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On the Formation of Anthocyan in the Petaloid Calyx of the Red Japanese Hortense.

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With one Plate.

I. Introduction.

It is well known that the different shades of red and blue observed in vegetable organs are due to the presence of anthocyan[1] in different stages of its development, the red pigment found, for instance, in the skin of many ripe fruits (Prunus, Pirus, Vaccinium, Vitis, etc.), in some young shoots (Acer, Ilex, Cinnamomum, Asparagus, etc.), and in various flowers (Pæonia, Pharbitis, Phlox, Portulacca, Celosia, etc.) being anthocyan in some stage of development.

Very little is known, however, of the chemical nature of anthocyan and, more especially, of the mode of its formation; a fresh investigation in these directions seemed, therefore to be highly desirable. Among the few known reactions of anthocyan

1) Marquart's anthocyan, and Fremy's and Cloëz's cyanin are identical.

may be mentioned the change of its colour from blue to red by the action of acids, this being looked upon as one of its characteristic properties.

Of the physical properties of anthocyan it is known that the red variety shows an absorption band at F and another at the end of G, that the violet variety shows one at D, with a feeble shade toward the green side and another at the end of the spectrum, and that the blue variety shows a broad absorption band beginning soon after D and continuing to F.[1]

The object of the present work has been to study the formation of anthocyan in vegetable cells and to ascertain the essential conditions under which this pigment is developed. Red Japanese Hortense seemed to offer suitable material for this study, because of the slowness with which anthocyan passes through the different phases of its development in this plant, and also because of the long duration of its blossoming period.

The different phases of the development of anthocyan in the petaloid calyx of Hortense may be briefly sketched out as follows :—

Colourless protanthocyan.
|
Yellow anthocyan.
/ \
Reddish anthocyan. Bluish anthocyan.
| |
Deep red anthocyan. Deep blue anthocyan.
\ /
Violet anthocyan in crystals.

All these colours have been observed under the microscope. The young calyx appears at first slightly greenish to the naked

1) Husemann, Th.—Die Pflanzenstoffe. Bd I. p. 259. (After G. Kraus).

eye, but its microscopical structure shows no colour. This green pigment in the epidermal cell-sap is present in such a very small amount that it is hardly recognizeable in a thin section. I intend to call anthocyan in this primitive stage colourless protanthocyan; from this yellow anthocyan is next derived. The latter is a true colour-generator. Zopf[1] studied the developmental phases of anthocyan in Fumariaceæ and observed that a colourless chromogen barely makes its appearance, then frequently a yellow pigment in some species, and lastly red anthocyan. His colourless „Chromogen," Wigand's[2] colourless „Cyanogen" and my protanthocyan are probably; identical, since they all consist of a certain tannin compound. Moreover, according to Harvey[3] the development of colour in flowers probably begins with green, which is followed successively by yellow, white and red, and ends with violet or blue. This is almost the same order as that in the case of the formation of anthocyan in red Hortense. How protanthocyan changes into the variously coloured anthocyans will be more fully discussed later on, but it may here be mentioned that both internal and external influences are active in producing these changes.

Of the existence of the blue or violet crystals of anthocyan, which I have found in the petaloid-calyx of red Hortense in the latest stage, only very little has hitherto known. Probably the "blue star" in the epithelial cells of the Delphinium petals[4] and the violet crystals in the epidermal cells of the coffee-berry

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1) Zopf, W.—Ueber die Gerbstoff und Anthocyan-Behälter der Fumariaceen und einiger anderen Pflanzen. 1886.

2) Wigand.—Einige Sätze über die physiologischen. Bedeutung der Gerbstoffes und Pflanzen. Farbe. (Bot. Ztg. 1862, 122).

3) Harvey, E. W.—Observation on the colour of Flowers. 1899.

4) Cf. Weiss, A.—Anatomie der Pflanze. 1878, p. 130, and Strasburger, Ed.—Das Botanische Practicum 1897, p. 122.

may be allied pigments. Zimmermann[5] describes the "pigment-secretion" as a certain compound of anthocyan with some unknown tannin substance. According to chemical examinations, however, the violet crystals, which I have observed, proved to be nothing else than those of anthocyan.

