A NOTE ON THE ANTHOCYANIN PIGMENTS OF THE PRIMROSE *P. ACAULIS*

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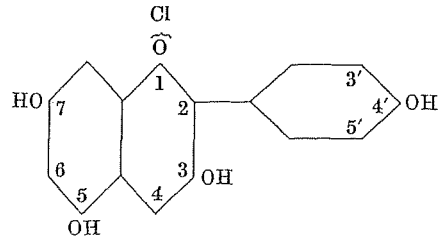
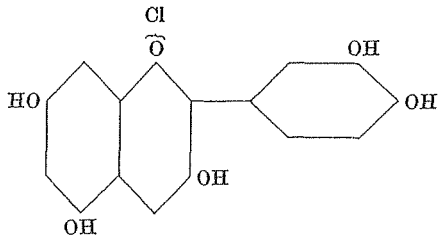
At the suggestion of Mr Buxton, who has kindly supplied the necessary samples of dried flowers, a brief preliminary investigation has been made into the chemical nature of the flower pigments of the primroses whose genetical relationships he has lately been studying (1931).

Although, owing to the small amount of material available, it has not been possible to make a complete analysis of these pigments, qualitative tests on the semi-pure acid extracts have given definite evidence that at least one of the causes of variation in flower colour is due to a difference in the constitution of the anthocyanin pigment present[1].

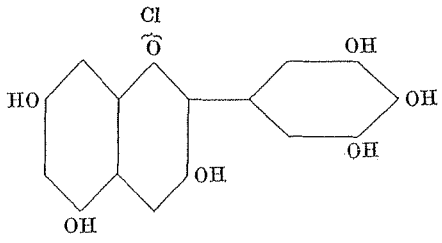
[1] The anthocyanin pigments, which have been isolated as hydrochlorides, fall into the following three groups:



Pelargonidin chloride (aglucone)
Pelargonin group (glucosidal in flowers)



Cyanidin chloride (aglucone)
Cyanin group (glucosidal)



Delphinidin chloride (aglucone)
Delphinin group (glucosidal)

The pigment of the carmine and claret flowers was found to be a very pure form of the monoglucoside primulin, while the blue flowers contain a diglucosidal pigment whose identification has not yet been completed. Two types of magenta flowers, containing ivory and yellow flavone respectively, have also been examined. In each case primulin was found to be the chief pigment present, but the acid extracts differed slightly both in colour and reactions from those of the carmine flowers. The full chemical explanation of this difference has yet to be found.

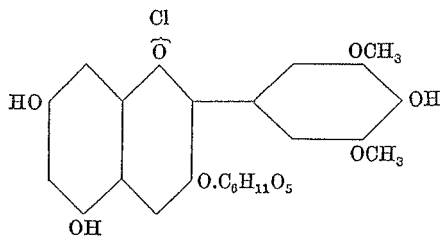
In each case the pigments were extracted from the dried and ground flower petals with a minimum quantity of 1·0 per cent. aqueous hydrochloric acid, and shaken repeatedly with a large excess of ethyl acetate to remove as much flavone or other impurity as possible.

The carmine, dark carmine and claret flowers, which contain no flavone, all yielded very pure extracts giving reactions resembling, in every respect, those of primulin chloride the 3-monoglucoside of the dimethyl delphinidin, malvidin, which has already been isolated from the flowers of *Primula polyanthus* (1930).

Owing to the large amount of flavone present in the blue flowers, it was not found possible, while working on such a small scale, to purify the pigment sufficiently to give a colour reaction with sodium carbonate other than a clear emerald green. However, such qualitative tests as were possible showed quite definitely that the principal anthocyanin in the blue flowers was not identical with that of the carmine ones. In the first place, both before and after purification, a distinct difference in the colour of their acid extracts was apparent, those from the blue flowers being magenta, while the carmine ones gave a cherry red. Secondly, the distribution number between amyl alcohol and 0·5 per cent. aqueous hydrochloric acid was relatively high in the monoglucosidal

The hydroxyl groups act as auxochromes, the colour tending from red to blue with their increase. Variation in the colour of the natural glucosidal pigments is also influenced by the methylation of one or more of the hydroxyl groups at 3′, 5′ and 7, and by the nature and position of attachment (3 and/or 5) of the glucosidal residue.

