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# *Studies on the Insoluble States of Anthocyanin in Rose Petals, I. The Insoluble State of Anthocyanin and its Relationship to Petal Color, together with a new Instance of this Relationship.*

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**Abstract**

The upper epidermal cells of certain cultivars of black roses, *e.g.* Charles Mallerin, Josephine Bruce, Bonne Nuit and so on, occasionally hold in the cell vacuoles massive structures colored purple or purplish-red.

It was observed histochemically that these massive structures showed a color change from either purplish-red or from purple to red with dilute hydrochloric acid and to bluish purple with a weak solution of sodium hydroxide. From these results the massive structure found in the red rose petals can be regarded as the insoluble state of anthocyanin.

In the surface view of those petals having these massive structures in their upper epidermal cells, the numerous black spots, which are considered to be shadows of epidermal cells, are visible even in petals whose height/width ratio of the upper epidermal cells are within the range of the red petal ones. It can therefore be said that the insoluble state of anthocyanin present in the red rose petals may play an important role in the blackening effect on the petal color.

## Introduction

It is generally known that anthocyanins are present in solution in the cell sap. However, it is considered to be a rare occurrence when anthocyanin appears in insoluble states in plant cells or in tissues. According to BLANK's review[1], the insoluble states of anthocyanin can be classified by the following three types:

(A) a crystallized type in cell plasma (*e.g. Allium*) and in sap (*e.g.* juice of the blood orange).

(B) a stored type in the cell wall (*e.g. Sphagnum, Marchantia, Preissia* etc.).

(C) an "anthocyanophore"* type in the cell vacuole (*e.g. Erythrea, Fuchsia, Iris, Dianthus, Delphinium, Pulmonaria* and so on).

Very recently, the present author reported that the cell vacuole of the bluing petals of the red rose, in some cases, includes anthocyanin as a component of the blue spherule, the basis of which may principally consist of a tanninlike substance[3]. This example could be considered as another type of the insoluble state of anthocyanin.

As stated above, several observations have been made on the insoluble states of anthocyanins, but there has been relatively little information concerning their cytological and physiological investigations.

As a beginning step in detailed investigations on the insoluble state of anthocyanin, especially of the anthocyanophore type, the present paper is concerned with its significance relative to the petal color, thus providing a new example found in some petals of the so called "black rose".

## Materials and Methods

Plants used in the present observations were three cultivars of the black rose: Charles Mallerin, Josephine Bruce and Bonne Nuit. Their fresh petals were harvested from the flowers in the fully opened stage** and cut at 30 micra in thickness with a freezing microtome.

The histochemical treatment of the petal sections with acid or alkali was done in the following manner: The petal sections were first mounted on the slide in an aqueous medium and then a coverslip was placed on the mounted medium. After that either dilute hydrochloric acid or a weak solution of sodium hydroxide was drawn inward from one edge of the coverslip. The color change with either the acid or the alkali treatment in the massive structures was observed microscopically.

The reflectance measurements of the petals and the colorimetric calculations for the reflectance curves were made by the same procedure described earlier[5].

The surface views of the petals were photographed microscopically in the manner analogous to that described previously[6].

The anthocyanin content in the rose petals was estimated in the way reported earlier[7].

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* This term was first named by WEBER[2] for the pigment mass present in the cell vacuole of *Pulmonaria* corolla.

**See preceding paper[4] as to the explanation of the various opened stages in the rose flowers.

## Observations and Discussion

The upper epidermis of the rose petals used in the present observation, in some cases, shows an appearance such as indicated in the colored photos of Fig. 1, namely, some massive structures colored purplish-red or in a degree of purple, are found in the vacuoles of upper epidermal cells.

When the petal sections were treated with dilute hydrochloric acid, the purplish shade of the massive structure was reduced and the reddish tone was then heightened in reverse. During this treatment, the massive structures expanded rapidly over the cell vacuole. The treatment with alkali brought about the color change in the massive structures from purplish-red to bluish-purple. However, subsequent color changes were not observed because the massive structures disappeared rather quickly. From these changes in the color tone of the massive structure with the acid or alkali treatment, and also from past informations[3,9] indicating that such rose petals of the cultivars as used in the present investigation contain cyanin as the major anthocyanin, the involvement of anthocyanin in this massive structure is unquestionable. Hence, this massive structure can be considered as an example of the insoluble state of anthocyanin.

The insoluble state of anthocyanin illustrated in the present paper seems closely similar in shape to the anthocyanophore of *Erythrea* reported by LIPPMAA[8]. However, it remains uncertain whether the present example can be classified validly as the anthocyanophore type or not. The results of cytochemical tests on the basic substance of this massive structure together with observations on their development will be published elswhere.