In the following pages I shall describe the development of anthocyan in Japanese Hortense in four phases. In Kanazawa, the flowering period of this plant lasts about four months, beginning in June and ending in September. My observations were carried on during the years, 1900 and 1901.

II. Different Phases of the Development of the Petaloid Calyx.

THE FIRST PHASE.

Duration of observation: June 1—July 1. *External form.*—At the biginning of June most of the peripheral flower-buds swell and open gradually. (Fig. 1.) Now each young flower nutates upon a curved stalk and already carries four or five involved petaloid calyx-leaves. Among the latter, the one which is the lowest in position is the largest and entirely covers the other. At this stage, particularly in the largest calyx leaf, the predominance of hyponastic growth can be traced, but, later, epinastic growth becomes the more powerful. Soon afterward, each curved flower stalk tends to stand erect and the flower itself to open. Even in full bloom, each calyx-leaf, remains to the last phase without losing its spoon shape.

5) Zimmermann, A.—Die Botanische Mikrotechnik. 1892, p. 104.

When the flowers open the petaloid calyx appears not absolutely white but yellowish or slightly greenish, fading from the base towards the end. However, this colour is not due to the presence of chlorophyll, but to the slightly yellowish cell-sap. At this stage protanthocyan is already formed and distributed equally over the calyx-leaf by centrifugal propagation. (Fig. 2.) After undergoing some morphological and metabolical changes from external influences (especially sunlight) the calyx becomes glossy white and red anthocyan now begins to appear.

Microscopical Structure.—On cross section the upper and the lower epidermal cells show a similar flat form and hold from four to ten mesophyll (chlorophylless) cell-layers between them. The mesophyll layer consists of more or less irregular, loosely united cells, in intercellular space and contains crystals of calcium oxalate. Crystals of irregular shape are very abundant in the mesophyll and in the hypodermal parts. Some raphides occur in the middle part of mesophyll.

Looked at from above, the upper epidermal cells show less wavy outlines than the lower, and both are furnished with indistinct cuticular striations. The lower epidermal cells are provided with many stomata and deciduous unicellular hairs, which are covered with thick cuticular spots. The latter are entirely wanting on the upper side. In this phase each cell in the calyx-leaf contains besides protoplasma nothing but slightly yellow sap, and the whole tissue is very clear. At a later period this yellowish sap becomes much more conspicuous and is confined to both epidermal cells. (Fig. 11.). This is the stage at which protanthocyan passes into yellow anthocyan, the chemical reactions of which are given in the following table I.

Table I.

	Reagents.	Reactions of colourless or slightly yellowish anthocyan. (= protanthocyan).
Acids.	Hydrochloric acid.	No reaction (slightly red).
	Nitric acid.	"
	Sulphuric acid.	Yellow (all through).
	Acetic acid.	No reaction.
	Phosphoric acid.	"
	Salicylic acid.	"
	Tartaric acid.	"
Alkalis and Salts.	Ammonia.	Yellow.
	Caustic potash.	"
	Caustic soda.	"
	Lead acetate.	Yellow precipitate.
	Sodium carbonate.	Slightly yellow.
	Potassium bichromate.	Reddish brown precipitate (not all).
	Ferric chloride	Black (deep green) precipitate.
	Ferric chloride + potassium ferrocyanide.	Deep blue precipitate.
	Ferrous sulphate.	No remarkable reaction.
	Silver nitrate.	Brown precipitate.
	Millon's reagent.	Browinsh yellow.
	Iodine in potassium iodide.	No reaction.
	Quinine sulphate.	"
	Potassium cyanide.	Yellow.
	Flemming's solution.	No reaction.
	Chloral hydrate.	"
	× Naphtol + sulphuric Acid.	(Mesophyll violet).
	Alum.	Yellowish orange.
	Antipyrine.	No. reaction.
	Caffeine.	"

From these chemical reactions protanthocyan and yellow anthocyan may probably be considered as some allied compounds of tannin or modified phenol compounds, as Pfeffer[1] remarks. Indeed I have often detected a substance like protanthocyan in the epithelial cells of white flower petals of many other plants,

1) Pfeffer.—Pflanzen Physiologie. Bd. I. 1897, p. 496.

but this substance never develops later on into any coloured anthocyan. Now the question arises why in certain flowers protanthocyan precedes a coloured anthocyan while in some others it does not. The answer is to be sought, probably, in the hereditary disposition of each plant.

