CLXXXII. A SURVEY OF ANTHOCYANINS. I.

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Although the details of the constitution and properties of the anthocyanins can only become known as the result of their isolation in substance and the study of the pure individual pigments following the lines of the pioneering researches of Willstätter and his collaborators [1913–16], yet it is also true that much can now be gleaned from an examination of the colouring matters in solution and there are many obvious advantages attaching to this procedure.

For example, the formation and occurrence of anthocyanins can be instantly recognised by inspection and the development of a method of analysis is a necessary step towards the utilisation of this property in making attempts to solve the problems of the origins and functions of these pigments. Moreover, phylogenetic investigations are often facilitated by a knowledge of the anthocyanins of the varieties, and more rapid methods than those demanding the collection of kilograms of the dried petals are urgently required.

Finally a preliminary survey should afford evidence of the existence of interesting new types of anthocyanins, and thus pave the way for extensions of the large scale investigations.

In all cases we have employed cold 1 % aqueous hydrochloric acid in order to extract the coloured materials and the best results were obtained by using fresh flowers and by examining the solutions as soon as possible following their preparation.

Colour of flowers and of the extracts—co-pigments.

The colours of the flowers or fruits themselves do not afford trustworthy indications, but whilst orange-scarlet shades indicate the occurrence of a pelargonidin derivative no blue flower-pigments are derived from pelargonidin. On the other hand not very dissimilar bluish-red *Phlox*, crimson *Dianthus* and crimson-scarlet *Linum* contain anthocyanins derived respectively from pelargonidin, cyanidin and delphinidin.

More reliance can be placed on the colour of the acid extracts provided these are observed at the boiling-point of the solution. Under these conditions the order from orange-red to blue-red is that of anthocyanins based on pelargonidin, peonidin, cyanidin, malvidin and delphinidin.

The necessity for this precaution arises from the existence in the solutions of substances (co-pigments) which intensify and modify the colour. Willstätter

and Zollinger [1916] observed that the addition of tannin to a solution of oenin chloride in dilute hydrochloric acid intensified the colour and produced a change in tone giving a much bluer red. These authors also noticed that gallic acid had a similar but weaker effect and that the ferric reactions and alkali-colour reactions were also modified; finally they commented on the fact that the effect is specific for oenin and is not observed to a comparable extent in the case of cyanin solutions.

This is true for gallotannin (although malvin is as sensitive as oenin) but co-pigments exist for all the types of anthocyanins, although they have not yet been identified. Some preliminary experiments in this field are described in the experimental section, and we note here in illustration of specificity that the glucoside of 2-hydroxyxanthone is a powerful co-pigment for cyanin but not for mecocyanin, and that the isomeric glucoside of 4-hydroxyxanthone is far less active.

The phenomenon has little or nothing to do with salt formation and occurs in the presence of a large excess of mineral acid; it is evidently the result of the formation of weak additive complexes which are dissociated at an elevated temperature or by the action of solvents.

We have reason to believe that the formation of these complexes occurs in the flowers themselves and plays a most important part in producing variations of colour. Our interest in this aspect of the subject arose from an examination of the fuchsia. The violet inner corolla gives a bluer red acid extract than that obtained from the outer bluish-red petals, and this was at first regarded as a proof that the anthocyanins are different in the two cases. We still believe that this is part of the truth, but it was noticed that the blue-red solution from the violet petals became much redder on washing with amyl alcohol. Addition of light petroleum to the separated amyl alcohol and extraction of the mixture with 0·5 % hydrochloric acid furnished an extract which, mixed with the washed anthocyanin solution, reproduced the original blue-red solution. In this case the substance in the amyl alcohol was tannin, identified as derived gallic acid. Subsequently the phenomenon was found to be very general and some of the effects are surprisingly great and cannot yet be imitated with synthetic compounds.

Willstätter and Mallison [1915] found that the rose-coloured flowers of *Pelargonium peltatum* contained pelargonin, the colouring matter of the scarlet pelargonium, and concluded that the cell-sap of the bluer red variety must be the more alkaline.

The aqueous acid extract of the rose-coloured petals is, however, also bluish-red and acquires the colour of a pure pelargonin solution only on heating; on cooling the blue shade returns. The bluish-red phlox is another striking instance of the occurrence of a pelargonin-co-pigment complex.

It seems that great changes in the colour of varieties in a species are not brought about by changes in the p_H of the cell-sap, but rather by changes in the nature of the anthocyanin, including in this term the formation of complexes

with organic substances and possibly with metals such as iron. Haas and Hill [1929] have expressed a similar view.

An apparent exception is *Primula sinensis*, in magenta and blue varieties of which Miss R. Scott-Moncrieff has found the same anthocyanins. Here, we think that one of the genetic factors concerned is a quantitative one; the magenta flowers are much the more intensely coloured and the modifying substances present in low concentrations are unable to deal with the whole amount. In the pale-coloured rather violet-shaded blue flowers the pigment remains the same, but it is all modified according to the present hypothesis. We wish to emphasise that the evidence for the theory is that changes in colour are so often found associated with a change in the anthocyanin and that we speak only of closely related varieties or species. It is agreed that changes of p_H in the cell-sap occur at different stages in the life of the plant and also in representatives of distinct genera; for example, Willstätter and Everest [1913] made it very probable that the colouring matter of the blue cornflower is the potassium salt of cyanin, whereas the rose and dahlia contain the pigment in the form of an oxonium salt with an organic acid. It is obvious also that a genetic factor for flower colour may be concerned only with the development of a co-pigment, the anthocyanin remaining unchanged in composition and concentration.

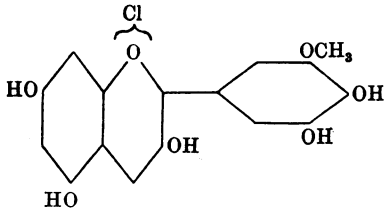
Methods of examination of the extracts and scope of the results.

The details are given in the experimental section, but in general we have observed the colour reactions with alkalis and ferric chloride and made considerable use of distributions between immiscible solvents.

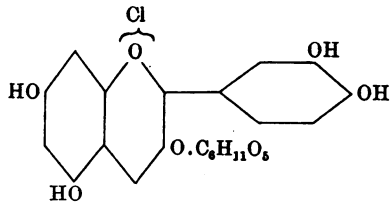
The value of this method was indicated by Schudel [1918] whose dissertation was, we understand, the result of work in Prof. Willstätter's laboratory. Willstätter and Schudel developed a technique for the separation of anthocyanidins, monoglycosidic[1] and diglycosidic anthocyanins and employed a similar process in the difficult isolation of betanin from the beet. We have made use of their observations in order to devise methods of purification of the anthocyanidins and anthocyanins and to assist in characterising the pigments.

Apart from the betanidin group and the yellow anthocyanin observed by Willstätter in the flowers of *Papaver alpinum*, it has not been found necessary to assume the existence of new anthocyanidins—we have not proved that such do not exist in the material examined, but we can accommodate our results without them. Nevertheless we are now of the opinion that petunidin chloride [Willstätter and Burdick, 1916] is essentially 3′-*O*-methyldelphinidin chloride (I) identical with the salt synthesised by Bradley, Robinson and Schwarzenbach [1930].

[1] The term "glycosidic" is employed when the nature of the sugar is unknown; "glucosidic" being used to denote a derivative of glucose.



