CXCVII. A SURVEY OF ANTHOCYANINS. II.

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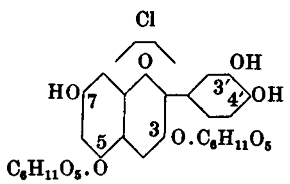
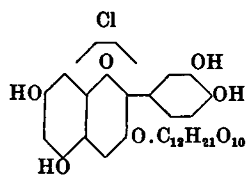
DURING the year following the publication of Part I [1931] of this series the progress of synthetical work has been rapid and the main diglucosidic anthocyanins have been synthesised and found to be identical with the natural products. Thus Dr A. R. Todd [1932] in collaboration with one of us, has synthesised hirsutin, malvin, cyanin, peonin and pelargonin chlorides, whilst the preparation of cyanidin 3-cellobioside, cyanidin 3 : 7-diglucoside and 5 : 7-diglucoside has made it clear that mecocyanin and keracyanin are in fact, as already assumed, cyanidin 3-biosides. The established formulae for cyanin chloride (I) and mecocyanin chloride (II) are here reproduced for convenience of reference to the numbering and nomenclature system.



(I)



(II)

The synthesis of cyanidin 3 : 7-diglucoside (MacDowell and Robinson; unpublished results) introduces a matter of which it is necessary to take cognisance, since it is difficult to distinguish this substance from cyanin by reactions in solution unless the p_H can be estimated accurately. Thus the class "3 : 5-dimonoside" may include some 3 : 7-dimonosides and ultimately special investigations will be made in order to determine whether the 3 : 7-isomerides occur in nature or not. In the meantime 3 : 5 is held to include 3 : 7.

Formerly the rhamnoglucosides were not differentiated from the monoglucosides on the one hand or from the diglucosides on the other, but we have now devised a simple test which enables the three classes to be sharply distinguished; we have also simplified the distinction of rhamnose from a simple pentose such as xylose but have not yet applied this to very many cases.

The system adopted in naming the anthocyanins is the following: *saccharide* is the most general term applicable to all sugars, monoses, bioses *etc.*; *glycoside* indicates a single unit of a hexose; *bioside*, a residue of a biose; *dimonoside*, two units of hexose separately combined in different positions of the anthocyanidin molecule; *pentoseglycoside*, a residue of a biose composed of an unidentified pentose and an unidentified hexose; rhamnoglycoside, a residue of a biose derived from rhamnose and an unidentified hexose.

Naturally we agree that it is very probable that most of the glycosides are glucosides and that most of the pentoseglycosides are rhamnoglucosides but exceptions are known to exist and therefore the more general terminology is retained.

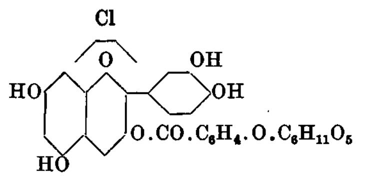
The methods used in the present investigation are the same as those described in Part I but attention must be drawn to a pitfall in connection with the recognition of complex anthocyanins (acylated anthocyanins). We have found that the test for complex character cannot be safely applied unless the anthocyanin is first purified as already described through the picrate or better by a method mentioned below. There is a substance present in crude plant extracts which is capable of condensing with the anthocyanin molecule in hot alkaline solution so that on reconstitution of the pyrylium salt it appears to be diglucosidic. Thus even synthetic monoglucosides, such as oenin or chrysanthemin, can be modified by treatment with hot aqueous sodium hydroxide and an extract of the skins of white grapes (for example) so that their distribution between amyl alcohol and dilute hydrochloric acid becomes that of a diglucoside. The only correction of Part I involved in this relates to the pigment of the copper beech which is really monoglucosidic and which has now been isolated and analysed.

The complex diglycoside test is perfectly satisfactory if applied to the purified pigments and fortunately the high distribution number of these complex pigments makes the purification relatively easy.

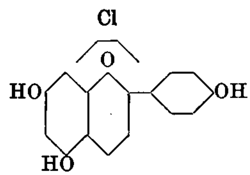
Experience of the application of the methods described in Part I has proved their adequacy but exceptional cases will always require special treatment and there is one hitherto unrecognised class of anthocyanins which must be specifically mentioned.

Hydrolysis by 15 % hydrochloric acid under the prescribed conditions is a drastic process and 30 seconds usually suffices to break down the anthocyanin into its constituent parts.

In several cases, however, it appears that sugar residues are eliminated, leaving an acylated anthocyanidin which is further hydrolysed on continued boiling with hydrochloric acid, or, more certainly, by treatment with aqueous sodium hydroxide followed by hydrochloric acid. The normal anthocyanidin reactions are obtained ultimately and the intermediate complex anthocyanidin may be a sparingly soluble precipitate or it may be recognised by special properties. Thus a substance which has been frequently encountered is a 3-acylated cyanidin and this has the curious property that it is largely extracted by the cyanidin reagent (*cyclo*hexanol-toluene 1 : 5) and not at all by the delphinidin reagent (picric acid-anisole-ethylamyl ether). The colour reactions of the anthocyanins which give rise to this 3-acylated cyanidin preclude acceptance of the view that the sugar residues are attached to other positions of the anthocyanidin nucleus; all the other phenolic hydroxyls, namely those in positions 5, 7, 3′ and 4′ must be free. Consequently the acyl group itself must carry the sugar residues, and this is quite feasible since such acids as *p*-hydroxybenzoic acid, *p*-coumaric acid and protocatechuic acid have been found in association with anthocyanins. Our hypothesis respecting the arrangement of the groups in these cases is illustrated by the expression III.



(III)



(IV)

This sequence of the side-chain groups does not, however, apply to the complex 3 : 5-dimonosides, such as monoardaein, because these give the diglycosides on hydrolysis and we may not substitute more than two of the phenolic hydroxyls on account of the colour reactions. The complex anthocyanidin glucosides, naturally, do not give anthocyanins on alkaline hydrolysis but are at once converted into anthocyanidins.

Some striking cases of the distribution of anthocyanins have been observed. A nasturtium was found to contain a pelargonidin derivative in the flower petals, a cyanidin derivative in the calyx and a delphinidin derivative in the leaves.

Three Pompon dahlias were selected at a nursery and again representatives of the three main types were diagnosed. The pelargonidin analogues of mecocyanin and keracyanin have been repeatedly encountered; they are among the more commonly occurring anthocyanins and we are making arrangements with a view to their isolation in substance.

Several new sources of betanin-like pigments have been found and, of these, *Bougainvillaea* is of special interest because one species appears to contain a monoglycoside of the group. This will greatly facilitate the investigation of what is perhaps the most interesting of all types of anthocyanins.

Some years ago one of us in collaboration with D. G. Pratt, A. Robertson and others synthesised various pyrylium salts allied to the flavones rather than to the flavonols because it appeared probable that these would ultimately be found to be natural products, but until quite recently this speculation was unjustified by the outcome of the present survey. Messrs Carter's Tested Seeds, Ltd., however, kindly sent us some flowers of *Gesnera fulgens* the colour of which would normally indicate pelargonidin 3-bioside or perhaps a carotenoid pigment. The pigment was soon recognised as an anthocyanin derived from apigeninidin chloride (IV) and owing to the stability and sparing solubility of this salt we were able to obtain about 10 mg. of the pure substance from a mere handful of the flowers. As the material could not be obtained in quantity we employed both *Gesnera fulgens* and *G. cardinalis* having found that the reactions of the anthocyanins were identical. The apigeninidin chloride was carefully compared with a specimen obtained by the method of Pratt, Robertson and Robinson [1927] and no divergences were observed.

Finally, we draw attention to the great stability of the majority of the anthocyanins in 1 % aqueous hydrochloric acid at the room temperature. Dr E. Fonseka very kindly sent us some specimens from Ceylon in the form of extracts and these gave the same results as freshly prepared solutions, the diglucosides suffering no partial hydrolysis. On the other hand some complex anthocyanins are extraordinarily readily hydrolysed, and the complex anthocyanin in *Centradenia grandiflora* (leaves) is converted into a normal diglucoside by the operation of purification through the picrate. Also some hydrangea anthocyanins and one derived from the chrysanthemum may be cited in this connection. The complex character is lost on merely keeping the solutions.