That the colourless protanthocyan becomes yellow by the action of alkalis, as stated in preceding table, is worth noticing, for it proves the presence of a tannin substance, as Wiesner[1] observed. The change of the colour of yellow anthocyan, when well developed, to a light red by the action of acids, is also of some interest. Moreover, the existence of intercellular air-spaces and the exceedingly abundant presence of the crystals of calcium oxalate at this stage is worth remarking.

The Second Phase.

Duration of observation: July 1—July 20. *External form.*—Each white calyx-leaf of the opened flowers now begins to colour, by the appearance of red spots from the distal end of its upper side. The colour proceeds along the margin centripetally over the surface (Fig. 3.4). In general, this propagation of colour occurs earlier on the larger calyx-leaf than on the smaller one, though at the end the whole upper surface becomes coloured, except the borders of the veins. For this development of colour it is necessary that the calyx should be exposed directly to the sunlight, the overlapped part of the upper side of a calyx-leaf and also the whole lower side being not at all tinged till the end of this phase.

1) Wiesner, J.—Einige Beobachtungen über Gerb- und Farbstoffe der Blumenblätter. (Bot. Zeitg, 1862, p. 389).

Microscopical structure.—It is noticeable that the epidermal cells of the upper side become much projected upwards, assuming the shape of a cap, whilst those of the lower side remain unchanged. The glossy-white appearance of the calyx-leaf which is observed at first is chiefly due to the abundant presence of air in the intercellular space and partly also to the reflection of light by the cap-shaped projection of the epidermal cells. Later, many epidermal cells containing red anthocyan, besides those containing the yellow variety, are found on the upper side, whilst on the lower side coloured anthocyan has not yet made its appearance. The microscopical study of the calyx-leaf of red Hortense certainly reminds one of that of the flower-petals of several other plants.

The cuticular radiating striations of the upper side become, finally, very distinct and the paralled striations of the lower side, commonest in epidermal cells of all plants, also become visible.

The air-spaces and the crystals of calcium oxalate show a tendency to gradually decrease and are mostly concentrated toward the hypodermal layers.

The chemical reactions of red anthocyan are shown in Table II.

Table II.

	Reagents.	Reactions of red anthocyan.
Acids.	Hydrochloric acid.	No reaction.
	Nitric acid.	Yellow.
	Sulphuric acid.	Yellowish brown.
	Acetic acid.	No reaction.
	Phosphoric acid.	"
	Salicylic acid.	"
	Tartaric acid.	"

Alkalis and Salts.	Ammonia.	Yellowish green.
	Caustic potash.	Green.
	Caustic soda.	"
	Lead acetate.	Green precipitate.
	Sodium carbonate.	Yellow (contracts into globular mass).
	Potassium carbonate.	Green (" " " ").
	Potassium bichromate.	Deep blue precipitate.
	Ferric chloride.	Black (deep blue) precipitate.
	Ferric chloride + potassium ferrocyanide.	Deep blue precipitate.
	Ferrous sulphate.	No reaction.
	Silver nitrate.	Brown precipitate.
	Millon's reagent.	Brownish yellow.
	Iodine in potassium iodide.	No reaction (contracts into a globular mass).
	Quinine sulphate.	No reaction.
	Potassium cyanide.	Violet yellow.
	Flemming's solution.	Violet, dark blue, brown.
	Chloral hydrate.	Dissolves out.
	α Naphtol + sulphuric acid.	Brown.
	Alum.	No reaction.
	Antipyrine.	"
	Caffeine.	"

Looking over the table it will be seen, in general, that acids do not produce any marked change in the colour of the red anthocyan but that alkalis change it into green. The presence of sugar in the mesophyll layer remains doubtful. Chlorophyll-grains have not yet appeared.

