CCXXVIII. A SURVEY OF ANTHOCYANINS. IV.

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THE scope and methods of this investigation have been sufficiently explained in earlier Parts of the series [1931; 1932; 1933] and only a few comments are needed here to supplement the results summarised in the sequel.

The progress of parallel synthetic investigations has now enabled the delphinidin and petunidin glucosides to be characterised; the result goes to show that the majority of delphinidin diglycosides may with confidence be classed as delphinidin 3 : 5-dimonosides.

A problem arises in connection with the so-called complex anthocyanidins. These substances are often obtained by hydrolysis of anthocyanins in crude plant extracts, and they are undoubtedly acylanthocyanidins because they yield the anthocyanidins by hydrolysis with alkalis followed by reconstitution with acids. A characteristic example is derived from the dahlia "George Ireland." Its behaviour to our "delphinidin reagent" is that of delphinidin itself, but unlike this anthocyanidin it is largely taken up in the "cyanidin reagent." As the "oxidation test" also indicated delphinidin we mistook this substance for that anthocyanidin in our first examination of it. The difficulty is that the anthocyanin of the dahlia is cyanin, and it is hard to find a position for the acyl group which will fit in with the facts. Possibly the acyl group is introduced in the course of the hydrolysis itself by a migration from sugar hydroxyl to phenolic hydroxyl.

In diagnosing petunidin there are two dubieties: in the first place, admixture of cyanidin or delphinidin is difficult to detect, and in the second place, petunidin is hard to distinguish from a mixture of delphinidin and cyanidin. As far as possible these doubts have been resolved by fractionation of the pigment, but it is not always feasible to do this and "petunidin" should be taken to imply the possibility of delphinidin-cyanidin mixture, especially when it is not the only anthocyanidin present. We have noted a number of cases in which petunidin is present without admixture and no doubt arises in those examples.

We would point out that in Part I no distinction was made between pentose glycosides and monosides; as far as possible the doubtful cases are being reviewed. Pentose-glycoside is used as a generic term to include both aldopentose glucosides (for example, primeverosides) and rhamnoglucosides.

The effect of co-pigments on the distribution numbers of anthocyanins may be very large (compare *Primula sinensis*) and we have included a brief account of some experiments made in collaboration with Mr E. A. H. Roberts, showing a connection between the colour-change and effect on distribution numbers brought about by the addition of papaverine hydrochloride to solutions of chrysanthemin chloride and oenin chloride. Narcotine is a very weak co-pigment for these anthocyanins and correspondingly its effect on the distribution number is slight.

We imagine that the co-pigments combine with the anthocyanins in solution and in so doing break up the associated complexes. Natural co-pigments, more readily soluble in water than in amyl alcohol, behave similarly and greatly reduce the distribution number.

SUMMARY OF RESULTS.

Abrus precatorius. The scarlet seed-coatings contain an interesting anthocyanin, the investigation of which is hindered by the presence of a large proportion of other substances soluble in amyl alcohol and in water. The coatings were separated and extracted with 1 % methyl alcoholic hydrogen chloride, and the anthocyanin was precipitated from the solution by means of ether. This process was twice repeated using ethyl alcoholic hydrogen chloride and the pigment could then be analysed. It contains two anthocyanins, one of them diglycosidic and the other most probably complex (that is, acylated) diglycoside. According to our tests the anthocyanidin is delphinidin but this is always mixed with another aglucone, possibly cyanidin, but more probably acylated delphinidin. The anthocyanin colour-reactions (violet in sodium carbonate and unusual stability towards sodium hydroxide) do not tally with those of known delphinidin derivatives. It is undoubtedly worthy of study in substance and a preliminary investigation has been made by N. Ghatak (*Bull. Acad. Sci.*, U. P. Allahabad) who regards the anthocyanin (found: C, 50.1; H, 5.4 %) as monoglycosidic and cites the distribution number, 11.1, 10.4. From the description it is evident that the pigment was not isolated in a pure condition.

Abutilon insignia. Brownish crimson flowers, richly pigmented; cyanidin 3-pentoseglycoside.

