1. Metallo-Anthocyanins. I

Reconstruction of Commelinin from its Components, Awobanin, Flavocommelin and Magnesium

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Commelinin is a blue metallo-anthocyanin^{**)} first isolated by one of us (K.H.)¹⁾ from the flower of *Commelina communis* L. var. *hortensis* Makino. As has been reported earlier,²⁾ this compound is composed of three components, awobanin,³⁾ flavocommelin⁴⁾ and magnesium in the molar ratio of 2:2:1, among which magnesium and flavocommelin are assumed to be responsible for the stable cobalt-blue color shown by the compound. Whereas our experiments have provided unequivocal evidence for the presence of magnesium in the compound, there are some workers who do not recognize, or give no due consideration to, this fact. Recently, T. Goto *et al.*⁵⁾ reported on the formation of commelinin from a mixture of awobanin and flavocommelin, throwing doubt on the necessity of the co-existence of magnesium for the formation of the compound. The experimental results described below will give further evidence for our earlier statements together with some new findings concerning the molecular make-up of the compound in question.

The first step of our experiments was to obtain, from a mixture of awobanin, flavocommelin and magnesium, a stable blue complex in the form of crystals, whose physical and chemical properties were identical with those of its natural counterpart.

Preparation of crystalline awobanin and flavocommelin. Awobanin was prepared from crystalline specimen of natural commelinin by treatment with 2% HCl (in the form of chloride) and purified in the usual way. The compound was finally obtained from a mixture of ethanol and acetone (1:1) containing 0.1% HCl in the form of slender, red needles. Crystallization was repeated twice. On paper chromatograms using various solvent systems, the crystalline material gave a single spot corresponding to that of the anthocyanin which had been liberated from natural commelinin by acid treatment. The

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^{**)} This term was first proposed by K. Hayashi in "The Chemistry of Flavonoid Compounds" (ed. by T. A. Geissman, Pergamon Press, 1962).

absorption maxima were at 279, 300 and 538 nm in 60% methanol containing 2% HCl. Isolation and crystallization of flavocommelin were performed according to the method described previously.⁴⁾

Reconstruction of commelinin from awobanin, flavocommelin and Mg acetate. The anhydro-base of awobanin, which was derived from its chloride as described before,⁶⁾ was mixed with an aqueous solution of flavocommelin and Mg acetate. The final solution contained the three components in the molar ratio of ca. 1:4:1. During the process of mixing, the color of the solution changed from purple to blue. The solution was evaporated to dryness *in vacuo*, and the residue obtained (deep blue in color) was dissolved in a small amount of water. Evaporation of the solution *in vacuo* and dissolution of the residue in water were repeated several times, and the final solution obtained was subjected to column chromatography using Sephadex LH-20 (1.5×20 cm). On gradual application of water as an eluant, a fraction of stable blue pigment rapidly moved downward, being clearly separated from other fractions of different colors. Such a phenomenon was never observed when the original mixture was devoid of magnesium and/or flavocommelin.

Crystallization and some properties of the blue pigment. The pigment in the blue fraction mentioned above was purified by repetition of Sephadex (LH-20) column chromatography (2.5×35 cm), using water as eluant. Further purification was made nearly in the same way as reported previously.²⁾ Finally, the blue pigment was separated from aqueous ethanol in the form of rectangular prisms or cubes (see Fig. 1a–b) with a copper-like reverberation. Crystallization was repeated twice.

Fig. 1. Crystals of commelinin (synthesized).

On paper chromatograms developed with neutral solvents, the pigment showed a single blue spot which agreed with that of natural commelinin. The R_f -values obtained with Tōyō No. 51 filter paper at room temperature were 0.66 with 60% EtOH and 0.46 with 60% iso-PrOH. When chromatographed with acidic solvents, *e.g.*, AcOH/

HCl/H₂O (3:1:8, v/v), the spots of awobanin and flavocommelin came out separately. The existence of Mg in the molecule was irrefutably confirmed by means of atomic absorption spectrophotometry. As shown in Figs. 2 and 3, the UV and IR absorption spectra showed the identity of the natural and synthetic pigments. On paper electrophoresis, both pigments were characterized by rapid migration of the spots toward the anode, namely +20 mm in 45 min. at room temperature under the following conditions: 0.8 mA/cm, 250 V, in 0.1 M acetate buffer (pH 4.80) on Tôyô No. 50 filter paper.

Fig. 2. Absorption spectra of natural and synthesized samples of commelinin (300 mg/l in 0.05 M acetate buffer of pH 4.80, path-length of 1 mm). ---- natural; — synthesized.

Fig. 3. Infrared absorption spectra of natural and synthesized samples of commelinin (KBr). ---- natural; — synthesized.

Molar ratio of the three components. Quantitative measurements of the three components were performed by the spectrophotometric method,²⁾ and the results are shown in Tables I and II. As may be seen from the tables, the molecular ratio of awobanin, flavocommelin

and Mg contained in the synthesized samples was practically equal to 2:2:1, a value which corresponds satisfactorily with that found for natural commelinin.

Table I. Quantities of awobanin, flavocommelin, and Mg in synthesized samples of commelinin

Samples used (mg)	Awobanin (chloride)	Flavocommelin	Mg
6.827	62.83 %	39.91 %	0.757 %
7.233	63.00	39.47	0.742
6.554	62.63	38.15	0.750
4.146	—	—	0.808
6.903	62.75	38.71	0.976
6.738	62.39	38.91	0.961
Mean	62.72	39.03	0.832

Table II. Amounts of the three components contained in synthesized and natural samples of commelinin

Components	Molecular or atomic weight	Number of molecules or atoms	Theoretical values	Experimental values (average) of commelinin	
				Natural	Synthesized
Awobanin (as chloride)	809.0	2	58.02 %	60.71 %*	62.72 %
Flavocommelin	608.5	2	43.64	39.83**	39.03
Mg	24.3	1	0.87	0.70**	0.83
K	39.1			0.86**	
Total (MW. 2788.5)			102.53	102.10	102.58

* Values obtained in the present study, using the calibration curve for awobanin chloride

** Values reported previously.²⁾

The results presented above indicate convincingly the identity of the synthesized and natural samples of the blue pigment, and provide conclusive evidence that Mg does exist in the molecule of commelinin. It may be added that our preliminary experiments have revealed that some other metals such as Zn, Cd, Mn, Co or Ni are also effective for the formation of the stable pigment complexes showing absorption spectra, whose maxima are slightly shifted from, but patterns are almost identical with that of natural commelinin. Experimental details of these experiments will be reported in the near future.

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