The Third Phase.

Duration of observation: July 20—August 1.

External form.—The majority of the flowers of red Hortense now tend to nutate around their stalks, though some of them are not yet wholly reddened on the upper side of the calyx. Each calyx-leaf becomes turned upside down. This process which is physiologically produced by the epinastic growth of the flower stalk may from a biological point of view be described as a phenomenon of "gamotropism."[1] Being exposed directly to the sunlight, the lower side of the calyx, now in an uppermost position, turns red beginning at the base and ending at the periphery in a way just opposite to that observed in the case of the upper side. The red colour on the lower side is not so bright as it is on the upper side, but is rather dark except at the veinal surface (Fig. 7.). Besides, the darkness of the red colour is increased with the concentration of the spot and with the appearance of green chlorophyllgrains. In the meantime, all parts of the calyx-leaf on both sides, hitherto whitish, become noticeably green (Fig. 7,8.).

Microscopical structure.—On the upper side the number of red coloured epidermal cells and the intensity of the red colour are both gradually increased. Some slightly blue cells are often met with in the hypodermal layer. On the other hand, the red cells on the lower side are mainly observable in its hypodermal layer consisting of idioblastic irregular cells, but partly also in its epidermal layer, the cells of which mostly remain colourless. Such colouration of the hypodermal cells may be characteristic of the calyx-leaf of Japanese Hortense, for it is not usually found in the flower-petals or petaloid calices of other plants, which contain anthocyan. The multipolar horns of the idioblastic cells of the entire mesophyll layer gradually become prominent and, at the same time, the layer appears more spongy, the intercellular spaces being more conspicuous. In the normally healthy state (i.e. when not diseased or injured) the red idioblasts are confined to the hypodermal layer and never go any deeper. This condition

1) Ludwig, Fr.—Lehrbuch der Biologie der Pflanzen. 1895, § 74.

differs greatly from that of the autumnal red colouration of leaves.

Crystals of calcium oxalate decrease still more than in the preceding phase. Some raphides are rarely found in the middle part of the mesophyll layer.

Chlorophyll-grains begin to appear, at first, in both hypodermal cells and later extend toward the middle of the mesophyll. The formation of starch, accompanying the chlorophyll-grains, is soon afterwards observed. That chlorophyll-grains appear later than red anthocyan has been noticed by former investigators[1] in many cases, and this fact is no doubt to be explained from a teleological point of view.

The chemical reactions of deep red and blue anthocyans are given in Table III.

Table III.

	Reagents.	Reactions of deep red anthocyan.	Reactions of blue anthocyan.
Acids.	Hydrochloric.	No reaction.	Red.
	Nitric.	"	"
	Sulphuric.	"	Red, brown, yellow.
	Acetic.	"	Red.
	Phosphoric.	" (contracts into a globular mass).	"
	Salicylic.	"	"
	Tartaric.	"	"
Alkalis, and Salts.	Ammonia.	Deep green (blackish).	Green.
	Caustic potash.	Green (then yellow).	Green (soon afterward yellow).

1) Pick, Bedeutung des rothen Farbstoffs bei Phanerogamen. (Bot. Cent. Blatt. Bd. 16. p. 281, 1883). Kerner, Pflanzen Leben. 2. Aufl. Bd. I. p. 470. Stahl, (Annales du Jardin Botanique de Buitenzorg. Vol. XIII. p. 137-216, 1896).