Experimental.

Notes on methods. The purification of anthocyanins through the picrates is often a very wasteful process and frequently cannot be applied at all; *cyclo*-hexanone is the best solvent. An alternative is to take advantage of the large effect of saturation of the solutions with salt on the distribution to amyl alcohol and butyl alcohol. Rosenheim [1920] was the first to suggest the use of butyl alcohol as a solvent for anthocyanins. The anthocyanin can thus be taken up in one of these solvents and returned to dilute hydrochloric acid by addition of a small proportion of benzene or light petroleum and successive extractions with small volumes of 1 % acid. The method can be applied as often as desired and the aqueous solutions can be washed with ethyl acetate or other organic solvent. An example of the process is the isolation of a crystalline anthocyanin from the leaves of the copper beech.

In preparing a solution of an anthocyanidin for the cyanidin reagent test, care must be taken to exclude ethyl alcohol and its derivatives, and to see that the acid concentration is 1 %. This is secured by repeated washing of the amyl alcohol containing the anthocyanidin with 1 % hydrochloric acid before adding benzene and extracting with the acid solution. The amyl alcohol must later be thoroughly removed by washing with benzene.

Test for pentoseglycosides (rhamnoglucosides and the like). At very low concentrations of the anthocyanin a distinction between pentoseglycoside and monoglycoside is possible by means of the distribution to amyl alcohol. The colour of the latter tends to become more apparent than that of the aqueous layer in the case of monoglucosides whilst with rhamnoglucosides it is the aqueous layer in which the colour appears to be more persistent. In practice the distinction is very easy to observe.

Similarly a distinction between diglycosides and pentoseglycosides can be obtained at low concentrations by means of the distribution to amyl alcohol.

Both appear almost zero (colourless organic layer) but on the addition of salt to the aqueous layer the pentoseglycoside is largely extracted by the amyl alcohol and the diglycoside distribution is almost unaltered. This applies even to delphinidin derivatives and pelargonidin derivatives.

Tests for rhamnose and aldopentose (xylose). The anthocyanin solutions must be purified as completely as possible by the method mentioned above and by repeated extraction with organic solvents. If anthoxanthins are present it is hardly good policy to conduct this test; the absence of anthoxanthins is indicated by normal, typical alkali-colour reactions of the anthocyanin.

The solution is mixed with an equal volume of concentrated hydrochloric acid and distilled, the distillate being examined for the presence of furfuraldehyde or methylfurfuraldehyde. These can be differentiated by the acetic acid aniline test or by a modification of the naphtharesorcinol test [Tollens and Rorive, 1908; Votoček, 1897]. We have found that the two aldehydes give entirely different reactions when their dilute aqueous solutions are added to a solution of naphtharesorcinol in 5 % ethyl alcoholic hydrogen chloride. The conditions are not critical and may need modification to suit special cases. We have convinced ourselves in this way that the pelargonidin 3-pentoseglycoside in the gloxinia is a rhamnose derivative and we confirmed Nolan and Casey's statement [1931] that their sambucicyanin is derived from an aldopentose and not from a methylpentose.

The following experiment may serve as a guide in applying this very characteristic reaction.

Distil rhamnose or xylose (0·025 g.) with concentrated hydrochloric acid (5 cc.) and water (5 cc.) and collect 5 cc. To 1 cc. of distillate add 5 cc. of acetic acid and 2 cc. of aniline. Xylose gives a very intense crimson and rhamnose gives a much weaker, browner orange-red.

Add 1 cc. of the distillate to 10 cc. of 5 % alcoholic hydrogen chloride containing 5 mg. of naphtharesorcinol. Xylose gives an immediate orange-yellow (without blue tinge), this quickly becomes darker and duller brown and later violet and acquires a most intense green fluorescence. Rhamnose gives at once a stable orange-red, eosin-red in thin layers. The colour does not change in the course of a few hours, but on long standing it changes to reddish-violet and the solution acquires green fluorescence; at this stage the solution from xylose is violet-blue and the fluorescence is much the more intense of the two.

Summary of results.

Acalypha bispida. Hydrolysis for a short time affords an acylated anthocyanidin; hydrolysis for several minutes under the usual conditions, or alkaline hydrolysis, affords cyanidin. The reactions are those of a 3-substituted cyanidin. The distribution number (amyl alcohol) is high and the anthocyanin is probably a 3-acyl cyanidin saccharide.

Acer dissectum atropurpureum, cyanidin 3-monoside; *A. palmatum atropurpureum*, *A. dissectum versicolor* and *A. campestre Schwedleri* contain the same anthocyanin, all in the leaves.

Achimenes. Flowers of Sutton's "Mauve Queen" give a co-pigmented solution containing malvidin 3 : 5-dimonoside; "Magnet," magenta flowers, contains the same pigment.

Aethionema grandiflora. Bluish-pink flowers contain delphinidin diglycoside with a slight admixture of cyanidin derivative.

Ajuga reptans. Violet-blue flowers contain a complex delphinidin saccharide. Acid hydrolysis gives an acylated anthocyanidin, but if the solution is first treated with sodium hydroxide, hydrolysis by hot hydrochloric acid affords delphinidin.

Allium cepa. The red skins of "Sutton's Blood Red" onion contain cyanidin 3-pentoseglycoside.

Allium narcissiflorum. The violet-red flowers contain a complex (acylated) cyanidin 3-monoside. The original pigment has an abnormally high distribution number and after purification and hydrolysis with sodium hydroxide it yields a cyanidin 3-monoside.

Alstroemeria aurantiaca. Orange-red and orange varieties are chiefly coloured by carotenoids, but these are accompanied by cyanidin 3-bioside.

Amherstii nobilis—pelargonidin 3-pentoseglycoside.

Ampelopsis hederacea—cyanidin 3-monoside in the red leaves.

Anemone pulsatilla. The blue-violet flowers contain delphinidin diglycoside (correction of Part I).

Anthurium scherzerianum. The vermilion leaves and the orange-scarlet flowers are coloured by pelargonidin 3-pentoseglycoside; the leaves also contain a cyanidin derivative in small relative amount.

Antirrhinum majus. The purple leaves of the variety "Defiance" (orange-scarlet flowers) contain pelargonidin 3-pentoseglycoside and the purple leaves of "Monarch" (crimson flowers) contain cyanidin 3-pentoseglycoside. The latter is probably Scott-Moncrieff's [1930] antirrhinin which is identical with the keracyanin of Willstätter and Zollinger [1916]. The flowers of "Monarch" were also examined and the presence of antirrhinin confirmed.

Apium graveolens. Violet-red leaf-stalks of "Sutton's Giant Red" celery appear to contain a complex cyanidin diglycoside, short hydrolysis of which furnishes an acylated anthocyanidin. The peculiar feature is that the distribution number is very low, corresponding to a normal diglycoside. The reactions approximate to those of the 3-bioside type but are not identical with them; for example, the colour with sodium carbonate is violet-blue rather than blue-violet.

Aquilegia alpina. The red-tinged blue flowers contain a delphinidin diglycoside. Another variety, deep violet-blue flowers, gave the same results. There is not much co-pigment in evidence.

Ardisia crispa. The bluish-red berries contain a cyanidin 3-glycoside, the distribution number of which appears to be lower than that of chrysanthemin chloride. As much anthoxanthin is present, a possible explanation is combination of anthocyanin and co-pigment in the aqueous layer.

Aster. A. Amellus var. Riverslea has deep heliotrope or blue-violet flowers containing delphinidin diglycoside. "Colwell Beauty" has a similar but paler shade and the delphinidin derivative in this case is mixed with cyanidin (probably). The Michaelmas daisy "Mrs T. J. Wright," light plum-coloured flowers, is coloured by a cyanidin 3 : 5-dimonoside.

A. sinensis, violet-blue flowers, contains a delphinidin diglycoside contaminated by a methylated derivative or by a cyanidin diglycoside.

