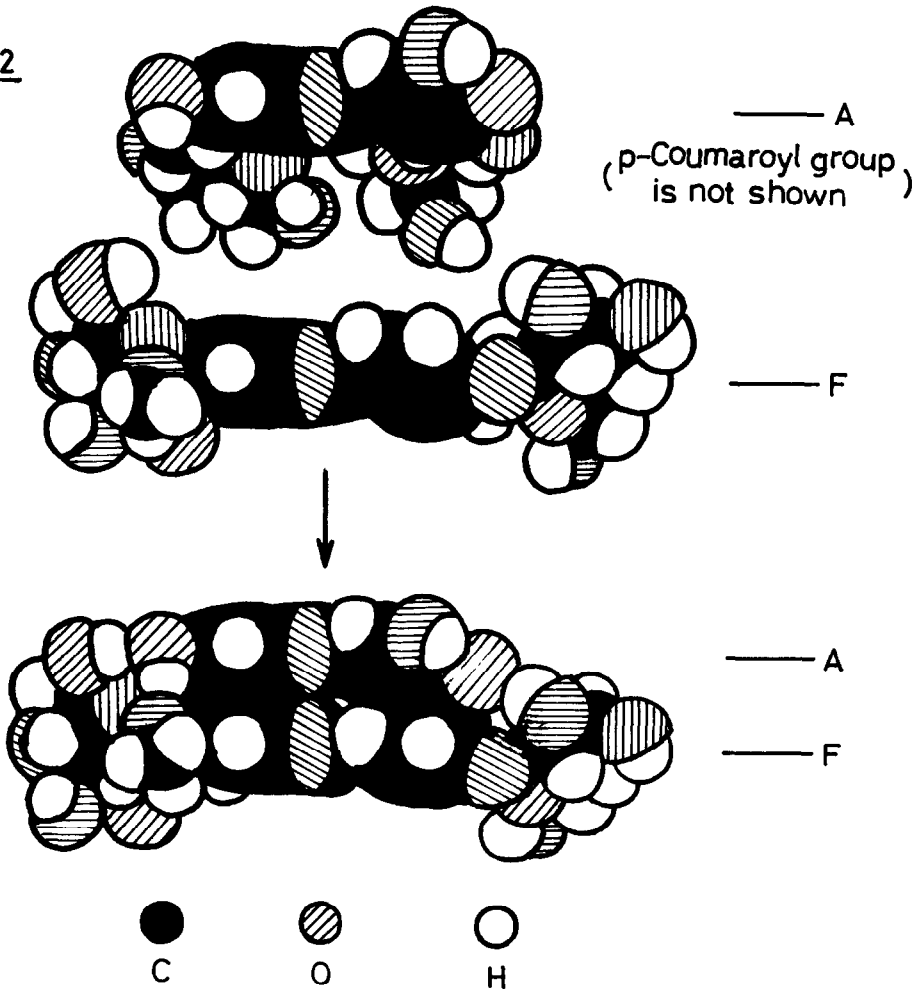


FIG. 2



had no effect on the stability of commelinin, supporting that Mg^{2+} is not an essential component of the complex formation. Rate of decomposition in neutral aq. solution and electrophoretic behavior¹⁶ of the synthetic commelinin were completely identical with that of natural one.

REFERENCES AND FOOTNOTES

- ¶ This paper was presented at The 20th Symposium on the Chemistry of Natural Products, Sendai Japan, October, 1976; Preprint pp. 59-66.
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 - 10) K. Takeda and K. Hayashi, Proc. Japan Acad., 53, 1 (1977); K. Takeda, ibid., 53, 257 (1977); He prepared commelinin containing Mn, Co, Ni, Zn, or Cd in place of Mg.
 - 11) Nature of the negative charge(s) has not yet been clarified.
 - 12) Preliminary results of mol. wt. determination in 3M NaCl (commelinin is stable in this solution¹³) by centrifugation method showed that composition of commelinin is around $(AF)_2 \sim (AF)_3$.
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 - 15) Addition of large amounts of $MgCl_2$ ($> 2M$) effects the complex formation possibly by salt effect.¹³
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