	Lead acetate.	Deep green precipitate.	Green (precipitate ?)
	Sodium carbonate.	Dark green (contracts into a globular mass).	Greenish yellow.
	Potassium carbonate.	Black (deep green) (contracts into a globular mass).	„
	Potassium bichromate.	No reaction.	Slightly red.
	Ferric chloride.	Black precipitate.	Momentarily red, then black.
	Ferric chloride + potassium ferrocyanide.	Deep blue precipitate.	Momentarily red, then black and blue precipitate.
	Ferrous sulphate.	No reaction.	No reaction.
	Silver nitrate.	„ (Violet, brown, dark).	„ (slightly brown).
	Millon's reagent.	Reddish brown precipitate, (later dark brown).	Momentarily red and then reddish brown.
	Iodine in potassium iodide.	No reaction (contracts into a globular mass).	No reaction (slightly black).
	Quinine sulphate.	Violet, green, yellow, (granules dissolve later).	Violet, green, yellow. (granules dissolve later).
	Potassium cyanide.	Green, yellow (contracts into a globular mass).	Slight green, yellow.
	Flemming's solution.	Violet, them black, brownish yellow.	Momentarily red, then slightly black, brownish yellow.
	Chloral hydrate.	No reaction.	No reaction.
	α Naphtol + sulphuric acid.	Dissolves.	Slightly red (dissolves).
	Alum.	No reaction (slightly violet).	No reaction.
	Antipyrine.	No reaction.	„
	Caffeine.	„	„

Here the presence of sugar mostly around the fibro-vascular bundle of the calyx-leaf can be demonstrated.

The Fourth or Last Phase.

Duration of observation: August 1—September 1.

External form.—Flowers standing erect can no longer be found. The calyx-leaves nutate and are soon reddened on the lower side. A very quick and remarkable development of red anthocyan on some injured parts of the calyx-leaf is particularly noticeable, and is most apparent on the upper greenish side. (Fig. 9.). This phenomenon is in entire agreement with the observations made by Linsbauer, Ludwig and Molisch. Now when the red colour of the lower side attains its maximum, it appears as a somewhat dark red, whilst on the veinal surface it is a rather distinctly crimson red. This dark red colouration is chiefly due to the mixing of the epidermal deep red, and the hypodermal blue, anthocyan. (Fig. 10.).

In the course of the process of the degeneration of the calyx-leaf the interveinal mesophyll parts become dark brownish, then gradually dry up with the decrease of the sap, and finally die out. After the removal of the destroyed mesophyll parts there still remains the brownish skeletal vein-net of the calyx-leaf. This dead calyx-net is not deciduous till the spring of the next year.

Microscopical structure.—Both red and blue anthocyans now become much more concentrated in all the cell-sap. Nevertheless, the colouring matter is still to be found in some cases in its various younger stages—colourless, yellowish and of a pale red. In this phase it is worth noticing that violet or bluish crystals and refractive globules appear in the outer layers of the calyx-leaf. (Fig. 12.).

Many of the violet crystals appear granular and measure about 5 μ on the average. Some of them have the form of a needle shaped prism, 7–9 μ long. They are found in the hypodermal or epidermal cells on both sides of the calyx-leaf. Their chemical reactions completely agree with those of the blue or violet cell-sap, and hence these crystals must be regarded as those of anthocyan. (Fig. 14). They dissolve in hydrochloric, acetic, and other acids to form a red solution. With sulphuric acid, however, no sooner are they dissolved, than they reappear as red brownish amorphous granules. Potash dissolves them into a pale green solution, whilst chloral hydrate dissolves them without any special change of colour. They are, no doubt, identical with Zimmermann's "pigment secretion" and Krœmer's[1] "violet chromatophore," which are generally considered as some tannin compounds of anthocyan. Hitherto the crystal form of anthocyan in a living cell has been but little known. Besides the two writers above named, Husemann[2] had enumerated the plants in which "pigment corpuscles" (Farbkörper) occur. Strasburger's "blue star," consisting of short needles of crystallized anthocyan, is found in many cells of the blue coloured calyx-leaf of the Delphinium-flower. It is identical with Zimmermann's "pigment secretion."