Aubrietia deltoides (compare Part I). "Fire King," reddish-violet flowers, contains a 3 : 5-dimonoside of cyanidin.

Begonia. Leaves of several varieties varying from brownish-red to bluish-crimson in colour were all found to contain cyanidin 3-bioside. *B. Margaretacea* and *B. rubrum* gave pure 3-bioside; "Gloire des deceaux" afforded a mixture of bioside and complex diglycoside and some evidence of contamination by the latter was also found in the leaves of the varieties "Professor Grieve," "Gloire des Ardennes" and *B. rex* var. *Versuive*, especially the latter.

Berberis aquifolium. Young leaves, faded leaves and purplish leaves in winter contain a cyanidin 3-pentoseglycoside.

Berberis Darwinii. Purple-blue berries appear to be coloured by petunidin monoglycoside and this is also true of the berries of *B. Stenophylla.* The anthocyanidin contained traces of malvidin or cyanidin or both. On the other hand the orange-red berries of *B. Farreri* and "Winter Cheer" contain a 3-glycoside of cyanidin and the berries of *B. vulgaris* contain the 3-glycoside of pelargonidin. It will thus be seen that the berries of species of *Berberis* embrace an unusually wide range of anthocyanins, all of which are monoglucosidic.

Bertolonia Houtteana. The bluish-red leaves yield an anthocyanin with an abnormally high distribution number. This was purified by the salt-amyl alcohol process and then proved to be a complex malvidin 3 : 5-dimonoside.

Bougainvillaea glabra. The pale pinkish-heliotrope bracts contain a pigment of the betanin group but one yielding acid solutions even bluer in tone than those of betanin. Moreover the distribution number is that of a monoglycoside. The variety "Mrs Butt" contains a diglycosidic pigment of the same group; this closely resembles betanin but its solutions are not quite so blue-toned.

Brassica campestris Rapa. The tops of purple-coloured turnips gave strongly co-pigmented anthocyanin solutions. The pigment proved to be resistant to hydrolysis but tallied with cyanidin 3 : 5-dimonoside in other respects.

Brunfelsia grandiflora, deep heliotrope, contains cyanidin diglycoside with a malvidin derivative.

Caladium bicolor. Crimson leaves, cyanidin 3-pentoseglycoside.

Calceolaria. The bluish-red flowers of the variety "Victoria Prize" contain cyanidin 3-pentoseglycoside.

Camassia esculenta. The violet-tinged blue flowers contain a delphinidin diglycoside.

Campanula muralis, violet-blue flowers, and *C. pusilla,* pure blue flowers, contain a delphinidin diglycoside; the former contains also a second anthocyanin, probably based on cyanidin.

Cattleya labiata Lindl. The magenta flowers give strongly co-pigmented solutions of cyanidin 3 : 5-dimonoside.

Ceanothus veitchianus. Lavender-blue flowers contain delphinidin diglycoside.

Centradenia grandiflora. The bluish-red leaves yield a co-pigmented solution of an anthocyanin having a high distribution number. After purification through the picrate, alkaline hydrolysis afforded malvidin 3 : 5-dimonoside. The pigment is therefore a malvidin complex diglycoside.

Centranthus ruber. The anthocyanin of the rose-pink flowers did not tally with known types in regard to distribution number and alkali-colour reactions; the anthocyanidin is cyanidin.

Cereus grandiflorus. The anthocyanin of the coral-red flowers closely resembles betanin in all its properties; it is perhaps a shade more blue-toned in acid solution.

Cheiranthus Cheiri. The bluish-magenta flowers of the variety "Ruby Gem" give a highly co-pigmented solution of cyanidin 3 : 5-dimonoside. A considerable quantity of this pigment was isolated in the crude condition some years ago from a red wallflower; this has been crystallised and identified as cyanin chloride by careful comparison with an authentic specimen. The Siberian wallflower (*Erysimum asperum*) produces anthocyanin-free flowers.

Chionodoxa Luciliae, blue flowers, contains a complex diglycoside of delphinidin.

Chrysanthemum. Brown-red flowered varieties like "In Memoriam" and "Enton Beauty" appear to contain a complex monoglycoside of cyanidin whilst the blue-red flowered varieties such as "Rose Chochod," "Rona," "Exmouth Pink" and "October Rose" contain a complex pentoseglycoside of cyanidin. In both cases the acyl group is readily eliminated, either by keeping the solutions in 1 % hydrochloric acid or by preserving the petals themselves. The purple leaves of the varieties "Mrs W. H. Webber," "Golden Seal" and "Source d'Or" contain a complex monoglycoside of cyanidin.

Clerodendron fallax. The reddish-orange flowers contain pelargonidin 3-bioside.

Colchicum autumnale. Malvidin 3-bioside.

Columnea Banksii. A hybrid with orange-scarlet flowers was found to contain pelargonidin 3-bioside.

Convolvulus minor, blue flowers, contains a delphinidin diglycoside and traces of methylated delphinidin derivatives.

Cordyline terminalis (Wartelli). The deep bluish-red leaves contain a complex 3 : 5-dimonoside of cyanidin. The distribution number is, however, quite low and the acyl group is only recognised by the fact that in presence of salt the pigment can be extracted from aqueous solutions by amyl alcohol. After hydrolysis by means of sodium hydroxide, good cyanin-type reactions were obtained and the distribution number remained low even when the solution was saturated with salt.

Cornus sanguinea. The blood-red twigs contain a cyanidin 3-saccharide. The tests for complex diglycosidic character gave results difficult to interpret.

Cosmea bipinnata. The magenta flowers gave a highly co-pigmented solution and the anthocyanin is mainly peonidin 3-bioside, but it is mixed with an anthocyanin based on cyanidin and probably also a delphinidin derivative such as malvidin.

Cotoneaster frigida. The red berries contain cyanidin 3-pentoseglycoside.

Cotoneaster Simmonde. Pelargonidin 3-pentoseglycoside and a cyanidin derivative in smaller relative amount.

Crambe maritima. The purple heads of sea-kale gave a highly co-pigmented solution of a cyanidin 3 : 5-dimonoside.

Crataegus. The red berries of *C. coccinea, macracantha, orientale,* contain cyanidin 3-monoglycoside. The flowers of *C. oxyacantha fl. pleno Rosea* also appear to contain a 3-monoglycoside of cyanidin (correction). In dilute solution the apparent diglycosidic character is due to the presence of impurities; after purification the anthocyanin is clearly monoglucosidic. However, the young red shoots were found to contain cyanidin 3-pentoseglycoside.

Crocus vernus. Violet-blue flowers are coloured by delphinidin diglycoside.

Cydonia Japonica. Pink flowers contain a 3-pentoseglycoside of cyanidin with a small relative proportion of a pelargonidin derivative. A variety with salmon-scarlet flowers was found to contain pelargonidin 3-pentoseglycoside.

Cytisus scoparia. The variety "Dorothy Walpole" with brownish blue-red flowers contains cyanidin 3-glycoside.

Dahlia. "Coltness Gem," orange-scarlet, contains pelargonidin 3 : 5-dimonoside and much co-pigment. "Lady Aileen" gave the same results. Pompon "Bacchus" (scarlet), "Glow" (salmon) and "George Ireland" (mauve) gave, respectively diglycosides (3 : 5) of pelargonidin, cyanidin and delphinidin.

Daphne cneorum. Co-pigmented cyanidin pentoseglycoside.

Daucus carota. Purplish tops of "Sutton's Favourite" contain cyanidin 3 : 5-dimonoside.

Delphinium nudicaule splendens. The scarlet flowers are coloured by pelargonidin 3-bioside.

Dianthus barbatus, crimson, was confirmed as cyanidin 3-glycoside (not pentoseglycoside). A salmon-pink was again found to be pelargonidin 3-glycoside, and search for a pelargonidin 3 : 5-dimonoside (found in the carnations) has failed up to the present. On the other hand a very blue-shade magenta (or purple) gave solutions of cyanidin 3 : 5-dimonoside.