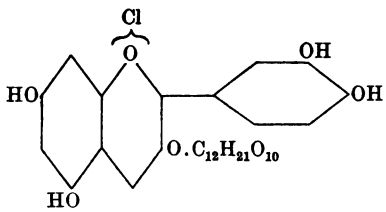
(I)



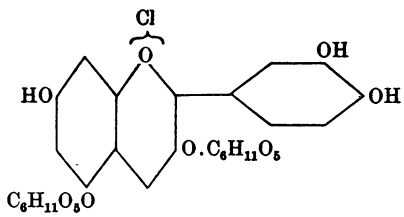
(II)

The methods we have employed throw little light on the nature of the carbohydrate groups in the anthocyanins, but in most cases we can give the position of attachment as the result of comparisons with pure natural or synthetic anthocyanins of known constitution. The simplest type of anthocyanin is represented by chrysanthemin chloride (II) which is cyanidin 3-monoglucoside.

The diglycosides can apparently be placed in one of two groups; either they are 3-biosides like mecocyanin (III) or they are 3 : 5-dimonosides like cyanin (IV). The arguments on which the constitution of cyanin are based will be fully described in papers of the series "Experiments on the Synthesis of Anthocyanins," published in the *Journal of the Chemical Society*. The evidence indicates that the 3-monosides are the basis of the anthocyanins, the other groups being subsequently attached to the molecule. Thus no 5-monoside is known. Furthermore this view affords some explanation of the changes in sugar-position type which occur, for example, in the sweet peas, the gladioli and the carnations.



(III)



(IV)

In addition, the carbohydrate residues may be acylated in the class of complex diglycosides.

Some interesting anthocyanins, not yet isolated in substance, have been encountered. The pelargonidin analogue of mecocyanin is rather widely distributed, it occurs *inter alia* in orange-red poppies, scarlet cineraria, gloxinia, tropaeolum and red-flowered anemones. Complex diglucosides of pelargonidin and delphinidin have been studied in detail by Willstätter and Mallison [1915], by Willstätter and Mieg [1915], by Karrer and Widmer [1927], and by Kondo [1931]; we have found a complex diglycoside of cyanidin in the leaves of *Coleus* and a complex diglycoside of malvidin in the flowers of *Salvia virgata nemorosa*.

Experimental.

Nature of petunidin chloride. Some comparisons of petunidin chloride with synthetic 3′-*O*-methyldelphinidin chloride were carried out by Bradley, Robinson and Schwarzenbach [1930], and no serious divergences were noted but an equimolecular mixture of delphinidin and malvidin chlorides gave almost identical results. It is remarkable that we have since found this to be true of many distribution-ratio experiments. A small specimen of finely crystallised petunidin chloride, for which we are greatly indebted to Prof. Willstätter, was available and the following experiments were carried out.

Malvidin chloride (4·002 mg.) was dissolved in 30 cc. of ethyl alcohol (1000 cc. of which contained 1 cc. concentrated hydrochloric acid) and the solution made up to 250 cc. by means of 1 % hydrochloric acid. This solution (30 cc. at 21·5°) was placed in a stoppered cylinder and shaken with air; 10 % aqueous sodium hydroxide (25 cc.) was then introduced, the liquid shaken once and immediately acidified with hydrochloric acid (10 cc.). The recovered anthocyanidin was taken up in amyl alcohol and colorimetrically estimated, using a standard prepared from malvidin chloride (0·860 mg.), ethyl alcohol (10 cc. containing hydrochloric acid as above) and amyl alcohol to 100 cc. The recovery was 78 %. Now, on performing this experiment with a solution of petunidin chloride (4·012 mg.) the recovery was quite small and indicated less than 10 % of malvidin in the sample. The recovery from both delphinidin and 3′-*O*-methyldelphinidin was less than 5 %. Using equivalent solutions of delphinidin chloride and malvidin chloride it was found that a mixture of 28 cc. of the delphinidin solution and 2 cc. of the malvidin solution gave slightly greater recovery than the natural petunidin chloride. A mixture of 7 % malvidin chloride and 93 % 3′-*O*-methyldelphinidin chloride could not be distinguished in its behaviour in the above experiment from natural petunidin. Various further tests were made with the remainder of the solutions and no divergences were noted. For example, the "93 %" and the "natural" solutions were each mixed with an equal volume of aqueous sodium acetate; the violet solutions were identical. On now shaking with 6 volumes of amyl alcohol the colour base separated as a violet film at the interface and in each case this exhibited a beautiful steel-blue reflex. In view of the facts that Willstätter and Burdick [1916] showed that petunidin yields delphinidin on demethylation and has approximately the correct methoxyl content, the only possible alternative to the methyldelphinidin hypothesis was that the substance might be an equimolecular mixture of delphinidin and malvidin. The experiments now recorded dismiss this possibility and the correctness of our conclusions was confirmed by comparisons of the extraction of equivalent solutions (50 cc.) of the "natural" petunidin chloride and of 3′-*O*-methyldelphinidin chloride (in 1 % hydrochloric acid) by successive portions (15 cc.) of the amyl ethyl ether-anisole-picric acid reagent for delphinidin mentioned below. The course of the extraction was very similar in the two cases; the pigment was gradually

removed without that sharp distinction between consecutive extracts which is characteristic of mixtures of malvidin and delphinidin. As anticipated, the first extract from the "natural" was a little more deeply coloured than that from the "synthetic"; otherwise the graded series were identical.

Tests on the original solution. The observations of the colour and of the change, if any, on heating have already been mentioned. The colour of a dilute solution of cyanin chloride is taken as standard in speaking of orange-red or blue-red.

If the colour is orange-red a small volume of the solution should be diluted with alcohol; peonidin derivatives then become much bluer and to a much greater extent than pelargonidin derivatives. In this, as in many other cases, comparison with authentic specimens is essential, and in the absence of crystallised homogeneous anthocyanins certain flower extracts will serve. For pelargonin the scarlet pelargonium; for peonin, a purified (see below) extract from the deep red peony; for cyanin, the blue cornflower; for malvin, *Epilobium* or the red-violet annual *Clarkia elegans*; for mecocyanin, any bluish-red poppy; for pelargonidin 3-diglycoside, orange-red nasturtiums; for chrysanthemin, crimson carnations; for callistephin, scarlet carnations; delphiniums and violas will give a supply of delphinidin derivatives. A portion of the original solution is shaken with amyl alcohol in order roughly to estimate the distribution number; a second extract should also be made in the case of anthocyanins of high distribution number. In the latter case, also, a test must be made for complex diglycosides. The solution is boiled in a test-tube to expel air and, whilst hot, 20 % aqueous sodium hydroxide is added drop by drop, until the colour changes through green to yellow or brownish-yellow. After actually boiling for a few seconds, concentrated hydrochloric acid is carefully added to the hot solution until the reconstituted anthocyanin indicates that the solution is acid; one more drop is then added and after 15 seconds the liquid is cooled and the distribution number again observed. In the case of monoglycosides there is no change and, although anthocyanidin is not produced under the conditions described, a second shaking is carried out for confirmation. In the case of complex diglycosides the distribution falls almost to zero.

The colour obtained on the addition of sodium acetate to the original solution is observed; this is not affected by the presence of anthoxanthins. The colours given by the pure anthocyanins are the following: callistephin, rather dull brownish violet-red; pelargonin, bright bluish-red; peonidin 3-glycosides, similar to callistephin but brighter; peonin, red-violet; cyanin, violet; mecocyanin (chrysanthemin), violet-red; malvin, bright violet; oenin, dull violet; delphinidin glycosides, blue-violet to blue. The reaction is subject to much interference, for example, from the presence of iron and/or tannin

In the case of quite clean extracts it may be desirable to test the response to the ferric reaction. Sodium carbonate is gradually added until the colour changes towards violet or blue; acid conditions are then restored by the addition of just sufficient 0·5 % hydrochloric acid. A positive reaction on the

addition of a drop of neutral ferric chloride is a deep violet coloration, replacing and more intense than the anthocyanin red. Any residual red tinge represents a negative result and the violet must be pure-toned and free from green or brown.

Examination of the anthocyanidin.