A peculiar body, the so-called refractive globule, is found constantly in this last phase within each epidermal cell of the calyx-leaf of the Japanese Hortense. (Fig. 13.). It appears, at first, as numerous fine drops around a nucleus, which gradually unite into one large refractive globule, measuring 4–5 μ. It is stained by alkannin, coloured a light brown by sulphuric acid, and is insoluble in alcohol, hydrochloric and acetic acids. This body closely resembles Krœmer's "strong refractive globules" found in the coffee-berry, and only differs from it in being almost insoluble in chloral hydrate and alcohol (5: 2). Aqueous ammonia produces no reaction with it. Probably it may be a certain

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1) Krœmer, K.—Ueber das angebliche Vorkommen von violetten Chromatophoren. (Bot. Cent. Blatt. Nr. 41. Bd LXXXIV. 1900).

2) Husemann, Th.—Die Pflanzenstoffe. 1882, Bd I, p. 259.

protein substance combined with some fatty bodies. It is different from oil drops.[1]

Chlorophyll-grains increase much in the mesophyll and, later, starch-grains of large size appear in abundance; soon afterward the chlorophyll-grains begin to degenerate.

Crystals of calcium oxalate decrease remarkably in amount and tend to disappear. Raphides, though very few, remain unchanged.

III. Summary.

1). When anthocyan has completed its development, chlorophyll-grains begin to appear in the mesophyll layer of the calyx and, at a later period, deposit a large amount of starch-grains.

2). After the appearance of the red colouration on the upper side, the flower-stalk nutates downward to expose its lower side directly to the sunlight. In this position, the lower side also assumes a reddish tinge.

3). The flower remains open for about four months in summer, and the calyx-leaf is persistent throughout this period.

4). Protanthocyan from which yellow, red, deep red, or bluish anthocyan and also bluish violet crystals are derived, consists of a certain colourless tannin compound.

5). Protanthocyan becomes yellow or green by alkalis.

6). In the red Hortense, the blue colour appears later than the red, and bluish crystals are often met with within reddish or purple cell-sap. Such variations as is well known depend on the different degrees of acidity.

7). The essential factors which bring about the change of

1) Rywosch, S.—Einiges über ein in den grünen Zellen vorkommendes Oel und seine Beziehung zur Herbstfärbung des Laubes. (Ber. Deutsch. Bot. Ges. 1897. p. 195).

protanthocyan into a coloured anthocyan in the red Hortense seem to be:—

a). Sunlight.—Those calyx-leaves which are situated in the shade or are overlapped by others never become red. An exceptional case of rare occurrence in which the formation of anthocyan is independent of sunlight was observed by Zopf in the root of Parnassia palustris. Also the red pigment which occurs in such an underground organ as the sweet-potato or the red raddish seems to have little need of sunlight for its formation.

b). Acidity.—It has been generally ascertained by previous observers that the red pigment can not exist without an acid cell-sap in the living state.

The crystals of calcium oxalate with the aid of intercellular air-spaces may serve as a protecting medium to the delicate young calyx-leaf against strong sunlight, as they reflect the light and hinder a too speedy transpiration. Schimper[1] states that leaves which have grown in the full sunshine are richer in crystals of calcium oxalate than those grown in the shade. But the calyx-leaf differs from ordinary green leaves in this respect, since these crystals are formed before chlorophyll granules appear.

c). Tannin and sugar.—Many observations render it probable that the mother substance of anthocyan is a peculiar tannin matter. The calyx-leaf of the red Hortense, however, never produces red anthocyan in the absence of sunlight, though it contains tannin. The root of Parietaria diffusa produces red anthocyan only in its tannin idioblasts. Hence some plants appear to require light more than tannin, and others tannin more than light, for the development of anthocyan.

1) Schimper, A. F. W.—Ueber Kalkoxalatbildung in dem Laubblätter., (Bot. Zeitg. 1888, p. 83).

