

## XL.—*The Colouring Matter of the Scarlet Pelargonium.*

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UP to the time when the present work was commenced, our knowledge of the nature of the colouring matter of the scarlet pelargonium (*Pelargonium zonale*) was confined to the variety known as "Scarlet Meteor" (grown in Europe), from the petals of which it was isolated by Willstätter and Bolton (*Annalen*, 1915, **408**, 42). It was shown to occur in the petals, to the extent of 6·6—7·1 per cent. of the dry weight, in the form of an oxonium salt of the diglucoside of pelargonidin, namely, pelargonin (probably pelargonin tartrate). It is also of interest to note that this glucoside was the first of the anthocyanin pigments to be obtained in a crystalline form (Griffiths, *Chem. News*, 1903, **89**, 249). Willstätter and Bolton have also shown (*Annalen*, 1915, **408**, 149) that pelargonin is the colouring matter of the cactus dahlia and the pink cornflower (blue cornflowers contain cyanin in the form of its potassium salt; Willstätter and Everest, *Annalen*, 1913, **401**, 189).

When boiled for a few minutes with 20 per cent. hydrochloric acid, pelargonin yields dextrose and the true, sugar-free pigment, pelargonidin.

Pelargonidin has been obtained synthetically by Willstätter and Zechmeister by the action of trimethoxycoumarin on magnesium anisyl bromide; on treating the product with hydrochloric acid, a tetramethyl ether was obtained, which, on conversion to the free phenolic compound by demethylation, yielded an oxonium salt that proved to be identical with pelargonidin chloride (*Sitzungsber. Preuss. Akad. Wiss. Berlin*, 1914, 886).

In the present research, it is shown that another variety of *Pelargonium zonale*, namely, "James Kelway" (grown in Australia), contains this same glucoside, *pelargonin*; it occurs in the petals as an oxonium salt of pelargonin to the extent of about 6 per cent. of the dry weight of the petals. The plants from which the flowers were gathered were purchased from Messrs. Shepherd and Sons, of Sydney.

### EXPERIMENTAL.

#### *Water Content of the Fresh Petals.*

In order to obtain some idea of the water content of the petals, a sample was dried to constant weight at 100°; the loss in weight was found to be 88·27 per cent.

*Isolation of the Anthocyanin Pigment.*

For the isolation of the pigment, the two methods employed by Willstätter and Bolton were tried, namely, extraction with glacial acetic acid and extraction with 96 per cent. alcohol.

The extraction with glacial acetic acid was carried out by keeping about 200 grams of the fresh petals in contact with this solvent in a closed bottle for a month, with occasional agitation; after filtration, the residue was further extracted with fresh acid for several days and again filtered. To the united filtrates, which had a dark red colour and faint green fluorescence, were added one-tenth of the volume of alcoholic hydrogen chloride (containing 15 per cent. of hydrogen chloride), and then 2 volumes of ether, whereby most of the anthocyanin pigment was precipitated as a reddish-brown mass; after being kept for some hours, the precipitate of crude anthocyanin chloride was collected, washed with ether, and dried in the air.

In the second method, 150 grams of fresh petals were extracted with 96 per cent. alcohol in a similar manner to that described above. As observed by Willstätter and Bolton, the extraction was much more complete than with acetic acid; these authors also state that the alcoholic extract, and the petals lying therein, become decolorised after having remained for some days. In the present instance, beyond a slight decrease in the intensity of the red colour of the extract, no such decoloration was observed. It is possible that the acidity of the cell-sap is greater in the petals of this variety of *Pelargonium* than in those of the variety used by Willstätter and Bolton, and consequently pseudo-base formation is retarded; this point was, however, not examined.

To the filtrate from the extracted petals 2 per cent. of alcoholic hydrogen chloride (containing 20 per cent. of hydrogen chloride) was added, followed by 2 volumes of ether, which caused the precipitation of the colouring matter, as chloride, in the form of a red, flocculent precipitate; this was collected, washed with ether, and dried in the air.

The precipitates obtained by both these methods were united, dissolved by boiling for a short time with methyl alcohol (containing 2 per cent. of hydrogen chloride), filtered hot to remove insoluble impurities, and the filtrate was mixed with one-fifth of its volume of 10 per cent. hydrochloric acid; on cooling, the anthocyanin chloride separated in bulky masses of slender needles, which were recrystallised in the same manner, washed with a small quantity of cold methyl alcohol, and dried in the air. The bronze-coloured product was at first taken to be callistephin, a monoglucoside of

pelargonidin, which Willstätter and Burdick obtained from the petals of the purplish-red aster, by reason of the description of its crystalline form given by these authors (*Annalen*, 1916, **412**, 149), but on further examination it was readily seen that this could not be the case.

#### *Identification of the Anthocyanin Chloride.*

*Crystalline Form.*—Hair-fine, red needles, which in bulk form a bronze-coloured mass when dry (Willstätter and Bolton describe their pigment as crystallising in long, thin, red needles) (Found : in air-dried material, loss in a vacuum desiccator at 40 mm. pressure = 8.39 per cent.). Willstätter and Bolton found that pelargonin chloride, when dried in a vacuum over sulphuric acid, lost 10.25 per cent. (=  $4\text{H}_2\text{O}$ ).