Agaricus. The pink parts of young mushrooms contain leuco-compounds yielding a water-soluble pigment recalling betanin in its colour reactions.

Allium giganteum. Violet flowers; cyanidin 3:5-dimonoside.

Ampelopsis hederacea. The outer parts of the stems contain leuco-anthocyanin → cyanidin. The blue-black berries contain a complex anthocyanin based on cyanidin, together with a little malvidin.

Anemone fulgens. Bright bluish red flowers; petals, pelargonidin 3-glucoside (distribution number of purified anthocyanin, 33, agreeing with callistephin); anthers complex delphinidin diglycoside.

Aquilegia hybrida. The purple leaves contain cyanidin 3:5-dimonoside. *A. alpina*, blue-violet flowers; delphinidin 3:5-diglycoside. *A. canadensis*, brownish pink flowers; pelargonidin 3-monoside. *A. sibirica*, violet flowers; complex delphinidin diglycoside.

Asparagus. The purplish tips contain a cyanidin diglycoside.

Aubrietia deltoidea. A series of flowers ranging from blue-violet to red-violet ("Church Knowle," "Dr Mules," "Aubrey Prichard," "Excelsa," "Fire King," "Gloriosa") all contained cyanidin 3:5-dimonoside. The colour variations must be due to co-pigments as the solution from "Dr Mules" becomes blue and that from, say, "Gloriosa," becomes violet on the addition of sodium acetate (compare Part I).

Beeswax. The presence of leuco-anthocyanin → cyanidin was detected.

Begonia. "Prince Eugene," bluish pink flowers; cyanidin 3-bioside.

Bignonia speciosa. Violet flowers; based on cyanidin and probably complex diglycoside.

Boronia elatior. Rosy carmine flowers; malvidin 3:5-diglycoside with a trace of Fe⁺ anthocyanin.

Bougainvillea. A variety with scarlet bracts collected in Madeira was found to contain a betanin-like anthocyanin. This gives solutions that are much less blue-red than those from other *Bougainvillea* or from *Beta* and affords an indication of the existence of more than one betanin type.

Browallia demissa. Blue flowers; complex diglycoside of delphinidin, highly co-pigmented.

Brunfelsia calycina var. *macrantha*. Violet flowers; mixture of malvidin 3:5-dimonoside and complex dimonoside.

Buddleia colvilei (Himalaya). Rosy magenta flowers; cyanidin pentoseglycoside or complex glycoside.

Burchellia capensis. Orange flowers; cyanidin 3-pentoseglycoside.

Butea frondosa. The gum contained leuco-anthocyanin → cyanidin.

Cactus. Heliocereus speciosus. Crimson; betanin-type anthocyanin.

Calceolaria. "Albert Kent" hybrid, brown centres; cyanidin 3-bioside; brownish violet flowers, petunidin 3-pentoseglycoside was indicated by the tests. The anthocyanin was proved to be not complex.

Calendula tagetes. Reddish brown petals; cyanidin 3-pentoseglycoside.

Callistemon citrinus var. *splendens.* Crimson stamens; co-pigmented cyanidin 3 : 5-dimonoside.

Camellia japonica. Bright bluish red flowers; very clean cyanidin 3-monoside. The solution gives the normal reactions without any treatment.

Canna indica. The scarlet flowers and the leaves contain cyanidin 3-pentoseglycoside and much anthoxanthin, especially in the leaves.

Catechu (pale). A little leuco-anthocyanin $\rightarrow$ cyanidin.

Cattleja Skinneri. Reddish violet flowers; co-pigmented cyanidin diglycoside, apparently Fe^- in the anthocyanin condition (compare *Senecio*).

Celosia cristata. Bluish red flowers contain a betanin-type pigment (also noted by R. Willstätter, private communication); yellow flowers contain a water-soluble, yellow pigment not extracted by ethyl acetate but soluble to some extent in amyl alcohol; scarlet flowers contain a mixture of these colouring matters.

Centaurea montana. Blue flowers with purple centre; a mixture of cyanidin 3 : 5-dimonoside [Willstätter and Everest, 1913] and complex diglycoside of cyanidin.