The presence of sugar in the calyx-leaf of Hortense is observed only in the earlier phases of its development. Sugar may have no close connection with the formation of anthocyan.

d). Mechanical injury.—Mechanical stimuli hasten the formation of red anthocyan from protanthocyan. When the calyx surface is scratched with a nail, the injured part in a few days becomes much more intensely red than the healthy part. (Fig. 9.). This test is effective only in the later stages of the growth of the calyx-leaf. It is already known that any diseased or injured part of leaves or fruit may easily develope red anthocyan, and Molisch[1] has recently observed a sudden development of a carmin red pigment on making a nail-scratch on the surface of a leaf of Schenckia blumenaviana. Ludwig and Linsbauer[2] also noticed the formation of coloured anthocyan under the influence of mechanical injury.

e). Soil.—The formation of anthocyan in the calyx of Hortense is probably more or less influenced by the nature of the soil. Schübler,[3] Darwin,[4] Molish,[5] Miyoshi[6] and others all ascertained that the colours of the anthocyan of Hydrangea-flowers are much influenced by the chemical nature of the soil. DARWIN observed that alum directly influences the colour of Hydrangea-

1) Molisch, H.—Ueber ein neues, einen carminrothen Farbstoff erzeugendes Chromogen bei Schenckia blumenaviana, K. Sch. (Ber. Deutsch. Bot. Ges. Bd. XIX. Heft 3).

2) Linsbauer, Ludwig.—Einige Bemerkungen über Anthocyanbildung. (Oesterreichische Bot. Zeitg. Jahrg. LI. 1901).

3) Schübler.—Untersuchung einer Erde welche die Eigenschaft hatte, die gewöhnlich rothblühende Hortensia speciosa blau zu färben. (Schweigger's und Meineke's Jahrb. d. Chem. u. Physik, 1821).

4) Darwin, C.—The variation of animals and plants under domestication. Vol. II. p. 267. (from the Journal of Hort. Soc., Vol. i, p. 160).

5) Molisch H.—Der Einfluss des Bodens auf die Blüthenfarbe der Hortensien. (Bot. Zeitg. 1897. p. 58).

6) Miyoshi, M.—Ueber künstliche Aenderung der Blüthenfarben. (Bot. Cent. Blatt. Bd. LXXXIII. No. 11. p. 345).

flowers. The chemical process that occurs in the conversion of protanthocyan into coloured anthocyan is, however, not yet cleared up.

In conclusion, I wish to express my obligations to Prof. Miyoshi for his helpful advice during the course of my investigation.

March, 1902, Fourth High School, Kanazawa, Japan.

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ON THE FORMATION OF ANTHOCYAN IN THE PETALOID CALYX OF THE RED JAPANESE HORTENSE.

PLATE.

Explanation of Figures.

1–10. Different stages of the formation of anthocyan in the petaloid calyx of the red Japanese Hortense, approximately in natural size.

1. Young state of the flower.

2. A little later stage of the flower.

3, 4, 5. Progressive appearance of anthocyan from the peripheral end of the calyx in a centripetal way.

6. Red colouration of the upper side of the calyx, with the exception of some overlapped parts and immediate vicinity of the veins. Most of the flowers begin to nutate at stages of figs. 5–6.

7. The lower side of the calyx of a somewhat dull red colour.

8. The same (only one leaf), much more advanced.

9. The upper side of the calyx, on which red anthocyan has only slightly developed but which has been remarkably reddened on the mechanically injured parts.

