*The leucoanthocyanine-reaction in the petals of white garden roses**

By Hitoshi YASUDA

Department of Biology, Faculty of Liberal Arts and Science Shinshu University.

(Received Nov. 30, 1960)

Bate-Smith[1] pointed out that leucoanthocyanines were widely distributed as leucocyanidin in the higher plants except *Rosaceae* and a few species of *Legminosae.* The anthocyanidin-type of leucoanthocyanine in some species of wild roses is left unknown, although the existence of leucoanthocyanine has been confirmed in them.[2]

The investigation reported here was performed in order to decide this type of the leucoanthocyanine in the petals of white roses, especially their garden varieties, as a fundamental study of the significance of its existence.

Generally, anthocyanidin-type of leucoanthocyanine is identified microanalitically by comparing the solution of crystal of anthocyanidin with the red solution obtained by boiling the organ or tissue of the plant with hydrochloric acid, regarding the R_f value by paperchromatography, the type of absorption curve and wave length of its peak. In the cases of white garden roses, however, the general procedure was found, in the experiment of the reporter, quite impossible to be applied to identify them. This seemed to be caused by some substances which were contained besides leucoanthocyanine and were also coloured on boiling the plant materials with hydrochloric acid.

The author reported in this paper on these disturbing substances, on the method for removing them and on the anthocyanidin-type of leucoanthocyanine in the petals of white garden roses decided by this method.

Materials and Experimental Methods

1) *Materials*

Nine varieties of white garden roses—*Caledonia, Virgo, Blanche Mallerin, White Swan, Misty Morn, Summer Snow, Elisabeth Faurax, Cl. Mrs. Hervert Stevens* and *Cl. White Gold*—were used in this experiment. These varieties had been cultivated on fertile fields for 2—5 years.

2) *The preparation of extract from petals.*

About 10g. of white petals were boiled with about 100 ml. of water for 10

* A part of this work was delivered at the 25th Annual Meeting of the Botanical Society of Japan, November, 1960.

minutes in Erlenmeyer flask. The content of the flask was cooled to room temperature, and then filtered. The filtrate was used through this experiment as extract of petals.

3) *Separation by ion exchange resin.*

The ion exchange resin used in this experiment was an OH-type of Amberlite IR-45 (weakly basic anion exchanger). 100ml. of the resin was stuffed in a glass tube which was 2 cm. in diameter and 35 cm. in length. The length of resin was 26cm.

The first elution mentioned in this paper means that 100 ml. of extract of petals is eluted and then the same volume of water is eluted through the resin; and the second elution means the next eluting procedure where N/10 sodium hydrooxide is used as eluant.

In both cases, the liquid dropping from the bottom of the resin was fractionated by automatic fraction collecter, each fraction being about 10 ml.

4) *The identification of flavone.*

The fractions, in which flavone might be contained, were collected and neutralized with acetic acid. The precipitation separated by adding 10% solution of basic lead acetate to the collected fraction was gathered up by centrifuge and suspended to 80% methylalcohol. Lead was removed by filtration after passing hydrogen sulphide through the suspension, and the filtrate was concentrated in vacuo. Flavone was crystalized when the liquid was stood for about 24 hours in a sulphuric acid desiccator.

The aglycone of flavone was obtained through hydrolysis of the flavone in about 20% sulphuric acid.

The melting points of the crystals obtained thus were measured.

The kind of carbohydrate in the residue of the hydrolysis was decided from R_f value of the paperchromatography recommended for the separation of carbohydrate by Partridge, using n-butanol 4, acetic acid 1, water 5 as eluting solvent[3]

5) *The identification of anthocyanidin.*

The fractions in which leucoanthocyanine might be contained were collected and boiled in the 15% hydrochloric acid. The anthocyanidin obtained in this way was extracted with small amount of iso-amylalcohol, and the absorption curve of red alcohol-layer was drawn by using Beckmann spectrophotometer. On the other hand, the paperchromatography was carried out in order to decide the R_f value of anthocyanidin by Bate-Smith's solvent: n-butanol 1, 2N hydrochloric acid 1, and by Hayashi's solvent[5]: acetic acid 5, 36% hydrochloric acid 1, water 5.