In some cases it may be necessary to purify the anthocyanin before submitting it to hydrolysis, but normally this is not the case. The solution is mixed with rather less than an equal volume of concentrated hydrochloric acid and boiled for 30 seconds (unless experiment indicates that a longer period is required). The solution is extracted with amyl alcohol and the upper layer separated and washed with water and then with 1 % hydrochloric acid. A small volume of the 1 % acid is then added in order to take up the oxonium salt after the addition of much benzene. The amount of benzene necessary to decolorise the upper layer will give some information; delphinidin requires 3–4 times the volume of the amyl alcohol; petunidin about 4–5 volumes; cyanidin, 5–6 volumes; malvidin, 5–6 volumes; peonidin, 8–9 volumes; and pelargonidin, 10–11 volumes. These figures are approximations and refer to 1 volume of the amyl alcohol solution with 3 volumes of 0·5 % hydrochloric acid. The filtered anthocyanidin solution is again extracted by amyl alcohol and the process repeated; finally, the aqueous solution of the anthocyanidin is washed three or four times with a large excess of benzene in order to remove traces of amyl alcohol.

The colour of this solution compared with that of authentic specimens gives valuable information. The following tests are carried out. A small portion is extracted with amyl alcohol and sodium acetate added; after observation a drop of ferric chloride is added and the tube gently shaken. A portion is shaken with an equal volume of a mixture of cyclohexanol (1 vol.) and toluene (5 vols.) (the cyanidin reagent); the concentration of anthocyanidin in this test should not be lower than 4 mg. in 100 cc. The upper layer is decanted into a narrow tube for observation.

A portion (concentration about 3–4 mg. in 100 cc.) is shaken with air and half its volume of 10 % sodium hydroxide added. This is immediately followed by concentrated hydrochloric acid and amyl alcohol. The recovery of anthocyanidin in this "oxidation" test is noted.

A portion (or several portions of different concentrations) is shaken with a 5 % solution of picric acid in a mixture of amyl ethyl ether (1 vol.) and anisole (4 vols.) (delphinidin reagent). (Note on recovery: amyl ethyl ether and anisole are difficult to separate by distillation, so that it is convenient to use the Abbé refractometer. The volumes % and the refractive indices are connected by a relation that is sufficiently linear for practical purposes and the following data may be of service—anisole, n, 1·5161; 4 : 1-mixture, n, 1·4902, ethyl amyl ether, n, 1·3902.)

The various anthocyanidins exhibit the following behaviour:

Pelargonidin. Amyl alcohol-sodium acetate, violet-red; no change with

ferric chloride; largely extracted by the cyanidin reagent and completely by the delphinidin reagent; not destroyed in the oxidation test. Pelargonidin is recognised by the colour of its acid solution and by the colour reactions of the anthocyanins derived from it.

Peonidin differs chiefly from pelargonidin in the colour of its acid solutions and in the colour reactions of related anthocyanins.

Cyanidin gives a reddish-violet solution when sodium acetate is added to the amyl alcohol extract over water and ferric chloride changes the violet to a bright blue colour. This is apt to be confused with malvidin containing a trace of ferric-reacting anthocyanidin. Cyanidin is fairly stable in the oxidation test; it imparts a rose-red colour to the cyanidin reagent (malvidin, a very weak mauve) and its extraction by the delphinidin reagent is not complete from dilute solutions. In order to distinguish cyanidin from impure malvidin it is best to perform the ferric reaction without sodium acetate using a carefully washed amyl alcohol solution diluted with ethyl alcohol. It is only when the anthocyanin is a 3 : 5-dimonoside that confusion with malvidin is a possibility; the reactions of cyanidin 3-glycosides are characteristic.

Malvidin gives a slightly bluer violet in the amyl alcohol-sodium acetate test than cyanidin and ferric chloride does not change it. Pure malvidin is, however, of rare occurrence. The oxidation test leaves malvidin largely unchanged and it is not extracted by the cyanidin reagent, but completely by the delphinidin reagent.

Petunidin gives a violet-blue in the amyl alcohol-sodium acetate test and pure blue after the addition of ferric chloride. It is destroyed in the oxidation test, not extracted by the cyanidin reagent and has a lower distribution than cyanidin in the delphinidin reagent. It is best recognised by successive extractions of a solution with small portions of the delphinidin reagent.

Delphinidin gives a blue solution in amyl alcohol on the addition of sodium acetate; it is destroyed in the oxidation test, not extracted by the cyanidin reagent or by the delphinidin reagent.

Hirsutidin is rare and if it occurs in any of the solutions tested it would be included under malvidin. It gives reactions similar to those of malvidin.

Colour reactions of the anthocyanins.

Pelargonin (and possibly other pelargonidin 3 : 5-dimonosides) is easily recognised. It gives a violet coloration with aqueous sodium carbonate and this becomes greenish-blue on the addition of acetone. Decisive confirmation is obtained by addition of $\frac{1}{4}$ volume of concentrated hydrochloric acid to the solution and boiling for $\frac{1}{2}$–1 minute; then on extracting with amyl alcohol a green fluorescence due to pelargonenin will be observed.

Pelargonidin 3-glycosides, for example, callistephin, give a violet-red coloration with sodium carbonate and this is rather stable towards sodium hydroxide. No other anthocyanin-type gives a similar reaction.

Peonin (3 : 5-type) gives a blue coloration with sodium carbonate. Peonidin 3-glycosides do not occur in the sequel, but the sodium carbonate reaction is a rich violet unchanged by sodium hydroxide.

Cyanin gives a rich pure blue coloration with sodium carbonate, unstable to sodium hydroxide, whereas mecocyanin (chrysanthemin) gives a blue-violet with sodium carbonate changing to pure blue with sodium hydroxide.

Malvin (3 : 5-type) gives a bright greenish-blue with sodium carbonate, whilst oenin (malvidin-3-glucoside) gives a blue-violet unchanged by sodium hydroxide.

The delphinidin glycosides have not been distinguished with the aid of their colour reactions in the absence of synthetic standards.

Very frequently the anthocyanin had to be purified before satisfactory reactions could be obtained.

Solutions of diglycosides could be exhaustively extracted with amyl alcohol and occasionally this was sufficient. Otherwise the pigment was taken up in amyl alcohol-acetophenone (2 : 1) in the presence of picric acid. This reagent was introduced by Willstätter and Schudel [1918]. The separated, filtered organic layer was agitated with 1 % hydrochloric acid and diluted with ether. Picric acid was then carefully removed from the aqueous solution by further extraction with ether. Monoglycosides were similarly purified by extraction with cyclohexanone and picric acid or with ethyl acetate and picric acid; the extracts were precipitated with light petroleum and the anthocyanins taken up in 1 % hydrochloric acid. The solution was usually extracted with benzene, then with cyclohexanone and again with benzene. Occasionally the process was repeated.

Summary of results.

Achillea Kelwayi. Complex diglycoside.

Aconitum napellus. Delphinidin diglycoside; contains much anthoxanthin.

Aesculus hippocastanum. The pink flowers contained a cyanidin 3-glycoside which required exhaustive purification. The distribution was intermediate suggesting a rhamnoglucoside or the like.

Althaea rosea. Various hollyhocks were examined and the results suggested mixtures of malvidin, petunidin and delphinidin mono- and di-glycosides. Delphinidin and malvidin were present beyond doubt and the occurrence of petunidin was inferred from the behaviour of the anthocyanidin on successive extraction with the delphinidin reagent.

Anchusa italica (Dropmore var.). The mauve buds and blue flowers gave the same results. The anthocyanin is essentially a delphinidin diglycoside, but the behaviour of the anthocyanidin with the delphinidin reagent indicated some methylation of the pyrogallol nucleus.