Dianthus. "Spark," deep-crimson flowers, contains cyanidin 3-pentoseglycoside. The alpine *Dianthus,* "Bee's Ruby," with bluish-crimson flowers, contains a cyanidin 3-saccharide with distribution intermediate between monoglucosidic and pentoseglucosidic. Among varieties of *D. caryophyllus* we have found that the bluest shaded flowers contain a cyanin-like anthocyanin. Thus "Mrs Hamilton Fellowes" (deep bluish-rose), "Wivelsfield Claret Improved" (intense bluish-crimson and brownish-crimson) and "Homeric" (deep bluish-red) all contain cyanidin 3 : 5-dimonoside.

Dictamnus fraxinella caucasica. The reddish-violet flowers contain a delphinidin pentoseglycoside contaminated by a cyanidin derivative.

Dielytra spectabilis, rose-red, is coloured by cyanidin 3-bioside; a bluer red variety gave the same results but a greater proportion of co-pigment was present.

Dracaena terminalis. Bluish-red leaves of the variety "Lord Wolseley" contained a complex diglycoside based on a mixture of anthocyanidins in which malvidin appeared to predominate.

Erica gracilis. Purplish-red flowers contain a 3-glycoside of cyanidin.

Erinus alpinus. Delphinidin diglycoside.

Erysimum linifolium. Cyanidin 3-bioside with much anthoxanthin.

Fagus sylvatica. The apparent complex diglycoside character of the pigment of the leaves of the copper beech was due to the phenomenon mentioned in the Introduction. The purified anthocyanin is cyanidin 3-monoglycoside.

The substance was isolated in the following manner. An extract of the leaves (collected at the end of May in Oxford) in 1 % hydrochloric acid was saturated with salt and shaken with an equal volume of amyl alcohol. Each 1000 cc. of the amyl alcoholic solution was mixed and agitated with benzene (500 cc.) and 1 % hydrochloric acid (100 cc.) and the separated aqueous solutions were thoroughly extracted with ethyl acetate. The solutions were then saturated with salt and the pigment transferred to butyl alcohol and from this solution to the minimum of 1 %

hydrochloric acid after the addition of sufficient light petroleum (about one-third to one-half vol.). The aqueous solution was mixed with acetic acid (2 vols.) and precipitated with ether (giving a more concentrated solution), the process repeated (syrup) and again repeated (solid precipitate), the crude pigment being then collected by means of a centrifuge. This material was washed with ether and triturated with not too much saturated aqueous picric acid when, on keeping in the ice-chest, the orange-red picrate crystallised almost completely. The substance was collected centrifugally, washed, and converted into chloride by solution in methyl alcoholic hydrogen chloride and precipitation with ether. The salt crystallised at once when 5 % ethyl alcoholic hydrochloric acid was added to its concentrated solution in 0·5 % aqueous hydrochloric acid. It was recrystallised in the same way and obtained in reddish-brown prisms with a weak green glance. (Found in material dried at 110° in a high vacuum over phosphoric anhydride: C, 52·1; H, 4·4. $C_{21}H_{21}O_{11}Cl$ requires C, 52·0; H, 4·3 %.) The colour reactions in a range of buffered solutions of graded p_H [Robertson and Robinson, 1929] agreed for ten solutions exactly with those of chrysanthemin and idaein and in every respect the anthocyanin has the properties of a cyanidin 3-glycoside. Examination of the distribution number, however, indicated that it is substantially idaein rather than chrysanthemin. (Found: 15·5, 15·3 for 5·0 mg. in 25 cc. of *iso*amyl alcohol and 25 cc. of 0·5 % hydrochloric acid, the solvents being previously equilibrated.) At the concentration used, the distribution number of idaein chloride [Willstätter and Mallison, 1915] is about 14 and that of chrysanthemin chloride about 19. The beech pigment, therefore, appears to be idaein mixed with a small proportion of chrysanthemin. A similar mixture of glucoside and galactoside of delphinidin and malvidin was found by Karrer and Widmer [1927, 1] to occur in the bilberry. Finally the pigment was dissolved in 0·5 % hydrochloric acid and 5 % ethyl alcoholic hydrochloric acid added; one-half of this solution was dusted with chrysanthemin chloride and the other half with idaein chloride. The latter crystallised immediately but not the former. The pigment is therefore idaein.

Ficus carica. Cyanidin 3-monoglucoside (fruit).

Fragaria virginiana. The monoglucosidic character of the pelargonidin 3-glycoside of the strawberry was confirmed.

Fritillaria Meleagris. The dull reddish-purple flowers contain a cyanidin 3-bioside, the reactions of the original solution conforming to the standard type. For this reason it seemed worth while to institute a direct comparison with mecocyanin and a solution of this anthocyanin (1·95 mg.) was diluted with 1 % hydrochloric acid until it matched the *Fritillaria* extract (this contained 5·55 mg. in 100 cc.). The solutions were both washed twice with equal volumes of amyl alcohol and the distribution number was then found to be 4·8 in both cases.

Fuchsia triphylla. Cardinal-red flowers contain pelargonidin 3 : 5-dimonoside. The crimson under-surface of the leaves is coloured by malvidin 3 : 5-dimonoside.

Fulminaria augustina azurea. Blue flowers, tinged with pink, contain delphinidin diglycoside.

Gentiana sino-ornata. The rich blue flowers contain a delphinidin complex diglycoside. Karrer and Widmer [1927, 2] found that gentianin, the pigment of *G. acaulis*, is a *p*-hydroxycinnamoyl derivative of a delphinidin monoglucoside.

Geranium ibericum. The violet-blue flowers give very clean extracts (suitable for standards of colour reactions) of malvidin 3 : 5-dimonoside; *G. grandiflorum* gives the same results.

G. armenum, reddish violet, also contains the malvidin 3 : 5-dimonoside but an anthocyanidin with a positive ferric reaction is also present in small relative amount. Since the anthocyanidin recovered after the oxidation test is Fe –, the second pigment is probably not a cyanidin derivative but may be based on petunidin.

G. sanguinea, deep bluish-pink flowers, contains malvidin 3 : 5-dimonoside.

Gesnera fulgens. The reddish-orange flowers contain an anthocyanin which is difficult to extract but is slowly yielded to 1 % hydrochloric acid. The solution is orange-yellow and gives a red-crimson coloration on the addition of sodium carbonate. On hydrolysis the anthocyanidin is obtained in the usual way; it gives yellow acid solutions and its properties at once recalled those of apigeninidin chloride with which it is in fact identical. It was obtained by hydrolysing the anthocyanin in crude extracts of *G. fulgens* and *G. cardinalis* and passing this to butyl alcohol and 0·5 % hydrochloric acid twice. The colour was too orange-red as the result of contamination with a second anthocyanidin and the latter was removed by addition of a few drops of ferric chloride and shaking the acid solution with air for 2 hours. The anthocyanidin was transferred to amyl alcohol and then to 0·5 % hydrochloric acid (minimum) in the usual manner after addition of light petroleum. The filtered solution was then concentrated in a vacuum and the oxonium salt precipitated as mercurichloride (slender orange-yellow needles). The chloride was regenerated in very dilute solution and finally an aqueous solution containing hydrochloric acid was concentrated and deposited crystals of the anthocyanidin chloride. Solution was again brought about by the addition of alcohol and heating, and the filtered liquid was mixed with 2 drops of concentrated hydrochloric acid and kept in the ice-chest. Orange prisms separated. (Found: C, 58·1; H, 4·3. $C_{15}H_{11}O_4Cl, H_2O$ requires C, 58·3; H, 4·2 %.) The substance was carefully compared with an authentic specimen of apigeninidin chloride and no differences of any kind in the behaviour of the natural and synthetic material could be observed. The colours in alkaline solutions and in alcohol were indistinguishable.

The distributions using 0·5 % hydrochloric acid with mixtures of *cyclo*hexanol and toluene (1 : 2, 1 : 3, 1 : 4, 1 : 5) were identical and characteristic; also noted were distributions to ethyl ether and *iso*propyl ether in the presence of picric acid and to ethyl acetate. The anthocyanin itself tallied closely in distribution number and other properties with apigeninidin 5-monoglucoside which has been synthesised in collaboration with Dr A. R. Todd. A separate account of this work will, it is hoped, be submitted.