Results and discussion

1) *The colour-development of extracts in various concentrations of hydrochloric acid.*

Colours, that appeared when the extracts obtained from nine varieties of white garden roses were boiled for 20 minutes in various concentrations of hydrochloric acid, were entirely the same in all varieties, and the general pattern of colourings in various concentrations is shown in table 1.

Table 1.

The general pattern of the colourings of the extracts on being boiled in various concentrations of hydrochloric acid.

The concentrations of hydrochloric acid (%)	The colours
1.0	colourless
2.0	colourless
5.0	yellow
7.5	deep yellow
10.0	deep yellow
15.0	deep yellow, and then deep orange

This table indicates that the leucoanthocyanines in the petals of white garden roses are unable to be developed in hydrochloric acid of less than 10%, but 15% hydrochloric acid can develop. In other words, the existence of leucoanthocyanine in these materials is not appreciable by the reacting condition of Bate-Smith[1)(2)] who used 2 N hydrochloric acid.

2) *Inquiry by ion exchange resin.*

It was difficult to identify the anthocyanidin-type of leucoanthocyanine in white garden roses from the deep orange liquid shown in table 1, because the absorption curve of the liquid was like B′ in fig. 3.

To use the ion exchange resin, however, was effective in obtaining clear absorption curve of anthocyanidin as shown in B~J of fig. 3.

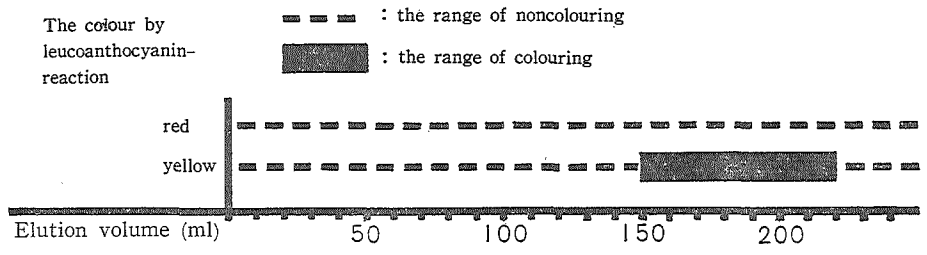

Fig. 1. The elution graph of *Caledonia* by the first elution.

(i) *The first elution.*

Each fraction of the first elution was boiled under 15% hydrochloric acid, and its colouring was observed. The elution graph shown in fig. 1 is the case of *Caledonia.*

As shown here, the red colour was not developed at all in any fraction of the first elution, while the deep yellow colour was developed strongly in the fraction between 150ml. and 220ml.

The faint yellow crystal obtained by the lead acetate method from this fraction was identified to be rutin from the following facts: that it was softened

