10. The Colouring Matter of *Shiso*.

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The pigment of *shiso* leaves (*Perilla Ocimoides* L. var. *Crispa* Benth)¹⁾ is extensively used for colouring *umeboshi* which is a kind of plum pickle favoured throughout Japan. The results of the chemical investigation of this colouring matter have been reported by Dr. Kaoru Kondo.²⁾ The powdered dry leaves were first treated with methyl alcoholic HCl, and the pigment, which was precipitated from the solution by addition of ether was dissolved in water, and precipitated again as a lead salt using lead acetate. The lead salt was decomposed by methyl alcoholic HCl, and from the resulting solution the colouring matter was reprecipitated by addition of ether, crystallized, and named perillanin. The compound was considered to be a derivative of delphinidin, in as much as the analytical results seemed to agree with a monoglucoside of delphinidin chloride, combined with protocathechuic acid as an ester, which could be separated from the glucoside by either heating with acid or treating with alkali. The acid was confirmed by its m.p. and the colour reaction.

The present authors have undertaken the study of the same pigment for the purpose of comparing it with other anthocyanins, which have been studied by them also. The investigation is reported here comprising some new results.

A dry powder, which was cautiously prepared from the leaves cultivated in Tokyo was soaked in cold petroleum ether to remove the yellow pigment. On separating off the yellow solution, it was then treated with methyl alcoholic HCl. From the resulting red solution, the chloride of the pigment was precipitated by addition of ether and then, from the aqueous solution of the chloride, the pigment was precipitated as its lead salt by lead acetate. From the lead salt, the colouring matter was isolated and purified. The colouring matter was treated with alkali in an atmosphere of hydrogen, acidified, and treated with ether, in which acid was dissolved leaving the pigment in the aqueous

1) Japanese special name "*Chirimen shiso*."

2) J. Pharm. Soc. Japan, 1931, 254.

red solution. From the etherial solution, P-coumaric acid was isolated, while protocatechuic acid which was noticed by Dr. Kondo, was not found present. The former was confirmed by its m.p. (208°) and its colour reaction with FeCl_3 (brown), other properties, and the analytical result, (Found C, 65.64; H, 5.06; Calc. C, 65.85; H, 4.88%). P-coumaric acid could be also obtained from the pigment treating it with acid. From the above red aqueous solution, the pigment was obtained in crystals with golden luster on purification, either in plates (Fig. 1), or needles (Fig. 2). The compound was found to be cyanin (cyanidin 3,* 5,* diglucoside¹⁾) chloride (Fig. 3); m.p. (d.p) of the crystals obtained, being 195° as the cyanin chloride from *dahlia* flowers (*Chishima*²⁾ and *Ubatama*). Moreover all the colour reactions toward Na_2CO_3 (blue), FeCl_3 aq. sol. (violet), solubilities (it was almost insoluble in cold water, or in dil.

aq. HCl, in these points it differs from Mecocyanin³⁾), and the analytical data were in agreement, (Found C, 46.82; H, 5.00;

Fig. 1. Cyanin chloride from *Shiso*.

Fig. 2. Cyanin chloride from *Shiso*.

1) R. Robinson and A. Todd: J. Chem. Soc. 1932, 2488.

2) Kôzô Hayashi: J. Bot. Japan 1933, 47, 396.

3) Mecocyanin chloride=3. Bioside of cyanidin chloride $\text{C}_{27}\text{H}_{31}\text{O}_{16}\text{Cl}_3\text{H}_2\text{O}$, Willstätter and Weil (Ann. 1916, 412, 231); A. Leon and R. Robinson (J. Chem. Soc. 1931, 2737).

$C_{27}H_{31}O_{16}Cl + 2\frac{1}{2}H_2O^{1)}$ requires C, 46.86 ; H, 5.21%).

Fig. 3.

Upon the decomposition by acid, from the above pure glucoside (m.p. 195°), one molecule of cyanidin and two molecules of glucose, which was confirmed by its osazone (m.p. 203°) were yielded quantitatively as anticipated. The estimation of the glucose was carried out by Pavy's method, in which the result agreed. Found 51.5% Calc. 52.0%. And the positions of the glucose are (3) and (5) as already indicated *.

The aglucone (brown crystal Fig. 4) was exactly proved to be cyanidin chloride ($C_{15}H_{11}O_6Cl + H_2O$), by its properties, analysis, (Found C, 52.84 ; H, 4.06 ; Calc. C, 52.86 ; H, 3.82%), and the following decomposition products. When fused with alkali, it gave phloroglucinol (m.p. 208° and all properties agreed); and protocatechuic acid (m.p. 197°, blue with $FeCl_3$). The protocatechuic acid was obtained, however in a better yield when the aglucone was oxidized with H_2O_2 in a sodium carbonate solution,—(Found C, 48.90 ; H, 4.55 ; $C_7H_6O_4 + H_2O$ Calc. C, 48.83 ; H, 4.65%).

Fig. 4. Aglucone of *Shiso* pigment (cyanidin chloride).

More confirmative colour reactions with Buffered solution²⁾ were observed. The results from the glucoside of *shiso* pigment and cyanin chloride (from *dahlia*) were quite identical, and agreed with the report on synthetic cyanin chloride,³⁾ and were distinctly distinguished from

1) Willstätter and Nolan (Ann. 1915, 408, 1); Willstätter and Mallison (Ann. 1915, 408, 147).

2) R. Robinson and A. Robertson: Biochem. J., 1929, 23, 35.

3) R. Robinson and A. R. Todd: J. Chem. Soc. 1932, 2496.

those of chrysanthemin chloride,^{1) 2)} obtained from natural pigments, and also from the report on cyanenin (synthetic; cyanidin 5 monoglucoside) chloride³⁾ and mecocyanin chloride⁴⁾ (natural).

As the results of estimation of distribution number, and of observation of absorption spectrum, the glucoside of *shiso* pigment was also proved to be quite identical with cyanin chloride.

In view of the above experimental results, the anthocyan of *shiso* is considered to be cyanin combined with P-coumaric acid.

From raw pigment, some quantity of crystalline cyanin free from P-coumaric acid could also be isolated, without treatment with acid or alkali.

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1) (Cyanidin, 3. monoglucoside); from reddish brown flower of *chrysanthemum indicum*.

2) Kuromamin; Kuroda, Wada: Proc. **9** (1933), 101, 517, and unpublished work.

3) A. Leon and R. Robinson: J. Chem. Soc. 1932, 2222.

4) S. Murakami, A. Robertson and R. Robinson: J. Chem. Soc. 1931, 2667.