*Behaviour with Amyl Alcohol.*—In respect to its distribution between aqueous acid and amyl alcohol, the pigment behaves as a normal diglucoside; the distribution number is between 1 and 2 per cent. (the monoglucoside, callistephin chloride, has distribution number 10, and the diglucoside, salvinian chloride, distribution number 50).

*Behaviour on Heating.*—When heated in a melting-point tube, the anhydrous pigment softens at about  $173^\circ$  and decomposes above  $180^\circ$  (anhydrous pelargonin chloride softens at  $175^\circ$  and melts and decomposes at  $180^\circ$ ).

*Solubility.*—The salt is sparingly soluble in 1—2 per cent. hydrochloric acid in the cold, but dissolves readily on warming, forming an orange-red solution.

The salt is very sparingly soluble in 5 per cent. hydrochloric acid in the cold, but readily in the hot, forming an orange-red solution. It is slightly soluble in water, forming an orange solution which quickly becomes violet (hydrolytic dissociation) and eventually colourless, due to the formation of the pseudo-base. In methyl alcohol it is slightly soluble in the cold and easily on warming, forming a red solution with a green fluorescence (difference from callistephin chloride); on cooling, the chloride crystallises in slender needles. The salt is even less soluble in ethyl alcohol. When a small quantity of the pigment is treated with ethyl alcohol, containing a little hydrochloric acid, the filtrate yields crystals which, under the microscope, appear as stellate clusters of thin, red needles.

When the anthocyanin chloride is boiled with a small quantity of 96 per cent. ethyl alcohol and filtered, the filtrate, on cooling, deposits minute crystals of the basic chloride, which, under the

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microscope, appear as small, violet needles displaying a yellow, metallic lustre.

*Behaviour with Reagents.*—The salt does not develop a coloration with ferric chloride (difference from cyanin chloride), yields a violet precipitate with lead acetate, sodium acetate, or calcium carbonate, develops a violet coloration, changing to greenish-red and finally yellow, with sodium carbonate, sodium hydroxide, or aqueous ammonia, decolorises a solution of sodium hydrogen sulphite, the colour being restored by acids, and very slightly reduces warm Fehling's solution.

With the exception of the crystalline form, these properties are identical with those of Willstätter and Bolton's pelargonin chloride; the anthocyan pigment contained in the petals of the scarlet pelargonium "James Kelway" is, therefore, the diglucoside pelargonin (pelargonidin [1 mol.] + dextrose [2 mol.]).

#### *Hydrolysis of Pelargonin Chloride.*

It is stated by Willstätter and Bolton (*Annalen*, 1916, **412**, 133) that if the hydrolysis of pelargonin be carried out under certain prescribed conditions, namely, by careful partial hydrolysis with cold concentrated hydrochloric acid, an intermediate monoglucoside, *pelargonenin*, will be produced. In the present investigation, no attempt was made to obtain this product, as the quantity of pelargonin chloride available was too small. Complete hydrolysis was therefore effected by boiling the glucosidic pigment with 20 per cent. hydrochloric acid for a short time; on cooling, the sugar-free pigment separated in reddish-brown leaflets, which were washed with cold 20 per cent. hydrochloric acid and dried in the air.

#### *Identification of the Anthocyanidin Chloride.*

*Crystalline Form.*—The chloride separates from dilute hydrochloric acid, on cooling, in red tablets.

*Solubility.*—It is sparingly soluble in cold hydrochloric acid, but dissolves readily when warmed, forming an orange-red or red solution, according to the concentration.

The salt is easily soluble in methyl or ethyl alcohol, forming red solutions with a violet tinge; these solutions are not precipitated by water (difference from cyanidin chloride).

On being shaken with amyl alcohol, an aqueous acid solution of the chloride gives up all the pigment to the alcoholic layer. If the red solution thus formed is shaken with an aqueous solution of an alkali acetate, it will become violet, whilst if shaken with aqueous

sodium carbonate solution, the colour will change to blue and pass completely into the aqueous layer.

The salt dissolves in water without separation of violet flocks (difference from cyanidin chloride), forming a red solution; this is decolorised on warming, the colour being restored by the addition of an acid.

*Behaviour with Amyl Alcohol.*—The distribution number with respect to amyl alcohol is normal for a non-glucosidic anthocyanin (namely, 100).

*Behaviour with Reagents.*—The salt does not develop a coloration with ferric chloride (difference from cyanidin chloride), gives a blue precipitate with lead acetate in alcoholic solution, acquires a blue colour with sodium carbonate, and effects the reduction of Fehling's solution to a larger extent than does the glucosidic pigment.

These properties are identical with those of Willstätter and Bolton's pelargonidin chloride.

On account of the small quantity of material available when this research was undertaken, no attempt was made to isolate the yellow sap pigments which may be present in the petals of this variety of pelargonium; this will be made the subject of a later paper.

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