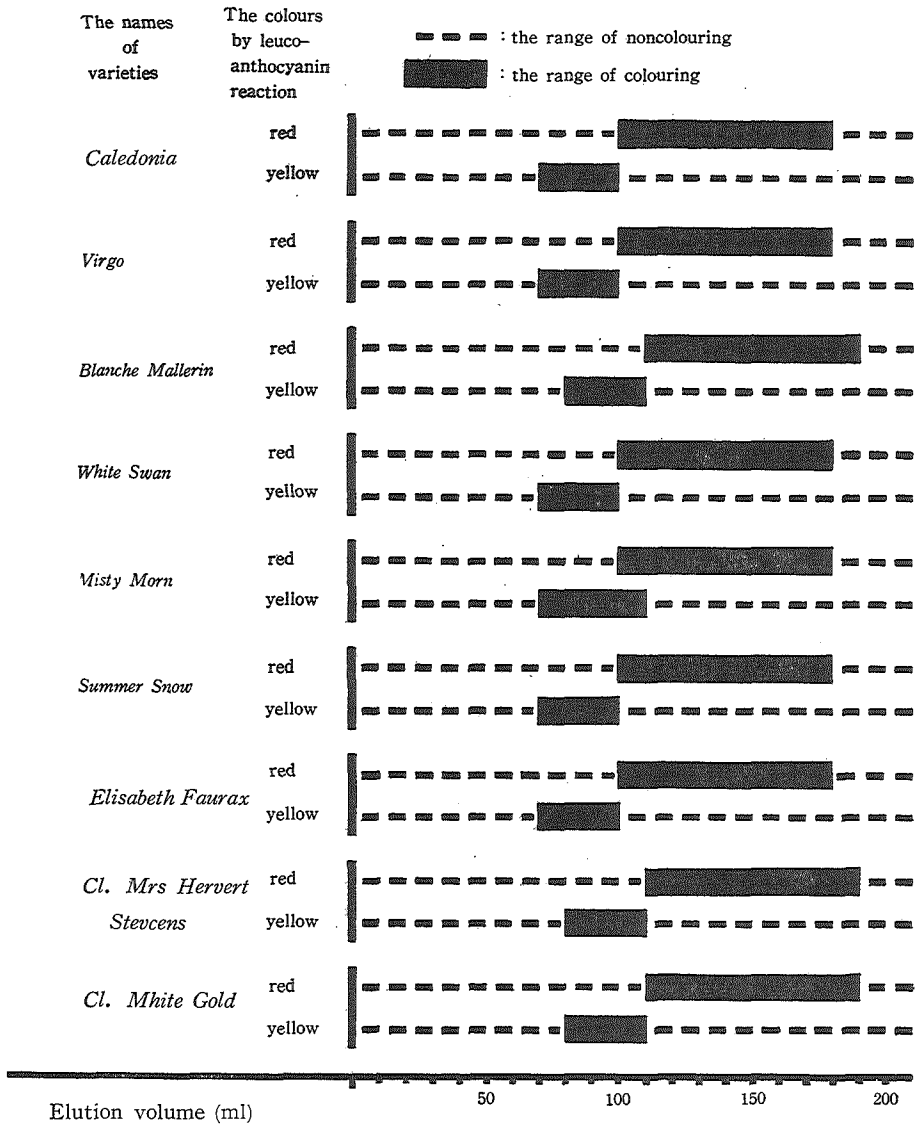

Fig. 2. The elution graph by the second elution.

at 183°C to 185°C and decomposed at 210°C to 218°C with bubbling, that the melting point of its aglycone was 305°C, and that the glucose and rhamnose were determined by paperchromatography from the residue of hydrolysis.