Anemone coronaria (St Brigid var.). Red flowers contained a pelargonidin 3-bioside and violet flowers contained cyanidin 3 : 5-dimonoside, possibly mixed with a malvidin derivative. The anthers of both varieties contain what appears to be the same anthocyanin characterised by a high distribution number. Further examination of the anemone is projected; this flower was studied before our system had been adequately developed.

Armeria maritima. Malvidin 3 : 5-dimonoside.

Arum maculatum. Anthocyanin absent.

Astilbe gloria purpurea. The reactions were much obscured by other substances but the anthocyanin proved to be a cyanidin 3-glycoside. The distribution was intermediate between that of mecocyanin and that of chrysanthemin.

Aubrietia deltoides. The purple flowers of the variety "Dr Mules" were found to contain a delphinidin diglycoside, whilst the rose-red Leichtlini was found to contain a cyanidin 3 : 5-dimonoside.

Begonia semperflorens. A fibrous-rooted bedder with crimson flowers gave a solution containing a cyanidin 3-bioside; the leaves of another variety with small pink flowers contained cyanidin 3-bioside.

Tuberous-rooted begonias have also been examined; the scarlet-flowered varieties contain pelargonidin 3-bioside and the crimson kinds, cyanidin 3-bioside.

Bellis (Rob Roy). A co-pigment makes the solution much too blue-red, but the anthocyanin is a cyanidin 3-bioside.

Berberis aquifolium. The deep purple fruits contain 3-monoglycosides of malvidin, delphinidin and probably also, petunidin. The anthocyanin gives a violet coloration in aqueous sodium carbonate and this becomes green on the addition of sodium hydroxide. Cyanidin is absent, but the reactions cited are those of cyanidin 3-glycosides, and it is evident that these changes may be observed in the delphinidin series also.

Buddleia amplissima. Delphinidin diglycoside.

Calceolaria. A magenta garden variety contained cyanidin 3-bioside and this was the main anthocyanin of a brown-red kind; in this case the cyanidin was partly replaced by pelargonidin.

Callistephus hortensis. A blue-violet Aster of the "Victoria" class was found to contain a delphinidin diglycoside. The characteristic pigments of other varieties are monoglycosidic. According to Fleming [1929] no true blue aster exists.

Campanula glomerata. Purplish-blue flowers contained a delphinidin diglycoside mixed with a small proportion of a reddening pigment. The variety "Telham Beauty" has blue-violet flowers and the anthocyanin was a delphinidin diglycoside.

Pink flowers of *Campanula Medium* were unexpectedly found to contain a pelargonidin 3-bioside; this anthocyanin is usually associated with a scarlet-coloured flower, the rose-pinks based on pelargonidin owing their colour to pelargonin or similar anthocyanin.

Carduus lanceolata. Cyanidin 3 : 5-dimonoside.

Centaurea nigra. The distribution number was zero, otherwise all tests showed that the anthocyanin was a cyanidin 3 : 5-dimonoside.

Chaerophyllum anthriscus. The purple leaves gave an extract containing a cyanidin 3 : 5-dimonoside.

Charies heterophylla. Delphinidin diglycoside.

Chrysanthemum coronarium. Pyrethrums such as "Countess Poulett," "Sutton's Blue Red," and "Mrs James Leake" were all found to contain a cyanidin 3-glycoside. There was a powerful co-pigment effect.

Chrysanthemum tricolor gave solutions having exactly the same properties.

Cichorium entybus. Delphinidin diglycoside.

Clarkia elegans. A coral pink variety yielded pelargonidin 3 : 5-dimonoside and a violet-flowered variety gave a very clean solution of pure malvidin 3 : 5-dimonoside. In this case the malvidin was free from any trace of a substance giving a positive ferric reaction.

Clematis Jackmani. The anthocyanin is a delphinidin diglycoside mixed with a relatively small proportion of methylated delphinidin derivatives.

Coleus Blumei. The leaves of several varieties were examined and all found to contain a complex diglycoside of cyanidin. The acid solution was very blue-red and developed a fine blue colour on the addition of sodium acetate.

The distribution was monoglucosidic, or even higher, and after purification the anthocyanin gave a reddish-violet with sodium acetate. The diglycoside resulting from the hydrolysis of the anthocyanin (cold sodium hydroxide in a hydrogen atmosphere) was purified and its distribution between butyl alcohol and 1 % hydrochloric acid was identical with that of cyanin. The whole of the reactions of the diglycoside supported the view that it is actually cyanin. So that coleusin is an acyl derivative of cyanin and the nature of the acid is being investigated.

Convolvulus sepium. Rose-heliotrope flowers contain a cyanidin 3-monoglycoside.

Crataegus oxyacantha fl-pleno rosea. The flowers are coloured by a cyanidin 3-bioside and the skins of the red berries contain a cyanidin 3-glycoside.

Dactylis glomerata L. The bluish-mauve flowers were separated and a cyanidin 3-bioside was diagnosed. The distribution is, however, intermediate between monoglucosidic and diglucosidic.

Delphinium. A number of the garden perennial varieties have been examined, for example, "Prince of Wales," "True Blue," "W. T. Ware," "Corry Ware," "Mrs Townley Parker" and *D. formosum*; also annuals derived from *D. consolida.* All gave evidence that delphinidin is the main anthocyanidin, but it is mixed in most cases with a small proportion of a methylated derivative.

The anthocyanins all exhibited the stability in aqueous sodium carbonate solution, which, Willstätter and Mieg [1915] showed to be characteristic of delphinin.

Dianthus barbatus. The bluish-red flowers of the type gave a blue-red acid extract and after exhaustive purification of the anthocyanin it was characterised as cyanidin 3-monoglycoside. The garden varieties (Sweet William) also furnished 3-monoglycosides; a crimson and also a black-crimson (*nigricans*) variety furnished a solution which could not be distinguished in distribution number tests from one of chrysanthemin chloride. A scarlet variety, however, was found to owe its colour to a pelargonidin 3-monoglycoside, probably callistephin and following that anthocyanin exactly in distribution number tests. These apparent monoglycosides were found to be such in fact, and do not owe their high distribution number to complex diglucosidic constitution.

Dianthus caryophyllus. We are greatly indebted to Mr E. M. Morgans for supplies of a number of varieties of carnations.

"Nigger" and "Topsy" (deep brown-red) owe their colour to a cyanidin 3-monoglycoside, the distribution number of which, after purification, was identical with that of chrysanthemin under the same conditions. "Brilliant," "Aviator" and "Spectrum" (scarlet) are similarly coloured by a pelargonidin 3-monoglycoside tallying with callistephin. "Mary Allwood" (salmon-red) also contains the pelargonidin 3-glycoside. But, surprisingly, "Improved Ward" (bluish-pink) contains a pelargonidin 3 : 5-dimonoside agreeing in distribution number (*n*-butyl alcohol) with pelargonin.

Dierama pulcherrima. Mauve-rose flowers contained a malvidin 3 : 5-dimonoside.

Digitalis purpurea. The anthocyanin is a cyanidin 3 : 5-dimonoside.

Dodecatheon integrifolia. Malvidin 3 : 5-dimonoside. The solutions contained much anthoxanthin.

Epilobium angustifolium and *E. hirsutum.* The willow herbs contain a malvidin 3 : 5-dimonoside and apart from the presence of a co-pigment these available flowers provide good standards for malvin-type reactions.

Erigeron speciosus. A very strong co-pigment effect was noted; the anthocyanin is a delphinidin diglycoside.