The variety "Orange King" also contains an apigeninidin monoglycoside but *G. zebrina discolor* (with yellow carotenoid) contains pelargonidin 3-pentoseglycoside.

G. isoloma hirsutum, orange-crimson and yellow, also contains the pelargonidin 3-pentoseglycoside.

The leaves and spine-like purple hairs of all species of *Gesnera* examined contain a diglycosidic anthocyanin based on an unidentified anthocyanidin. It is Fe + and probably not 3-substituted but it is not luteolinidin as was at first suspected. It may be the delphinidin of the flavone series and the synthesis of this substance will be undertaken.

Gladiolus. The purplish-red outer parts of the corms contain pigments which are not readily extracted by 1 % hydrochloric acid even on heating. When obtained in solution the pigment was found to be cyanidin 3-bioside but it may not exist in this form in the bulbs. Corms of "Jacob von Beeren" (purple flowers) and "War" (scarlet flowers) gave the same results, but the latter contained much anthoxanthin and the former very little.

Gloxinia. Carter's "Invincible Pride" and a bright cardinal-red flowered variety contain pelargonidin 3-rhamnoglycoside. The tests for rhamnose were carried out on the anthocyanin purified by the butyl alcohol process (thrice transferred).

Gynura aurantiaca. The bright purple hairs on the leaves contain a malvidin 3 : 5-dimonoside but the anthocyanin required drastic purification before the usual alkali-colour reactions could be observed.

Hedera helix. The purple stems and deep purplish-black berries contain a cyanidin 3-bioside; the same is true of the purple stems of a variety "Silver Irish."

Helianthemum vulgare. A pink-flowered variety, also "Rosy Gem" (bluish-red) and "Ben Venue" (pinkish-orange) were found to contain cyanidin 3-glycoside but the last named is coloured in part by a second anthocyanin tending to a bluer shade in the sodium acetate-amyl alcohol test of the anthocyanidin.

Helichrysum bracteatum. The brown-red to bluish-crimson flowers do not readily yield their anthocyanin and the petals were heated with 1 % hydrochloric acid. The pigment is a cyanidin monoglycoside but the usual colour reactions were too much obscured to allow of satisfactory allocation of sugar-position.

Heuchera sanguinea. The light red, slightly blue-tinged flowers contain a cyanidin complex 3-bioside. It yields an acylated anthocyanidin when hydrolysed for a short period.

Hibiscus rosa Silensis var. *Cooperii*. The bluish-red leaves contain cyanidin complex 3-saccharide and furnish an acylated anthocyanidin on careful hydrolysis.

Hieracium lima. The spotted leaves contain cyanidin 3-glycoside.

Hyacinthus. The purplish bulbs of the varieties "King of the Blues," "Lady Derby" and "Roi des Belges" contain an anthocyanin which is very difficult to extract by means of 1 % hydrochloric acid. Its distribution number approximates to that of an anthocyanidin. The anthocyanidin is cyanidin and after hydrolysis with sodium hydroxide the anthocyanin is cyanidin 3-pentoseglycoside. The alkali-colour reactions of the original are, however, too blue for exclusive 3-substitution and it should therefore be described as a complex cyanidin 3-pentoseglycoside and it probably bears an acyl group in position 5 or 7 of the phloroglucinol nucleus.

The pink flowers of "Lady Derby" contain a complex pelargonidin 3 : 5-dimonoside (salvianin-monardaein type of anthocyanin). "Pink Queen" and "Ella" (pink) gave the same results as "Lady Derby." If the petals are kept in the air for a week or if the solution in 1 % hydrochloric acid is kept for the same period, the acyl group is removed, the anthocyanin becomes much more readily soluble in 1 % hydrochloric acid and is no longer complex. The distribution and reactions then correspond to a normal pelargonidin 3 : 5-dimonoside. The flowers of "King of the Blues" (hot-house grown) contain a complex diglycoside of delphinidin which is relatively stable and the distribution number is unchanged when the solution in 1 % hydrochloric acid is preserved. On the other hand, the same variety grown in the open (at a later season) seems to produce a normal diglycoside with a very low distribution number.

Hydrangea hortensis. The varieties "Deutschland" and "Parzival" (pink) were found to contain a complex pentoseglycoside of delphinidin when examined in May. The flowers had probably been developed under hot-house conditions; later in the open garden the colour remained the same but the complex character had disappeared. Blue-flowered varieties "Mme A. Riverain" and "Vice-President Truffant" were coloured by delphinidin pentoseglycosides from the commencement. An intermediate shade *H. Rubis* had an intermediate distribution early in the season.

The purple leaves of "Vice-President Truffant" and the adjoining stems contain a cyanidin 3-bioside.

The purple colour developed in the leaves (var. Marechal Foch) by frost or starvation is a mixture of cyanidin 3-glycoside and cyanidin 3-bioside. These were separated and examined independently.

The purplish black stem of *H. cynoclada* gives a highly co-pigmented solution of cyanidin 3-bioside. This is possibly complex since unusually prolonged hydrolysis was necessary in order to obtain the anthocyanidin.

Crystalline acids occur free in hydrangea flowers. Early in the season we obtained one, M.P. 200°, from *H. rubis*; colourless flat needles (Fe –). Attempts to obtain more of this at a later period gave an acid (Fe –) crystallising from 1 % hydrochloric acid in colourless, hair-like needles, M.P. 179°. (Found: C, 60·6; H, 5·5 %.) This substance has not yet been identified.

Ilex Shepherdii (*I. aquifolium* var. *Hodginsii*). The red berries were extracted without bruising and contain a pelargonidin 3-pentoseglycoside and very much anthoxanthin.

Impatiens Holstii. The orange-scarlet flowers contain a pelargonidin complex 3 : 5-dimonoside, closely resembling monardaein. Another variety with deep bluish-rose flowers contains peonidin complex 3 : 5-dimonoside.

Impatiens biflora. The young red leaves contain cyanidin 3-pentoseglycoside.

Ixia orateroides. A reddish-violet variety is coloured by petunidin diglycoside, probably mixed with derivatives of malvidin and delphinidin.

Jacaranda ovalifolia. Delphinidin diglycoside.

Kochia tricophylla Childsii. This plant was examined when the autumnal colouring of the leaves had been produced by starvation. The foliage then contained a colouring matter of the betanin-class differing but little in its reactions from the beet pigment.

Lathyrus odoratus. Mr L. H. Stone of the John Innes Horticultural Institution has kindly submitted to us a number of sweet peas of interest from the point of view of genetics. We comment on the chemical aspect only.

"Colorado," orange, pelargonidin 3 : 5-dimonoside almost free from co-pigment; "Beatall," bluish-red, highly co-pigmented pelargonidin 3 : 5-dimonoside; "Charm," deep bluish-red, co-pigmented 3 : 5-dimonoside of pelargonidin; as in "Colorado" the distribution suggests admixture with a pentoseglucoside or other anthocyanin with a higher distribution number; "Ascot," bluish-pink, very strongly co-pigmented pelargonidin 3 : 5-dimonoside; "Flamingo," orange-scarlet, mixture of pelargonidin 3 : 5-dimonoside and pelargonidin 3-bioside; "Wonderful," slightly bluer scarlet than the last, the same mixture of anthocyanins; "Damask Rose," bright carmine, peonidin 3 : 5-dimonoside with co-pigment; "Honour," crimson, peonidin 3 : 5-dimonoside with less co-pigment and making the closest approach to a pure anthocyanin in the peonidin group; "Miss Hunt"—wings, magenta-blue, highly co-pigmented peonidin 3 : 5-dimonoside mixed with a smaller proportion of a diglycoside derived from a methylated delphinidin derivative and with one derived from cyanidin or petunidin; a mixture of malvidin and cyanidin derivatives or petunidin alone would fit the facts; "Miss Hunt"—standard, bluish-wine-red, contains the same anthocyanin as the wings but it is far less co-pigmented; "Pinkie," blue-pink, highly co-pigmented peonidin and malvidin 3 : 5-dimonosides; the flavone glycoside has powerful basic properties and gives an intense yellow colour with moderately concentrated hydrochloric acid; "Lord Nelson," deep violet-blue, petunidin diglycoside with a small proportion of malvidin derivative—possibly delphinidin in small amount but no cyanidin; "Purple," "Purple with modified wings" and "Purple Invincible" give similar results and contain, chiefly, petunidin diglycoside with rather more malvidin derivative than was found in "Lord Nelson"; "Copper,' coppery red-violet, also petunidin diglycoside with a little malvidin (less than the purples) and possibly some delphinidin; "Dobbie's Maroon" gives almost the same results as the purples but it is not strongly co-pigmented. *L. tingitanus*, deep violet, co-pigmented malvidin 3 : 5-dimonoside.