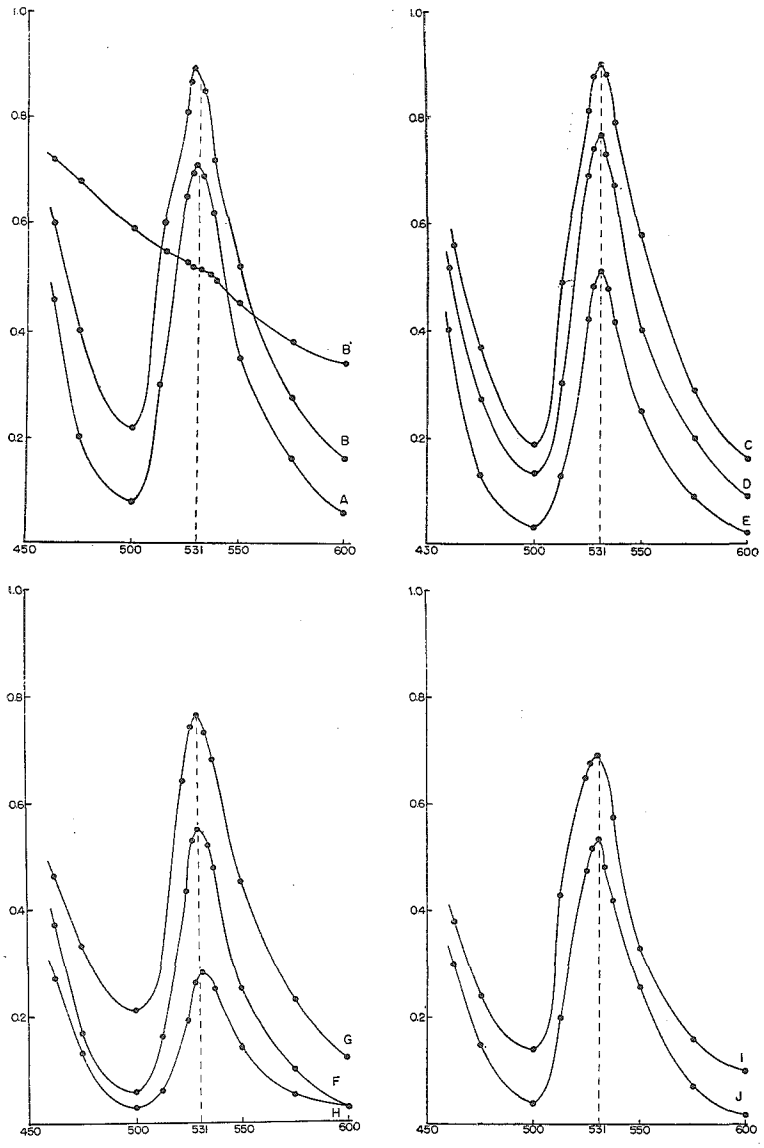

Fig. 3. The absorption curves of red liquids.

A: pelargonidin	B and B′: *Caledonia*
C: *Blanche Mallerin*	D: *Elisabeth Faurax*
E: *Summer Snow*	F: *Cl. Mrs. H. Stervens*
G: *Misty Morn*	H: *White Swan*
I: *Virgo*	J: *Cl. White Gold*

The vertical axis. : absorption degrees
The horizontal axis : wave length (mμ.)

From the other eight varieties, the same elution graphs as that from *Caledonia* were obtained, but the author in this paper omitted, for simplicity, the graphs of the other varieties.

(ii) *The second elution*

The elution graphs of the nine varieties obtained by the second elution were shown in fig. 2. It was generally observed in all cases that the yellow colouring slightly appeared within the range from 70 to 110ml. of the fraction volume, and the strong red colouring from 100–110ml. to 180–190ml.

Although the yellow substances were unable to be identified in all cases, because of their quantity too meager, it is probable that they were a sort of flavonoid, judging from the reaction to ferric chloride solution.

The absorption curves of red liquid in the nine varieties shown in fig. 3(B～J) were identical with that of pelargonidin itself (fig. 3A) in respect of the shape and the wave length of peak (531 mu): and also by the Bate-Smith's and Hayashi's paperchromatographies the R_f values obtained from the red liquids were approximate to that of pelargonidin itself. (table 2).

Table 2.

R_f values of pelargonidin and of the red liquids from varieties.

Materials	R_f values in	
	acetic acid 36% HCl water (5:1:5)	butanol-2 N HCl (1 : 1)
pelargonidin	0.55	0.78
Caledonia	0.53	0.80
Virgo	0.56	0.79
Blanche Mallerin	0.54	0.77
White Swan	0.54	0.78
Misty Morn	0.53	0.77
Summer Snow	0.55	0.79
Elisabeth Faurax	0.56	0.81
Cl. Mrs. Hervert Stevens	0.54	0.78
Cl. White Gold	0.55	0.79

From these results, it is clear that the red liquid obtained here is attributable to pelargonidin and that the leucoanthocyanine in the petals of white garden roses is pelargonidin-type.

Summary

The anthocyanidin-type of leucoanthocyanine in the petals of nine varieties of white garden roses—— *Caledonia, Virgo, Blanche Mallerin, White Swan, Misty Morn, Summer Snow, Elisabeth Faurax, Cl. Mrs. Hervert Stevens* and *Cl. White Gold*——was investigated.

The leucoanthocyanine was detected by boiling these petals with 15% hydrochloric acid, but the identification of anthocyanidin-type was disturbed by flavones such as rutin which coloured to deep yellow by boiling with hydrochloric acid.

The use of weakly basic anion exchanger, Amberrite IR-45, was effective in removing these disturbances and in obtaining the clear red solution which is suitable in identifying anthocyanidin by absorption curve and paper chromatography.

From the results of the leucoanthocyanine-reaction using this resin, the anthocyanidin-type of leucoanthocyanine in white garden roses is decided to be leuco-pelargonidin.

The writer wishes to thank Prof. B. Sakurai for his kind revision of the manuscript.

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