Fagus sylvatica. Leaves of the copper beech give blue-red extracts and were found to contain a complex cyanidin 3-bioside. The distribution number of this anthocyanin closely approaches that of chrysanthemin for amyl alcohol, ethyl acetate in presence of picric acid and butyl alcohol, all with 1 % hydrochloric acid. A considerable divergence was, however, noted when the organic solvent was *n*-butyl alcohol mixed with $\frac{1}{8}$ vol. of benzene. The beech anthocyanin had the higher distribution number. The complex nature of the pigment was proved in the usual manner and an attempt to identify the acid concerned will be made in due course. This is the first example of a complex diglycoside derived from a 3-bioside anthocyanin.

Fragaria vesca. The colour of strawberries is due to a pelargonidin 3-monoside.

Fuchsia. The purple petals of the inner corollae of several varieties (for example, "Rose of Castile") gave identical results which have already been described. The anthocyanin is mainly a malvidin 3 : 5-dimonoside but the alkali-colour reactions were not quite standard, being too red even after purification of the pigment through the picrate. The anthocyanidins concerned could be pelargonidin and peonidin only, since the ferric reaction was quite negative.

A clue was obtained by studying the bluish-red outer petals which were found to contain a peonidin 3 : 5-dimonoside mainly, mixed with a blueing component. We then found that the reactions could be perfectly matched by adding a little peonin to malvin for the inner petals or a little malvin to peonin for the outer petals. The use of pelargonidin 3-bioside from *Papaver orientale* gave a very poor match and pelargonidin 3 : 5-dimonoside cannot be present, since no pelargonenin-type fluorescence could be observed. The absence of pelargonidin from the fuchsia

anthocyanidins was confirmed by adding saturated aqueous picric acid (1 vol.) to a solution in 0·5 % hydrochloric acid (1 vol.) and shaking with a mixture (1 vol.) of *iso*propyl ether with 3 vols. of *iso*amyl ether. The upper layer was not coloured by the anthocyanidin, but after adding a few drops of a dilute solution of pelargonidin chloride and again shaking, the organic layer was coloured by anthocyanidin.

Carrying out the same experiment but using a mixture of equal volumes of *iso*propyl ether and *iso*amyl ether, the presence of peonidin was indicated in the anthocyanidins from both types of petals. This mixture distinguishes peonidin from malvidin in dilute solutions; in the former case the upper layer assumes a bluish salmon-red colour but malvidin is not extracted, although the picrate may be precipitated. The variety "Corallie" with long, tubular, salmon-red flowers was found to contain a pelargonidin 3 : 5-dimonoside.

Galega officinalis. The violet flowers contain a delphinidin diglycoside.

Geranium pratensi. Malvidin 3 : 5-dimonoside.

Geranium Robertianus. The leaves and stem furnished an extract of a cyanidin 3-bioside, whilst the bluish-pink flowers contained a malvidin 3 : 5-dimonoside.

Geum chiloënse. The varieties "Mrs Bradshaw" and "Orange Queen" gave the same results. The anthocyanin is a cyanidin 3-monoside but there are indications of the presence of a small proportion of a pelargonidin derivative.

Gladiolus gandavensis. This group exemplifies well our thesis that colour variations are usually due to the appearance of different anthocyanins. The salmon-pink varieties all contain a pelargonidin 3 : 5-dimonoside. In some cases, for example, "Flaming Sword," the alkali-colour reactions suggest admixture with a small proportion of a peonidin or cyanidin derivative. In the scarlet sorts, for example, "Scarletta," we find a pelargonidin 3-bioside. The distribution number is lower than that of callistephin but is higher than that of the pelargonidin bioside in the gloxinia and scarlet cineraria.

A crimson-flowered variety contained an anthocyanin, the distribution number of which was zero; it gave all the reactions of a cyanidin 3 : 5-dimonoside. The occurrence of a malvidin 3 : 5-dimonoside was noted in a purple-red variety (Jacob von Bengeren); this contained a trace of an anthocyanin giving a positive ferric reaction.

G. primulinus, a red-violet variety, was also found to contain a malvidin 3 : 5-dimonoside.

Gloriosa vivescens Rothschildiana. Specimens kindly supplied by Lord Rothschild were found to contain cyanin, which in this case was isolated and identified. There is also a bright yellow anthoxanthin.

Gloxinia. "Reading Scarlet" and "Beacon" contain a pelargonidin 3-bioside, whilst "King George" is coloured by the same pigment together with a small proportion of an anthocyanin giving more blue-red acid solutions. "Purple Sultan" and "Violet" yield diglycosidic anthocyanins based on malvidin but containing some cyanidin, petunidin or delphinidin derivative. The alkali-colour reaction was not precisely of malvin-type and it may be that this is due to the rare malvidin 3-bioside constitution or more probably to lack of homogeneity.

Hyacinthus orientalis. "King of the Blues" contains a delphinidin diglycoside and "Queen of the Pinks" contains a pelargonidin 3 : 5-dimonoside. Neither anthocyanin is homogeneous, the evidence being in favour of the presence of a cyanidin derivative in both violet-blue and pink flowers.

Hydrangea hortensis. Considerable interest attaches to a study of the pigments of these flowers on account of the popular idea that iron in the soil helps in the development of the blue colour. Unfortunately the blue kinds do not produce their pigment lavishly, but we have been able to form an opinion respecting the relation between the respective colouring matters.

A red-flowered variety, "Parzival," gave a bluish-red solution containing a co-pigment and the anthocyanin was a delphinidin derivative. As the distribution number seemed rather high for a delphinidin diglycoside, tests for complex diglycosidic character were made and these gave a positive result, the recovered diglycoside having a distribution number approximating to zero. The anthocyanin from a blue-flowered variety, "Maréchal Foch," had a much lower distribution number than that from the red flowers. It proved to be a delphinidin diglycoside. It is noteworthy that the colour from the red flowers is sensitive to co-pigments, whereas that from the blue flowers is much less so.

Iberis umbellata. The purple flowers contain a cyanidin 3 : 5-dimonoside mixed with a relatively small proportion of a malvidin derivative.

Ilex aquifolium. The skins of holly berries contain a pigment which tallies best with a pelargonidin 3-bioside; a further examination is necessary.

Iris Kaempferii. A bluish-purple variety of the Japanese Iris contained a malvidin 3 : 5-dimonoside mixed, probably, with a petunidin derivative. Several unnamed blue and violet varieties of the German Iris furnished solutions of delphinidin diglycoside much blued by co-pigments and the variety "Imperator" gave identical results.

Ixia crateroides. The cerise-coloured flowers contain a cyanidin 3 : 5-dimonoside and this is probably cyanin, since a comparison of the purified pigment with cyanin in respect of its distribution number (*n*-butyl alcohol—1 % hydrochloric acid) showed that these were identical.

Lathyrus. L. grandiflorus, crimson flowers, develops a mixture of diglycosidic anthocyanins; the anthocyanidins consist of malvidin (less than 40 %) and delphinidin, whilst the presence of petunidin may be inferred from the behaviour of the anthocyanidin solution on extraction with successive small volumes of the delphinidin reagent. *L. latifolius* is coloured by nearly homogeneous malvidin 3 : 5-dimonoside. *L. odoratus* provides one of the most interesting groups and we propose to study it in some detail; the solutions are clean and the petals are easily extracted.

For named varieties of Sweet Peas as also for Gloxinias, Cinerarias, Salpiglossis, *etc.*, we are very greatly indebted to Messrs Sutton and Sons of Reading. "2 LO," scarlet, and "Glorious," pink, gave solutions of a pelargonidin 3-bioside. "Crusader," bluish-pink, contains, however, pelargonidin 3 : 5-dimonoside. "Captain Blood," blood-red, gives solutions containing more anthoxanthin than usual in the group. Its chief anthocyanin is cyanidin 3-bioside, but this is mixed with a small proportion of pelargonidin 3-bioside. Cyanin and pelargonin types of anthocyanins were not present. "Damask Rose," bluish-red, contains a mixture of pelargonidin and cyanidin 3 : 5-dimonosides. The same is true of "Sybil Henshaw," bluish-red, but in this case the proportion of cyanidin derivative is higher than in "Damask Rose." A match for all the reactions was obtained using 70 % of cyanin and 30 % of pelargonin. No match could be made using mecocyanin. Doubtless a variety yielding good cyanin-type reactions free from pelargonin exists but, even although we have not yet encountered it, we consider the proof of the occurrence of cyanidin 3 : 5-dimonoside is satisfactory.