Thus primulin chloride:



or methylated delphinin in which the OH auxochromes at 3′ and 5′ are blocked and put out of action. The monoglucose —O—$C_6H_{11}O_5$ tends to reddening and the diglucose —O—$(C_6H_{11}O_5)_2$ to blueing.

carmine pigment, while the diglucosidal nature of the anthocyanin of the blue flowers was demonstrated by very slight solubility in the alcoholic layer. Thirdly, on addition of a saturated solution of picric acid to the carmine extract, abundant scarlet needle crystals, identical with those of primulin picrate, separated out, but no such insoluble picrate could be obtained from extracts of the blue flowers. Fourthly, the colour reaction of the latter with sodium acetate was violet blue, while both the carmine pigment and pure primulin chloride give a red violet.

It is therefore evident that the pigment of the blue primrose differs from that of the carmine flowers, and that it is a diglucosidal anthocyanin. The colour of the acid solutions points to the aglucone being either a methylated delphinidin, or, possibly, that pigment itself. It cannot be the pigment malvin, since, according to Willstätter and Mieg (1914), malvin picrate is comparatively insoluble in picric acid solution. Until the pigment can be isolated on a larger scale and in a purer condition, it will not be known whether the blue and carmine flower pigments differ in their aglucone as well as in their glucosidal residue.

The nature of the chemical difference between the pigments of the carmine and magenta flowers is still uncertain. The presence of primulin in the magenta types was ascertained by means of the distribution number and by the precipitation of a crystalline picrate identical with that of primulin, but it is evident that something, such as a second pigment, is also present, which modifies the colour not only in the flowers themselves but also in their semi-purified acid solution, the colour of which lies midway between those of the carmine and of the blue flower extracts. The possibility that a variation in pH is responsible for the difference in colour is ruled out by the fact that this difference can still be distinguished even in strongly acid solutions.

A more extensive investigation, isolation and identification of all these pigments is to be undertaken next spring, when enough material should be available to throw further light upon the factors influencing the colour of these pigments in the living plant. The presence of flavones in the blue and magenta flower extracts, even after considerable purification, rules out, for the time being, the use of such useful identification tests as colour reactions with sodium carbonate, ferric chloride and with a range of buffers.

The genetical significance underlying these chemical investigations is not altogether clear at this preliminary stage. The presence of the factor **S** appears to favour the production of the redder monoglucoside

primulin in place of the diglucosidal anthocyanin present in the blue flowers. The modification of the colour from carmine to magenta is probably represented by a second blueing factor, whose chemical nature is still undetermined. According to Mr Buxton's breeding experiments, one would expect this second factor to be present in the blue flowers, **BWs**, from which the factor **S** is absent, in which case the blue colour might be accounted for by this combined effect. At present no flowers of the constitution **Bws** are available for examination. It is probable that these will prove to be the dull purples obtained together with carmines on crossing violets *inter se*. If these should appear again next year, it will be possible to make certain of the chemical effect represented by the factor **S** alone.

The results obtained in these preliminary investigations do not support Mr Buxton's suggestion that differences in *p*H are responsible for the chief variations in flower colour, although the possibility that *p*H may play a small part cannot be ruled out.

The change in the colour of the red primroses on drying or on macerating, which he remarks upon, may indicate a change in *p*H, but in my opinion this is not a conclusive proof that the cell membrane of the living flower exercises "selective permeability." Changes due to chemical change, conversion from solution to solid, and also colloidal effects must be taken into account. However, it may be that in addition to the two chemical differences for which some evidence has been put forward in this note, the extremes in colour shown by the carmine and blue flowers are also due in part to a difference in hydrogen-ion concentration.

It is interesting to compare the anthocyanins of the primroses with those of *Primula sinensis*. Varying amounts of primulin have been isolated from the red, magenta, slaty and blue varieties of the Chinese primula, while the acid extracts of the magenta and blue flowers are in each case bluer than those of the red and slaty. The fact that the blue flowers of *P. sinensis* are not such a good blue as those of *P. acaulis* might be explained by the presence in the former flowers of a definite quantity of primulin, while the diglucoside forms the principal pigment of the latter. As in the primrose, the red and slaty flowers of *P. sinensis* contain practically no flavone, while the magenta and blue ones possess a considerable amount.

Further investigations on all these pigments are to be continued with a view to throwing further light upon the chemical nature of these Mendelian factors for flower colour.

My thanks are due to Prof. J. B. S. Haldane and Prof. R. Robinson for their help and advice, and to Mr Buxton for kindly supplying the dried flower samples.

I am also indebted to the Department of Scientific and Industrial Research for the grant which has enabled me to undertake this investigation.

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