Cercis siliquastrum. Pink petals; malvidin 3 : 5-dimonoside; the sepals contain a complex anthocyanin based on malvidin.

Cestrum purpureum elegans. Brownish red flowers; mixture of pelargonidin and cyanidin 3-pentoseglycosides.

C. Newellii. Rosy red flowers; the same mixture.

Cinchona rubraceae. The powdered bark contains leuco-anthocyanin $\rightarrow$ cyanidin.

Cinnamomum. The drug contains leuco-anthocyanin $\rightarrow$ cyanidin.

Clematis Durandii. The most blue-flowered variety examined; delphinidin diglycoside. *C. Kernesina*, the reddest kind tested; cyanidin diglycoside of unusual type. In both cases a part of the anthocyanin was difficult to hydrolyse. *C. Hendersonii* and *C. Viticella*, reddish violet and bluish violet flowers respectively; delphinidin 3-bioside and some cyanidin derivative in the former. The bluer kinds had more anthoxanthin co-pigment. The colour reactions of the anthocyanins in aqueous alkalis are violet and this in conjunction with relative stability to sodium hydroxide indicates the 3-substituted delphinidin type.

Clanthus Dampieri. Crimson flowers; chiefly pelargonidin 3-monoside with cyanidin 3-monoside as secondary anthocyanin.

Clivia nobilis. Pinkish orange flowers; pelargonidin 3-pentoseglycoside. *C. miniata*, orange-scarlet flowers; pelargonidin 3-saccharide, probably 3-bioside.

Cocos nucifera. The mesocarp contains leuco-anthocyanin $\rightarrow$ cyanidin.

Coleus shirensi. Violet flowers; complex delphinidin 3 : 5-dimonoside.

Colombia thyracanthus rutilans. Dull vermilion flowers, pelargonidin 3-rhamnoglycoside (the distribution tallied with that of the 3-rhamnoglycoside of the scarlet gloxinia).

Crambe maritima. The purplish tips contain a diglycoside anthocyanin very difficult to hydrolyse by hydrochloric acid in the usual way. After boiling with aqueous sodium hydroxide it was readily hydrolysed by hydrochloric acid, yielding cyanidin.

Crassula coccinea. Bluish red flowers, co-pigmented cyanidin 3-monoside.

Cyclamen. Karrer and Widmer [1926] found a malvidin 3-glucoside in *C. persicum* Mill. Other pigments are, however, present in some of the varieties. A mauve-red-flowered kind contained pure malvidin 3-monoside and very little anthoxanthin. Sutton's "Giant Crimson" is also of this type. Sutton's "Firefly," scarlet flowers, contained peonidin 3-pentoseglycoside. "Giant Salmon Scarlet" and "Silver-leaf Salmon" are of the same type. The acid and alkaline solutions are a little bluer than those of peonidin 3-glucoside (synthetic), but this is due to co-pigment and if tannin were added to the synthetic anthocyanin solution, a good match resulted. "Giant Mauve" contains malvidin 3 : 5-dimonoside.

"Hydrangea Pink" contains much anthoxanthin, and the results in regard to the anthocyanin were not clear; the anthocyanidin is malvidin. "Vulcan," deep red flowers; malvidin 3-monoside

which was directly compared, after purification, with oenin and found to have an identical distribution number and other properties.

Dahlia. The variety "George Ireland," mauve flowers, was stated in Part II [1932] to contain a delphinidin diglycoside. On hydrolysis the anthocyanidin has indeed some of the properties of delphinidin, but it is really a complex cyanidin. Cyanidin, showing the correct distribution and other properties, can be obtained only after a preliminary hydrolysis with sodium hydroxide.

Delphinium. "Los Angeles," pink flowers; pelargonidin 3-bioside of the type unstable to sodium hydroxide.

Dipterocarpus Zeylanicus. The bark contains leuco-anthocyanin $\rightarrow$ delphinidin and less cyanidin.

Echinops bannaticus. Lavender-blue flowers; delphinidin 3:5-dimonoside possibly accompanied by a cyanidin derivative.