It thus appears that the Sweet Peas contain both types of pelargonidin and cyanidin diglycosides and these are by no means all. "Bacchus," wine-red, possesses colouring matters based on methylated delphinidins, delphinidin itself appears to be absent. Cyanidin is also absent and we consider that the chief anthocyanins are malvidin and petunidin 3 : 5-dimonosides. The evidence for malvidin is the result of the oxidation test and the behaviour towards the delphinidin reagent; petunidin is indicated by the latter and by the positive ferric reactions, cyanidin and delphinidin being ruled out.

"Chieftain," light red-purple, contains chiefly malvidin 3 : 5-dimonoside and the same is true of "Royal Purple," red-purple; these two give weak ferric reactions as purified anthocyanin or as anthocyanidin. "The Flag Lieutenant," purple, and "Black Diamond," deep red-purple, furnish anthocyanins giving a strong ferric reaction; malvidin and delphinidin were proved to be present and cyanidin was absent. The existence of petunidin in the mixture is strongly suspected but we cannot put this forward with confidence. The anthocyanins are all diglycosidic and the alkali-colour reactions are of the malvin type.

"Blue Bird," violet-blue, contains a delphinidin diglycoside as the major anthocyanin.

Lavandula vera and *L. nana* both contain a delphinidin diglycoside.

Lavatera trimestris rosea splendens. This anthocyanin will be further examined; it appears to be based on petunidin. The oxidation test on the anthocyanidin shows that cyanidin and malvidin are absent, yet the ferric reaction is positive and the distribution with the delphinidin reagent is approximately correct for petunidin. Peonidin and pelargonidin are absent. Moreover, the reactions of the anthocyanin, which has a high distribution number, agree with the suggestion that it is petunidin 3-monoside. We have not excluded the complex diglycoside possibility in this case.

Lilium Martagon. The reddish-purple flowers and also the stems yielded a blue-red solution containing a co-pigment. The anthocyanin is a cyanidin 3 : 5-dimonoside.

Lilium umbellatum (Dahurian Lily) has orange flowers but the anthocyanin is a cyanidin

3-bioside. It required exhaustive purification on account of the presence of much anthoxanthin and other substances.

Linum. A crimson-scarlet-flowered annual variety (*grandiflorum* var. *rubrum*) was found to contain a delphinidin diglycoside. In this case we may be justified in stating that the anthocyanin is a 3-bioside, since the coloration in sodium carbonate is royal blue and not greenish-blue as usual. There seems to be little co-pigment in this case and for these reasons we propose to attempt the isolation of this anthocyanin. It is remarkable that a flower so far removed from blue should contain an anthocyanin based on unmethylated delphinidin. Nevertheless, a little cyanidin appears to be present.

Linum narbonense, blue, contains a delphinidin diglycoside and, by contrast with the preceding, this is a 3 : 5-dimonoside and gives a greenish-blue coloration in sodium carbonate; also the colour in sodium acetate is violet-blue, whereas in the case of the annual scarlet flax it was violet.

Lupinus polyphyllus. The varieties "Pink Perfection," "H. Marshall," reddish-violet, and "Downers Delight," dull brownish salmon-red, contained a cyanidin 3-bioside. "King of the Blues," reddish-blue, contains a delphinidin complex diglycoside; "Sutton's Saxe Blue," "Sutton's Ruby" and "Amethyst" from Brookside Nurseries, Oxford, give quite similar results. "Victoria," bluish-dark purple, is also a complex diglycoside and the results suggest that it is a petunidin derivative. This conclusion is provisional.

Lychnis chalcedonica. The anthocyanin is a pelargonidin 3-glycoside with a distribution number intermediate between callistephin and pelargonidin 3-bioside.

Lythrum salicaria. "Brightness" contains a malvidin 3 : 5-dimonoside and a trace of an anthocyanin having a positive ferric reaction.

Matthiola incana. Some single and double ten-week stocks have been examined and all the anthocyanins therein have high distribution numbers and were found to be complex diglycosides. A magenta single-flowered variety furnished an acyl derivative of a pelargonidin 3 : 5-dimonoside; this had a distribution number > 50. A violet-coloured variety contained an acylated cyanidin 3 : 5-dimonoside. A deep purple double-flowered kind furnished an anthocyanin with a distribution number nearer to that characteristic of a diglycoside but still too high. On hydrolysis this yielded an anthocyanin not to be distinguished in distribution and other tests from cyanin.

It is noteworthy that the solutions of all these complex diglycosides of *Matthiola* are much too blue-red and that heating the solutions has a relatively small effect. We consider that in these cases the attached phenolic acid residue exerts an intramolecular effect similar to the intermolecular effect of the co-pigments. Coleusin is under all circumstances much bluer red in solution than cyanin.

Myosotis dissitiflora. "Blue King" contains a delphinidin diglycoside.

Nepeta mussini contains a complex diglycoside of delphinidin; the only difficulty was that the sodium acetate-amyl alcohol test on the anthocyanidin gave rather too red a shade. Other tests, however, confirmed the diagnosis of delphinidin.

Nepeta okrani also contains a complex diglycoside of delphinidin, but in this case the evidence of contamination is still more definite; also the distribution number of the original anthocyanin is higher than in the case of that from *N. mussini*.

Nemophila insignis. Delphinidin diglycoside.

Nigella damascena. Malvidin 3 : 5-dimonoside.

Oenothera amoena (syn. *Godetia rubicunda*). A bluish-red variety gave diglycosidic anthocyanins, certainly 3 : 5-dimonosides, but apparently not homogeneous and derived from malvidin and cyanidin (or petunidin).

Origanum vulgare. The anthocyanidin is very definitely malvidin and it is found unmixed with other anthocyanidins; further the anthocyanin is diglycosidic. The characteristic malvin-type alkali-colour reaction could not be obtained and the colour with sodium carbonate was violet, but this is possibly due to impurities not removed in the cyclohexanone-picric acid process.

Papaver orientale bracteatum. The orange-scarlet flowers, after removing all the dark central parts, gave a solution of a pure pelargonidin 3-glycoside. The colour reactions matched those of callistephin. The distribution number, however, was zero and the distribution number with solvents other than amyl alcohol was also abnormally low. Perhaps this anthocyanin is a trioside. The epidermis of the dark portions gave a solution of cyanidin 3-bioside, doubtless mecocyanin

and the anthers also contained the cyanidin derivative. A pink variety, "Queen Alexandra," also contained a pelargonidin 3-bioside and the explanation of the colour change is probably to be found in the presence of a co-pigment.

Many other varieties of poppies have been examined and in general the pink and scarlet kinds (also the orange, *Papaver nudicaule*) contain pelargonidin derivatives, always 3-glycosides, whilst the deep red and blue-red varieties contain a cyanidin 3-bioside (mecocyanin). The darker portions are never free from mecocyanin and this may, or may not extend to other parts of the petals. A common form of the field poppy (*P. rhoeas*) has scarlet flowers, the petals of which (excluding the splashes) yield solutions containing both pelargonidin and cyanidin derivatives. Pink Shirley poppies give blue-red acid solutions, but these are co-pigmented and purification of the anthocyanin through the picrate yields normal pelargonin-like solutions.