In connection with the secondary anthocyanins of the sweet peas it will be necessary to institute experiments on a somewhat larger scale in the more interesting cases; we are unable to detect a slight admixture of delphinidin in petunidin.

Ligustrum vulgare. The dark purple berries contain an anthocyanin which on hydrolysis in the usual manner gives a complex anthocyanidin, very sparingly soluble in dilute hydrochloric acid. This is hydrolysed by aqueous sodium hydroxide to an anthocyanidin tallying with delphinidin except that the colour in the amyl alcohol-sodium acetate test was much too red; also the anthocyanidin was not completely extracted by ether-picric acid. It is possible that the hydrolysis was accompanied by side reactions.

Reinvestigation (after some months, hence the possibility of changes in the solution) showed that the anthocyanin was separable by the amyl alcohol-salt process into monoglucosidic and diglucosidic fractions. The former was found to be based on petunidin, the latter on delphinidin. It was certainly unfortunate that Grafe [1906; 1909; 1911] was led to select the berries of *Ligustrum* as material for his attack of the problem of isolation of anthocyanins but he appears to have obtained crystalline pigments.

Lilium lancifolium rubrum. The white flowers with bluish-red streaks contain petunidin pentoseglycoside and the anthers contain cyanidin pentoseglycoside probably in a solid form as the solution in 1 % hydrochloric acid is produced at once. It is particularly clean and if these anthers were readily accessible in quantity they would form a ready source of (probably) keracyanin.

Crimson bulbs of *L. l. Melpomene* contain a mixture of cyanidin 3-bioside and cyanidin 3-pentoseglycoside. *L. tigrinum*, orange flowers, was found to contain much carotenoid and petunidin pentoseglycoside.

Lilium umbellatum erectum and *L. umb. splendidum*, orange-red and scarlet flowers respectively, contain cyanidin 3-pentoseglycoside.

Limonium sinuata rosea. The pink flowers contain a delphinidin pentoseglycoside. *L. Suworowie*, rose-pink, is coloured by malvidin 3-glycoside together with a small proportion of a petunidin or delphinidin derivative.

Lithospermum prostratum. The bright blue flowers appear to contain an almost homogeneous petunidin diglycoside.

Lobelia erinus. The variety "Crystal Palace," blue, is coloured by a delphinidin diglycoside.

Lonicera sempervirens. Cyanidin 3 : 5-dimonoside. The bright amethyst berries of *L. nitida* contain a cyanidin 3-bioside.

Lotus corniculatus (bird's foot trefoil). The young buds are yellow, tipped bright red and contain cyanidin 3-glycoside.

Lycesteria formosa. The brownish-red berries and calyx contain cyanidin 3 : 5-dimonoside.

Lychnis dioica (red campion). The flowers contain a mixture of cyanidin 3 : 5-dimonoside and delphinidin diglycosides and the stems and calyx contain a monoglycoside of delphinidin with preponderating cyanidin 3-glycoside.

Lychnis viscaria. The bluish-pink flowers contain a mixture of diglycosides of cyanidin and delphinidin; a "Tom Thumb" variety gave similar results.

Magnolia Sennei also *Alexandrina* and *Rustica rubra*, reddish-violet to bluish-pink flowers, appear to contain peonidin 3 : 5-dimonoside. The anthocyanin is diglycosidic and the anthocyanidin is peonidin but the colour reactions are rather obscured, even after purification, by the presence of anthoxanthins.

Meconopsis Baileyi (blue Himalayan poppy) is coloured by cyanidin 3 : 5-dimonoside.

Meconopsis cambrica fl. plena. The orange flowers contain pelargonidin 3-bioside.

Metrosideros speciosus (Australian bottle-brush tree). The crimson flowers contain cyanidin 3 : 5-dimonoside with an admixed delphinidin diglycoside.

Musa coccinea. Pelargonidin 3-glycoside.

Muscari (grape hyacinth). Delphinidin diglycoside.

Naegelia. "Walmsgate Beauty" has a dull blue-crimson leaf coloured by a cyanidin 3-bioside.

Nemesia strumosa. "Suttonii," pink and crimson hybrids, contain a cyanidin 3-bioside. "Blue Gem" contains delphinidin pentoseglycoside. The orange shades are largely due to carotenoid pigments.

Nicotiana. "Sutton's Scarlet" contains a delphinidin pentoseglycoside.

Nymphaea gloriosa, bluish-crimson, is coloured by a delphinidin glycoside, possibly complex. *N. stellata odorata*, blue, contains a complex diglycoside of delphinidin. *N. Zanzibariensis*, pinkish-mauve, contains a delphinidin glycoside.

Ononis spinosa. The pink flowers contain a delphinidin pentoseglycoside.

Orchis mascula, violet, is coloured by cyanidin 3 : 5-dimonoside.

Paeonia. The young red leaves contain a cyanidin 3-bioside but the difficulty of hydrolysis and the formation of an acylated cyanidin indicate the presence of some complex diglycoside.

Papaver nudicaule. It has been confirmed that the anthocyanin of the orange flowers is mainly pelargonidin 3-bioside and not pentoseglycoside. The oriental poppies also contain biosides, but pentoseglycosides occur in some of the Shirley varieties.

Pelargonium. We have directed our attention to the nature of the anthocyanin in the bluer red varieties and find that it is usually a malvidin derivative. The salmon-pink Zonal Pelargonium, "Kovaleski," contains practically pure pelargonidin 3 : 5-dimonoside (pelargonin). Flower petals of "Henry Jacoby" contain chiefly pelargonin with some malvidin 3 : 5-dimonoside, but no cyanidin derivatives. The "Versailles" (Regal) variety "Madame de Monier," is likewise coloured by a pelargonin-malvidin 3 : 5-dimonoside mixture and cyanidin is absent. Petals of the flowers of *P. Veitchianum*, reddish-violet, *P. Bertiana*, reddish-mauve and *P. Cucullatum*, reddish-mauve, contain chiefly malvidin 3 : 5-dimonoside.

On the other hand, the flower stalks and calyx of "Henry Jacoby" contain cyanidin 3 : 5-dimonoside and the leaves of "Paul Crampel" also contain a cyanidin diglycoside.

Pentstemon heterophyllus, pure blue occasionally tinged pink, gives a highly co-pigmented extract containing a delphinidin diglycoside. *P. platyphyllus*, lilac, and *P. Garrette*, bright blue, gave similar results.

A violet-flowered variety contained a mixture of monoglycosidic and diglycosidic delphinidin derivatives and these were separated and examined independently.

Petunia. "Rosy Morn," deep pink, is coloured by peonodin 3 : 5-dimonoside.

Phaseolus multiflorus vulgaris. The flowers of the Scarlet Runner bean are coloured by pelargonidin 3-bioside. The reddish-purple seeds were placed in hot water for a short time and the coloured exterior parts then removed and extracted by means of 1 % hydrochloric acid. The anthocyanin is apparently a complex cyanidin pentoseglycoside but as it gives a nearly pure blue colour with sodium carbonate, there is some peculiar feature in connection with the arrangement of the attached groups. The brownish-violet seeds of the "Canadian Wonder" bean contain pelargonidin 3-glycoside.