The present author previously pointed out that the development of the blackish tone in red rose petals depends upon the shadows in upper epidermal cells[6]. According to this explanation, the shadows come from the characteristic structure of the petal surface, which is represented by the higher ratios of the height/width of the epidermal cells. Table 1 shows that two petal colors are distinguishable, one being red and the other black, even in the petals having similar pigment contents and having similar height/width ratios of epidermal cells, which are within the range of the ratios of red petals reported by the writer previously[6]. From this table it is clear that those petals with the insoluble state of anthocyanin in the upper epidermal cells exhibit the blackish tone. In this case, the height/width ratios of the massive structure are 2.0—3.0, being within the range of the ratios of the black petals.

Therefore, it is quite reasonable to consider that the insoluble state of anthocyanin, found in the plant materials used in the present observations gives a blackening effect to red petals. The surface view of a petal having the in-

Table 1 The effect of massive structure present in the upper epidermal cells of rose petals on the petal color.

| Petal color | Presence (+) or absence (−) of massive structure | Anthocyanin content ($\mu g/cm^2$) | Ratio of height/width | |
|---|---|---|---|---|
| | | | of upper epidermal cell | of massive structure |
| Black* | + | 170 | 1.40 | 2.0 |
| | + | 150 | 1.33 | 3.0 |
| Red** | − | 125 | 1.45 | —— |
| | − | 180 | 1.40 | —— |

I.S.C.C.-N.B.S. color names are
* very dusky purplish Red or very dusky Red Purple,
** dark purplish Red.

soluble state of anthocyanin fits the expectation posited by the consideration mentioned above; that is, the shadows of the epidermal cells (of the massive structures) evidently appear as numerous black spots (Fig. 2). This surface view is identical to that of the black petals of roses reported previously[6].

Up to the present, three processes for petal blackening in the red rose have been offered:

(A) an increase in the anthocyanin content[5,10],
(B) an accumulation of tannic substance[10],
(C) a characteristic structure of petal surface[9].

The instance reported in the present paper can be regarded as a special case of (C).

In some cases, the massive structure of rose petals may give a weak bluing effect to anthocyanin. Further study in this connection is in progress.

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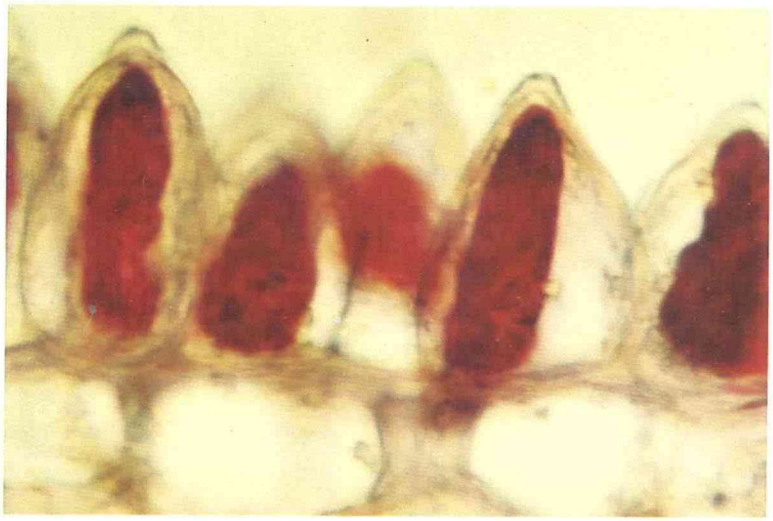

Fig. 1 Photomicrographs illustrating the insoluble state of anthocyanin present in the upper epidermal cells of rose petals.

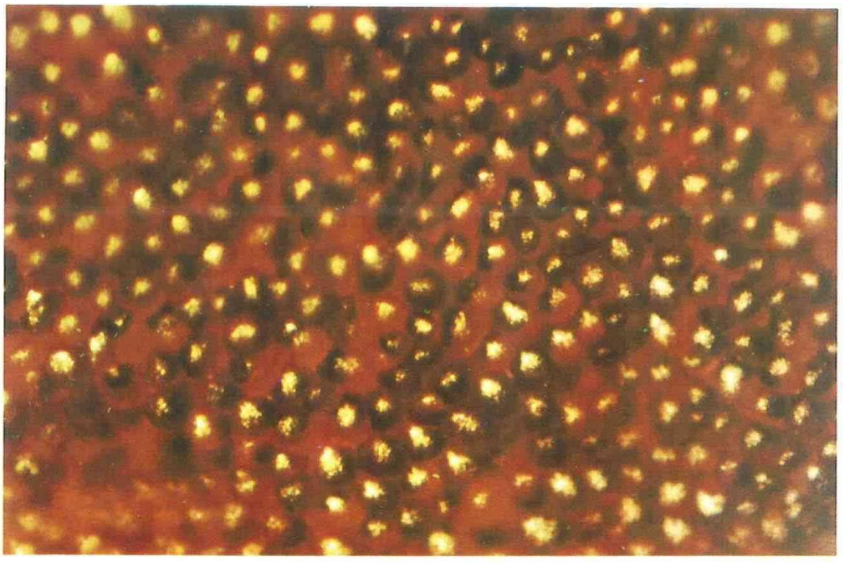

Fig. 2 Photomicrograph illustrating the surface view of red rose petals including the insoluble state of anthocyanin.