Mecocyanin [Willstätter and Weil, 1916] may, as usually prepared, be accompanied by an impurity; its alkali-colour reaction can be matched by a solution made by adding about 12 % of cyanin to chrysanthemin. On the other hand, this tendency to give slightly bluer reactions than chrysanthemin may be an inherent property of the substance.

Pentstemon. "Southgate Gem" and *isophyllis* gave scarlet-red solutions having similar reactions; a strong co-pigment was present. The anthocyanin is a pelargonidin 3 : 5-dimonoside, the distribution number of which is higher than that of pelargonin and lower than the complex diglycosides of this group.

Petunia. In view of our section on the existence of petunidin, it is interesting to note that permanganate-coloured petunias furnished an anthocyanin of the methylated delphinidin type, but this gave, on hydrolysis, an anthocyanidin consisting of malvidin to the extent of at least 57 %. The remainder was probably petunidin, but this could not be proved in the presence of so much malvidin. Willstätter and Burdick [1916] used a special blue petunia and their process of isolation may have had much to do with the successful removal of anthocyanins based on malvidin and delphinidin.

Phlox. A few varieties of herbaceous Phlox have been examined; they are remarkable for the efficiency of their co-pigments.

"Jules Sandean," pink, contains a pelargonidin 3 : 5-dimonoside and a trace of a cyanidin derivative; "Leo Schlageter," crimson-scarlet, contains the same anthocyanin with a little more cyanidin derivative. On heating, the solutions in 1 % hydrochloric acid became much more orange-red and the blue-red colour returned on cooling. This property was not destroyed by heating with sodium hydroxide until bright yellow, boiling the solution for a few seconds, and then reproducing the anthocyanin. The solution was still blue-red and the alternation of colour on heating and cooling could still be brought about. The co-pigment is therefore not an ester. A blue-violet "Iris Phlox" was found to owe its colour to a malvidin 3 : 5-dimonoside.

Potentilla Willmottiae. Cyanidin 3-bioside; the distribution number suggests the possibility of rhamnoglucoside. Other potentillas having a like colour or deep brown-red colour, gave similar results.

Poterium obtusum. The blue-red flowers contain a cyanidin 3 : 5-dimonoside.

Primula Juliana contains a malvidin 3 : 5-dimonoside and *P. sinensis*, "Reading Ruby," also contains this type of anthocyanin.

Prunus Avium. The skins of small black cherries contain cyanidin 3-monoside; the distribution number is higher than that of keracyanin [Willstätter and Zollinger, 1916].

Prunus communis. The skins of a deep purple plum were found to contain a cyanidin 3-monoside. The variety was "Rivers Early."

Prunus Pissardii. The leaves contain a cyanidin 3-monoside; there was no complex diglycoside.

Pyrus malus. The skins of the apples "Wine Sap" and "Jonathan" were found to contain a cyanidin 3-monoside. The flowers of "Niedzwetzkyana" also yield a cyanidin 3-monoside.

Rheum rhaponticum (Sutton's rhubarb). The colouring matter in the skin of the stem is a cyanidin 3-bioside.

Rhododendron indicum. A salmon-red azalea gave an extract singularly free from anthoxanthins and the colouring matter was a cyanidin 3-monoside. It was almost certainly chrysanthemin, since its distribution numbers under four sets of conditions were identical with those of chrysanthemin. The solvents employed were amyl alcohol, butyl alcohol with 20 vols. % of benzene, ethyl

acetate in the presence of picric acid in small and also in large concentration; all were put in competition with an equal volume of 1 % hydrochloric acid.

Ribes. Willstätter and Bolton [1916] found that the berries of *R. rubrum* L. contained a cyanidin diglucoside. This is a cyanidin 3-bioside and it is accompanied by a pelargonidin or peonidin derivative. A curious feature was the difficulty experienced in getting a positive ferric reaction with the original solution.

The skins of large red gooseberries (*Ribes grossularia*) contain a cyanidin 3-monoside and the black currant (*Ribes nigrum*) contains a cyanidin 3-bioside. In the latter case there is much co-pigment in the original solution and normally coloured solutions result from purification of the anthocyanin through the picrate. Cyanidin was the only anthocyanidin found, but it should be noted that a small percentage of delphinidin could not in any case be detected and the related anthocyanin would probably be eliminated in the purification process.

Rosa. Young red leaves of the rose contain a cyanidin 3 : 5-dimonoside, doubtless cyanin.

Rubus idaeus. Willstätter and Bolton [1916] diagnosed a cyanidin glucoside in the raspberry; it is a cyanidin 3-bioside. Loganberries also contain a cyanidin 3-bioside and in both cases the reactions represent a close approach to those of pure mecocyanin.

Rubus odoratus. An interesting anthocyanin occurs in the flowers. It has a high distribution number and on hydrolysis yields a cyanidin 3 : 5-dimonoside indistinguishable in its reactions and distribution between *n*-butyl alcohol and 1 % hydrochloric acid from cyanin. This complex cyanidin diglycoside is probably not identical with that found in *Coleus Blumei*; its solutions though blue-red are not markedly so as in the latter case, and the reactions with sodium acetate are strikingly dissimilar. The anthocyanin under consideration gives a violet-red and that from *Coleus* leaves almost a pure blue coloration. The difference must reside in the nature or mode of attachment of the associated acid and further investigation is projected.

Rumex acetosa L. Cyanidin 3-bioside.

Salpiglossis sinuata. Sutton's "Large Blue-flowered" and "Light Blue and Gold" contain a delphinidin diglycoside. The reactions of the solutions from "Velvet Red" and "Black Knight" agree best with the view that they contain mainly petunidin diglycosides.

Salvia splendens. The variety "Grahamei" has crimson flowers and as the more orange-scarlet types were employed by Willstätter and Bolton [1916] it is of interest to note that this variety, also, contains a complex pelargonidin 3 : 5-dimonoside.

Salvia patens. Delphinidin diglycoside, possibly complex.

Salvia pratensi. The anthocyanin appears to be not homogeneous; it is mainly a complex malvidin 3 : 5-dimonoside, but there is also a substance present which gives a ferric reaction.

Salvia virgata nemorosa. The interesting anthocyanin is a complex malvidin 3 : 5-dimonoside; it is very blue-red in acid solution and is relatively difficult to hydrolyse by means of acids, in which respect it resembles the anthocyanin of *Coleus*. The diglycoside obtained after alkaline hydrolysis and recovery by means of hydrochloric acid is probably malvin. The acid of the complex has been isolated and is being examined.

Saponaria. A scarlet-crimson annual variety gave a solution which contained a cyanidin 3 : 5-dimonoside and very satisfactory reactions were obtained with the original unpurified material.

Saxifraga sanguinea superba. The solution was blue-red and the anthocyanin had a high distribution number. It yielded cyanidin on hydrolysis but did not tally with any of the known types.

The reaction with sodium acetate is blue-violet even after purification through the picrate, and the violet colour with sodium carbonate becomes blue on the addition of sodium hydroxide, but not so readily as in the usual chrysanthemin-type cases. There is some novel feature in connection with this anthocyanin and next season a closer investigation is proposed.

Scabiosa arvensis. Delphinidin diglycoside.

Scrophularia nodosa. This figwort contains a cyanidin 3-bioside and much anthoxanthin.

Solanum dulcamara. The purple flowers contain a delphinidin diglycoside which gives a deep blue-violet with sodium acetate and so is probably a 3 : 5-dimonoside.