Phlox Drummondi. The examination of some hybrids was rendered difficult by the presence of co-pigments. Crimson flowers contain a petunidin glycoside. Bluish-crimson flowers also contain petunidin glycoside mixed with monoglucosidic derivatives of malvidin and delphinidin. Violet flowers, also magenta flowers, contain delphinidin diglycoside along with a malvidin (and possibly petunidin) derivative.

Plumbago carpentae. The bright blue flowers contain malvidin and delphinidin (or petunidin diglycosides and the reddish leaves contain cyanidin 3-glycoside or 3-pentoseglycoside.

Poinsettia euphorbia pulcherrima. The red leaves contain cyanidin 3-pentoseglycoside.

Primula. Malvidin 3 : 5-dimonoside has been found in *P. Burmanica,* bright violet-red, *P. vulgaris,* blue and lavender-blue, also in the variety *Wilsonii,* pale pink-heliotrope, in *P. Veitchii,* deep bluish-red, in *P. Juliana,* deep reddish-plum coloured, mixed with hirsutidin 3 : 5-dimonoside. The latter anthocyanin (very probably hirsutin) occurs in *P. denticulata,* mauve, and also in *P. frondosa* and *P. rosea.* "Mrs Neale" gives ambiguous reactions but is probably coloured by a malvin-hirsutin mixture.

P. Japonica (Miller's crimson) seems to contain a malvidin pentoseglycoside. *P.* "Aileen Aroon," brownish-red, is coloured by a mixture of malvidin diglycoside and pentoseglycoside.

Very interesting cases are those of *P. Forrestii,* violet, and *P. Waltoni,* deep brownish-violet, for these are coloured by malvidin 3 : 5-dimonosides in which, from the distribution phenomena, one of the sugars, or even both of them, should be a pentose or methylpentose. This would constitute a novel type since the pentoseglycosides have only been observed in the 3-substituted anthocyanidins.

Among the primulae coloured by hirsutidin 3 : 5-dimonoside is *crispata* which has blue-violet flowers, more blue-toned than hirsutin itself can possibly be at any p_H. This is an example of the influence of a co-pigment of the flavone glucoside type and the phenomenon can be reproduced *in vitro* with synthetic materials.

P. Cockburniana, orange, contains cyanidin 3 : 5-dimonoside; some brownish-red flowered Lisadell Hybrids were also found to contain mixed cyanin- and malvin-like anthocyanins. An orange-red *P. Bulleyana* contained cyanidin 3-bioside and it thus appears that the primulae contain a wide range of anthocyanins. They may also be almost free from these pigments as in the case of *P. Bulleyana,* yellow flowers, which contains only a trace of a malvin-type anthocyanin situated at the base of the corolla.

Prunus communis. Skins of the large blue Californian plum contain cyanidin 3-pentoseglycoside; those of the red South African plum and the light red Gaviota plum contain cyanidin 3-glycoside. This contrast between red and blue-coloured plums appears to hold generally.

P. persica appears to contain cyanidin 3-glycoside in the skins.

P. insititia (damson) has cyanidin 3-pentoseglucoside in the skins of the fruit. The Japanese cherry "Shidaro Sakura" has pink flowers coloured by a cyanidin 3-bioside.

Punica granatum. The pigment in the pomegranate (Californian origin) seems to be delphinidin diglycoside. All the tests indicated delphinidin but the colour of the anthocyanidin with amyl alcohol and sodium acetate was too red-toned. Nevertheless it must be delphinidin or a new anthocyanidin and most probably the former.

Pyracantha coccinea Lalandei. Cyanidin 3-pentoseglycoside.

Pyrus acerba floribunda. This bright red crab-apple contains cyanidin 3-glycoside. *P. acerba Aldenhamensis,* pink flowers, also contains cyanidin 3-glycoside.

Ramondia pyrenaica. The violet-blue flowers contain much anthoxanthin and a delphinidin diglycoside.

Rhododendron. Hardy species derived from *R. ponticum*: cyanidin 3-glycoside is found in most rose-red varieties.

R. yannense, bluish-red flowers, co-pigmented cyanidin 3-glycoside. "G. A. Simms," Doncaster, Ascot Brilliant and *R. Thomsoni,* crimson flowered, also contain cyanidin 3-monoside. "Charles Dickens" with flowers of a very blue-toned red shade, contains cyanidin 3 : 5-dimonoside and intermediate shades between *R. Thomsoni* and this are found to contain mixtures of the cyanidin mono- and diglycosides. Such are "William Austin," *R. arboreum coccineum* and "Mrs Thistleton Dyer." The anthocyanins are readily separable by the salt-amyl alcohol method and the bluer the shade of the red flowers the higher the proportion of cyanin-type anthocyanin was found to be. Thus the rhododendrons afford a further example of the correlation between the colour of flowers and the nature of the sugar side-chains. In any particular series of anthocyanins (all derived from the same anthocyanidin) it is generally true that the monoglycosides, pentoseglycosides and diglycosides make a series of increasing blueness of tone of red flowers.

In the case of the rhododendrons, however, the presence or absence of co-pigments is an additional factor having a large effect and in several cases it was found that a bluer red rhododendron contained less diglycoside than one not so blue-red because the bluer red flower also contained a higher relative amount of anthoxanthin, probably flavone glycoside in character.

In the Azalea section, *R. indicum*, "Mme van der Cruyssen," bluish-red flowers, contains cyanidin 3 : 5-dimonoside. The variety "Hindu Girl" and a similar unnamed variety were found to contain a mixture of cyanidin 3-glycoside and cyanidin 3 : 5-diglycoside. In each case the pigments were separated and examined independently. The occurrence of pure cyanidin 3-glycoside in salmon-red azaleas has been confirmed (not pentoseglycoside).

Violet and purple rhododendrons contain methylated delphinidin derivatives. *R. splendens*, purple, is coloured by petunidin diglycoside (trace of pentoseglycoside, possibly) containing a small relative amount of malvidin diglycoside.

R. Augustini (*orestrephis* is similar), blue-violet, contains a mixture of cyanidin or petunidin and malvidin 3 : 5-dimonosides, the latter predominating. *R. Californicum*, reddish-violet, is coloured by malvidin 3 : 5-dimonoside with a trace of a cyanidin or petunidin derivative. "Purple Splendour" was found to contain chiefly petunidin diglycoside and "Royal Purple" contains diglycosidic anthocyanins derived from malvidin (chiefly) and petunidin or delphinidin.

Rhodora canadensis (syn. *Rhododendron Rhodora*) contains a delphinidin glycoside in the heliotrope flowers and cyanidin 3-pentoseglycoside in the leaves (autumn).

Rhoeo discolor. The purple leaves contain cyanidin 3-bioside.

Ribes sanguineum. The rose-pink flowers of the flowering currant contain two anthocyanins which are readily separable by means of amyl alcohol (without salt). The fraction more soluble in amyl alcohol has almost the distribution number of an anthocyanidin but it is not extracted by ethereal picric acid from aqueous solutions. The anthocyanidin is cyanidin and the anthocyanin is evidently of a novel type. It gives a pure blue solution in aqueous sodium carbonate and on hydrolysis by hydrochloric acid evidence of step-wise removal of more than one group was obtained. Hydrolysis by means of alkalis appeared to give anthocyanidin and some normal monoglycoside. Further investigation on a larger scale is necessary. The more soluble anthocyanin is cyanidin 3-pentoseglycoside.

R. grossularia. The flowers of the gooseberry contain cyanidin 3-pentoseglycoside.

Rosa. The young red leaves of roses contain cyanidin 3 : 5-dimonoside and the haws contain no anthocyanin.

Rosmarinus officinalis. The flowers contain a complex diglycoside of delphinidin.

Rubus fruticosus. The stems and leaves of the blackberry, gathered in winter, contain cyanidin 3-saccharide.

Saponaria vaccaria. The pink flowers contain co-pigment and a cyanidin 3-bioside.