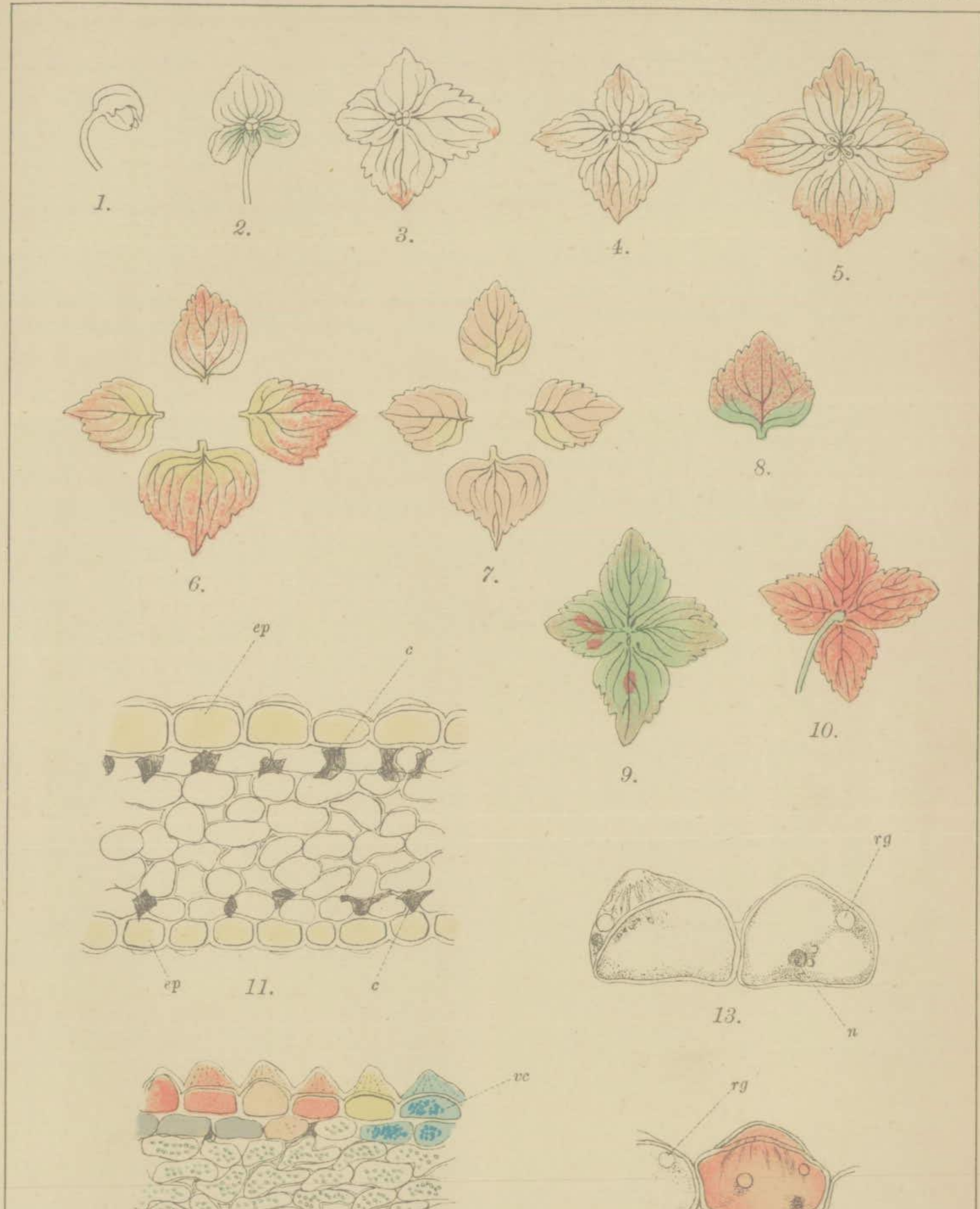
10. Maximum degree of anthocyan development on the lower side.

11 Cross-section of the calyx-leaf at a little later stage than that in fig. 2. × 420.
- ep. Epidermal cells enclosing protanthocyan.
- c. Crystals of calcium oxalate found in intercellular air-spaces of hypodermal parts.

12. Ditto, in the same stage as that in fig. 8, showing the fully developed state of anthocyan on the upper and the lower side. × 325.
- ch. Well formed chlorophyll-grains.
- v. c. Bluish violet crystals of anthocyan.

13. Epidermal cells of the upper side of the same, strongly magnified, showing their minute contents. × 925.
- n. Nucleus.
- rg. Refractive globules.

14. Epidermal and hypodermal cells of the upper side of the same. × 925.
- v. c. Violet crystals.
- ch. Chlorophyll-grains.



Ichimura ad Nat. del.