Senecio (*Cineraria stellata*). Deep blue hybrids contain a delphinidin diglycoside (distribution number zero), and the aqueous acid extracts become pure blue on the addition of sodium acetate. Similar results were obtained with named varieties, for example, "Forget-me-not Blue," "Royal

Blue," "Blue Gem," "Blue with White Zone" and "Duchess Star." On the other hand, the rich purple flowers give an extract that develops a corresponding blue-violet on the addition of sodium acetate. The distribution number is still zero and the anthocyanidin is delphinidin. Possibly the change is in the attachment of carbohydrate residues. Cineraria "The King" and "Brilliant Ruby" contain the same anthocyanin which has a low distribution number (but not zero), and appears to be a malvidin derivative mixed with a substance giving a positive ferric reaction. The reactions do not, however, tally with those of known types and this, together with the blue cineraria, will be further studied. Cineraria "Pink Pearl" and the large scarlet-flowered variety contain a pelargonidin 3-bioside unmixed with other anthocyanins.

Silene angelica. The flowers of this catchfly contain a malvidin 3 : 5-dimonoside mixed with a trace of a cyanidin derivative, and the stems contain a cyanidin 3-bioside with a trace of a malvidin derivative. Attention is directed to the fact that this distribution of a malvin-type and meco-cyanin-type of anthocyanin has also been observed in the *Geranium Robertianus* (Herb Robert).

Sistus purpurea and *Sistus* "Sunset" appear to contain a cyanidin 3-bioside, but the change of colour from violet to blue on adding sodium hydroxide to a solution containing sodium carbonate was obscured by impurities not removed by the ordinary processes.

Stachys sylvatica. Cyanidin 3-monoside.

Statice sinuata. The violet-blue flowers yielded nothing to 1 % hydrochloric acid and had to be ground with the solvent in a mortar. (Note: this is not usually a good practice unless the solutions are centrifuged, when it is time-saving.) The very blue-red extract was found to contain a delphinidin diglycoside unmixed with other anthocyanins.

Tricuspidaria dependens. The reactions of the anthocyanin from this crinodendron were much obscured but the anthocyanidin is cyanidin, the distribution number was very low and after exhaustive purification the cyanidin 3-bioside reactions were observed, although not in the usual unambiguous manner.

Trifolium. "Sutton's Early Red Clover" gives an extract containing a cyanidin 3-bioside.

Tritoma uvaria. The flowers do not contain anthocyanin.

Tropaeolum majus L. Willstätter and Bolton [1916] stated that the flowers contain a cyanidin derivative; this is true of the deep brown-red variety and the anthocyanin is a cyanidin 3-bioside. The common orange-scarlet variety contains a pelargonidin 3-bioside and the anthocyanins are thus like those of the poppy.

Tulipa Gesneriana L. This is a very complex group characterised by mixtures of anthocyanins. Willstätter and Bolton [1916] found that the anthocyanin of a dark red garden tulip was cyanin, occurring both in the petals and the anthers. We are quite prepared to accept this finding (especially in view of our experiences with the sweet peas and gladiolus), but have not ourselves encountered a similar variety. We are much indebted to Sir Daniel Hall for supplies of varieties of tulips and the present is an interim report; the examination of the tulips is much more difficult than that of most other groups.

The varieties "Orange Perfection," "D," "City of Haarlem" and "Inglescombe Scarlet" constitute a group in which the main anthocyanin is pelargonidin 3-bioside, but it is mixed with some cyanidin 3-bioside in all cases. A variety "Electra" is typical of a middle group in which the main anthocyanin is a cyanidin 3-bioside and the subsidiary pigment, present in substantial relative amount, is pelargonidin 3-bioside. Other members of this group are probably "Dorothy Ann," "Couleur Cardinal," "Proserpine," "Cecilia," "28" and "Mary Swan." "Venus," "Rhoda Backhaus" and "Eos" also contain cyanidin and pelargonidin 3-biosides, but in these cases we did not gain an idea as to which predominated. At the other end of the scale "William Copland" was found to contain a delphinidin diglycoside and similar varieties are "Daphne," "Lucy Lund," "Widor" and "916." The greater number of varieties are intermediate between the cyanidin 3-bioside group and the delphinidin diglycoside group. They are free from pelargonidin derivatives and comprise "Giant," "Isobel," "Velvet King," "Orange Girl," "Julietta," "Prancing Nigger," "47," "29," "Cecil Dolling," "Lavinia," "Philip de Commines," "Grey Dawn," "Perdita," "Zulu," "La Noire," "Sultan." All are diglycosidic and none is homogeneous. The anthocyanidins derived from "La Noire" and "Sultan" have been separated in some quantity and the result of their examination is to suggest that they consist of petunidin and delphinidin with a trace of malvidin, but work on this topic is still in progress. A summary of the results is that in the

tulips we find a group in which pelargonidin and cyanidin occur as 3-biosides and then apparently with a rather sharp separation a second group containing delphinidin derivatives.

The varieties "Grey Dawn" and "Perdita" were examined in "breeder" and in "broken" condition; in both cases the extracts from the former were bluer but no great differences could be detected in the anthocyanins.

Urtica dioica. The flowers contain a cyanidin 3 : 5-dimonoside.

Verbena, Crimson hybrid. A cyanidin 5-dimonoside was diagnosed, but this case presented some curious features in that hydrolysis was difficult and the solutions were much too blue-red.

Vicia cracca. Delphinidin diglycoside.

Viola gracilis and other violas contained a delphinidin diglycoside, doubtless violanin in many cases. The causes of colour variation in this family, apart from the presence of carotenoids and anthoxanthins, appear to be methylation of the delphinidin nucleus and the presence or absence of co-pigments. Thus "Pickerings Blue" gives a very blue-red acid extract and the redder-violet *Viola cornuta* yields a redder acid solution. But on heating the two solutions are identical in tone and the colour difference returns on cooling. The co-pigment present in "Pickering's Blue" can be removed and introduced into the solution from *Viola cornuta*; the situation is then reversed. Evidently the difference in colour in this case is purely a question of the co-pigment.

In other cases of very red-violet violas it was shown qualitatively by the micro-Zeisel method that the anthocyanidin is partly methylated. Cyanidin derivatives were not encountered in the violas.

Weigela (*Diervilla hybrida,* Eva Rathké). The crimson flowers contain a cyanidin 3-monoside.

Zapania nodiflora. Delphinidin diglycoside.

Some observations on co-pigments.

The following substances have little or no effect in low concentrations on the colour of dilute acid solutions of oenin chloride: alanine, asparagine, nicotinic acid, quinolinic acid, anthranilic acid, *m*-aminobenzoic acid, *p*-aminobenzoic acid, phthalic acid, benzilic acid, glucose, raffinose, maltose, starch, inulin, methylglycoside, rutin (possibly due to sparing solubility), euxanthic acid (same comment), helicin, pyridine, 2 : 4-dihydroxyquinoline, catechol, hesperidin.

The following substances have a slight blueing effect on oenin solutions. Tyrosine, 2-hydroxy-3-naphthoic acid, protocatechuic aldehyde, pyrogallol, orcinol, salicylic acid, *p*-hydroxybenzoic acid, arbutin, euxanthic acid, baptisin, 2-hydroxyanthraquinone, β-methylglucoside, β-naphthol glucoside, catechin. The following have a moderately powerful effect. *m*- and *p*-Hydroxybenzaldehyde, β-resorcylic acid, gentisic acid, protocatechuic acid, vanillin, phlorhidzin, aloin, quercitrin, 4-hydroxyxanthone glucoside. Aesculin, ethyl gallate and tannin have a strong effect and the most active compound tested was 2-hydroxyxanthone glucoside; it is far more active than the isomeride. 2-Hydroxyxanthone glucoside also exerts a strong deepening and blueing effect on the colour of anthocyanins derived from cyanidin and the order of efficiency is cyanin $>$ chrysanthemin $>$ mecocyanin. The action on pelargonin is to darken the solution but not to make it more blue-red. Quercitrin blues oenin but does not increase the tinctorial intensity. We are indebted to Dr A. Robertson for pure specimens of his synthetic glucosides.

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