Saxifraga decipiens bathoniensis. The red flowers contain two anthocyanins of which the more soluble in amyl alcohol appears to be a cyanidin complex saccharide. The complex anthocyanin can even be removed by extraction of an aqueous acid solution with *cyclo*hexanone. The anthocyanin, more soluble in dilute hydrochloric acid, is a cyanidin 3-bioside (not tested for pentoseglycoside) which is unusually unstable in alkaline solution. The saxifrage "Sir Douglas Haig" contains a complex anthocyanin which gives a complex anthocyanidin on partial hydrolysis. It is a 3-acylated-cyanidin saccharide (the nature of the sugar groups cannot be estimated in such cases) and this clear case may perhaps give a clue to that of the flowers of the currant (Ord. Saxifragaceae).

Saxifraga cordifolia purpurea (*Megasea cordifolia*). Strongly co-pigmented cyanidin 3 : 5-dimonoside.

Scilla nonscripta. The blue-bell pigment is a delphinidin diglycoside, and a pink-flowered variety contained cyanidin 3-bioside. The flowers of *S. nutans* also contained delphinidin diglycoside and the bulbs are coloured by a complex diglycoside of cyanidin.

Scutellaria violaceae. The violet-red flowers give a highly co-pigmented extract and the anthocyanin is a complex diglycoside of delphinidin.

Sedum spectabilis. Malvidin 3-pentoseglycoside.

Sempervivum arachnoideum. The pink flowers contain malvidin pentoseglycoside mixed with a derivative of another anthocyanidin (Fe +).

Senecio. The cineraria "Rainbow," bright crimson, contains a cyanidin diglycoside which has unusual colour reactions. The violet coloration with sodium carbonate does not give a clear blue on the addition of sodium hydroxide. This suggests substitution in position 3′ and it is therefore of interest that a ferric reaction could not be obtained with the anthocyanin itself. The anthocyanin may be cyanidin 3 : 3′-dimonoside but this is only a provisional hypothesis and further investigation is necessary. The anthocyanin is definitely diglucosidic and it is unlikely that it can be a complex diglycoside.

Solanum Melongena. The purplish skin of the fruit (aubergine) contains delphinidin pentoseglycoside.

Solanum tuberosum. "Sutton's purple Edgecote potato" contains malvidin 3 : 5-dimonoside with a little of a cyanidin derivative. The Congo potato (coloured through the tuber) contains a complex malvidin 3 : 5-dimonoside, the distribution number of which is similar to that of a monoglucoside. On keeping the solution in 1 % hydrochloric acid for 3 months, the acyl group was eliminated and the anthocyanin then gave the reactions and distribution properties of malvidin 3 : 5-dimonoside. The variety "Mr Bresce," pink, and "Cardinal," bluish-pink contained pelargonidin 3-pentoseglycoside and 3 : 5-dimonoside, respectively.

S. pseudocapsicum has berries devoid of anthocyanin and this is also true of *S. dulcamara* but in this case (Bitter Sweet) the stems adjoining the berries contain a delphinidin derivative.

Sophronitis grandiflora. The flowers of this orchid are coloured by pelargonidin 3-bioside.

Spathodea nilotica and *Sterculia lanceolata.* Pelargonidin 3-saccharide with a distribution intermediate between monoglucosidic and rhamnoglucosidic.

Streptocarpus Wendlandii, violet, contains malvidin 3 : 5-dimonoside. The hybrids "Brightness," red, and "Vivid," deep scarlet, contain pelargonidin 3-rhamnoglycoside. Sutton's "Giant" bluish-pink, contains peonidin 3 : 5-dimonoside and a magenta-flowered variety and also Sutton's "Giant," blue, contain malvidin 3 : 5-dimonoside.

Syringa. "Madame Lemoire," light heliotrope, is coloured by delphinidin pentoseglycoside in association with co-pigment. "Congo," reddish-purple, contains the same anthocyanin but relatively to the latter a smaller proportion of co-pigment. Other unnamed species have been examined and the anthocyanin was always found to be the same; the colour variation is due to changes in the concentration of the anthocyanin and of the co-pigments.

Thunbergia. The orange flowers of a hybrid were found to be devoid of anthocyanin but the dark purple corolla tube contained cyanidin 3-glycoside.

Thymus coccineus, bright reddish-violet flowers, contains cyanidin 3 : 5-dimonoside.

Tradescantia Zebrina pendula. The bright purple leaves contain cyanidin 3-bioside along with a delphinidin derivative. *T. virginica,* bluish-violet hybrid, is coloured by petunidin diglycoside.

Tropaeolum Majus. The diglucosidic character of the anthocyanin in the common orange climbing nasturtium has been confirmed and it is from this source that we hope to isolate pelargonidin 3-bioside in substance. *T. M. nana,* "Empress of India," orange-scarlet, has pelargonidin 3-bioside in the petals, cyanidin 3-bioside in the sepals and delphinidin diglycoside in the leaves. The variety "King Theodore," deep brownish-red flowers, contains delphinidin diglycoside in both petals and sepals. The occurrence of delphinidin in these flowers and leaves was not anticipated.

Verbascum Phoeniceum. The dark, dull purple flowers contain peonidin 3-glycoside. This anthocyanin is therefore of the type of oxycoccicyanin [Grove and Robinson, 1931] and may be identical with it.

Veronica Teucrium rupestris, deep blue flowers, contains a delphinidin diglycoside; *V. chamaedrys* contains a delphinidin glycoside.

Viburnum Tinus Laurustinus. The small hard blue berries were extracted by means of hot 0·5 % hydrochloric acid. The anthocyanin is cyanidin 3-glycoside.

Viola odorata. The single violet "Princess of Wales" contains a complex saccharide of delphinidin. A viola known to be a cross between *V. Bosniaca* and *V. cornuta* and possessing flowers having the bluish-pink colour of the former, was found to contain cyanidin 3 : 5-dimonoside. The brownish-red flowered viola "Arkwright Ruby" contains cyanidin 3-pentoseglycoside in the greater part of the petals and in the dark central portions this is mixed with some cyanidin 3 : 5-dimonoside. The colour is highly co-pigmented. Violanin [Willstätter and Weil, 1916] which is delphinidin rhamnoglucoside has been found to be the characteristic constituent of dark purple violas; the pure blue flowered kinds contain delphinidin diglycoside.

Vitis vinifera. The skins of a red South African grape, "Hanefoot," contain cyanidin 3-glycoside. "Colmar" grapes have a colour intermediate between this and the bluest grapes but the anthocyanin is oenin.

The leaves of *V. discolor* contain a malvidin diglycoside.

Wistaria chinensis. The flowers are coloured by diglycosides of methyldelphinidins, probably largely petunidin.

Zinnia. Bluish flame-coloured flowers contained pelargonidin 3 : 5-dimonoside and a variety with orange flowers gave evidence pointing to the same pigment, mixed with a small proportion of a peonidin or malvidin derivative.

Co-pigments.

Throughout our work we have again observed that the acid extracts of flowers are almost always more blue-toned than are the solutions of the pure anthocyanins and the phenomenon of co-pigmentation is most in evidence when the presence of anthoxanthins can be detected in other ways. The common co-pigments are the tannins and the flavone and flavonol glycosides and we have been able to reproduce most of the effects with these. In general, pelargonidin glycosides, especially 3-biosides, are not greatly co-pigmented, but there are some noteworthy exceptions to this statement (phlox, for example). A solution of tannic acid is a useful auxiliary for the detection of malvidin derivatives because the colour change is much more striking than with peonidin derivatives.

It was stated in Part I that rutin has a feeble co-pigment effect, but it was suggested that this might be due to insolubility. Actually rutin and quercitrin are powerful co-pigments.

It is of interest that papaverine and quinaldine exert a strong blueing and intensifying influence on the colour of oenin, chrysanthemin and malvin, whereas laudanosine and quinoline have very little action.

There is evidence that co-pigmented anthocyanins *in vivo* as well as *in vitro* are stabilised, for example, against the decomposing action of light and experiments on this phenomenon are in progress. It is perhaps on this account that the anthocyanins collected in winter, having, as it were, proved their power to survive, are often very difficult to investigate by reason of the large admixture with other substances. We have also reason to believe that complex anthocyanins tend to be formed when the pigment remains *in situ* for long periods.

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