Epacris miniata superba. Bluish red flowers; cyanidin 3-monoside.

Erica curviflora var. *Burchellii*. Cyanidin 3-pentoseglycoside.

Eriobotrya japonica. The testa contains leuco-anthocyanin $\rightarrow$ cyanidin.

Euonymus japonicus. The bluish red fleshy aril contains much anthoxanthin and a cyanidin derivative. The colourless testa becomes red in light and air and contains leuco-anthocyanin $\rightarrow$ cyanidin.

Fagus sylvatica. The green leaves that have been protected from light contain leuco-anthocyanin $\rightarrow$ cyanidin.

Gentiana campestris. Reddish violet flowers and stems; delphinidin 3-pentoseglycoside.

Geranium phaeum. Maroon flowers; the anthocyanin has a pentoseglycosidic type of distribution, and it is based on delphinidin and its methyl ethers. The colour reactions suggest a 3-saccharide.

Gladiolus Colvillei. "Spitfire," salmon flowers tinged blue-red; pelargonidin 3-pentoseglycoside.

Gloxinia speciosa. "Prince Albert," violet flowers; cyanidin 3-pentoseglycoside with some Fe^- anthocyanin. "Roi des Rouges" and "Kaiser Friedrich," crimson, contain pelargonidin 3-pentoseglycoside. The latter anthocyanin is rather stable towards aqueous sodium hydroxide.

Grevillea punicea. Vermilion flowers; cyanidin 3-pentoseglycoside.

Haemanthus Katherinae. Salmon-pink flowers; pelargonidin 3-bioside having an abnormally low distribution. As with the anthocyanin of *Clivia miniata*, the action of sodium hydroxide gives relatively slow decoloration to a greenish shade. That of *Papaver orientalis*, also pelargonidin 3-bioside, is immediately changed to yellow. The pelargonidin 3-bioside of the orange-scarlet *nasturtium* is much more stable. It is evident that there is some unexplained cause of this striking difference in behaviour.

Helipterum Manglesi. Bright pink flowers; cyanidin 3:5-dimonoside.

Helleborus. Rose-pink flowers contained leuco-anthocyanin $\rightarrow$ cyanidin and some colouring matter not soluble in dilute hydrochloric acid.

Hydrangea hortensis. The ordinary red-flowered varieties such as "Parzival" appear to contain complex delphinidin diglycoside early in the season; later on this complex character is not observed. Some faded red flowers were extracted with 1 % hydrochloric acid and the pale-coloured solution discarded; on heating the residue with more 1 % hydrochloric acid, a considerable amount of leuco-anthocyanin $\rightarrow$ complex cyanidin was obtained. The pigment of blue flowers under similar conditions was much more stable.

Impatiens minimum. Garden hybrid with pinkish violet flowers; complex malvidin 3:5-dimonoside. Rosy scarlet flowers; complex peonidin 3:5-dimonoside.

Ipomaea Horsfalliae var. *Briggsii*. Bluish red flowers; pelargonidin 3:5-dimonoside. *I. tricolor*, sky-blue flowers which become rosy mauve in a few hours and are then acid to litmus; malvidin 3:5-dimonoside.

Iris miranda. Bluish violet flowers; complex delphinidin diglycoside. *I. Kharput*, violet; also complex delphinidin diglycoside, but not identical with *I. miranda* unless there is a very large co-pigment effect. The distribution number of *I. Kharput* is the higher, and with sodium acetate it gives a violet coloration, *I. miranda* a blue coloration. "Rose Unique," reddish mauve flowers; complex petunidin diglycoside; the colour reactions closely resemble those of the *Iris Kharput* pigment.

Ixia crateroides. Red flowers; cyanidin 3:5-dimonoside with much anthoxanthin.

Kalanchoe flammea. Orange-scarlet flowers; carotenoid along with cyanidin 3:5-dimonoside. *K. globulifera coccinea*, scarlet flowers; cyanidin 3-saccharide, very much anthoxanthin obscured distribution ratio observations.

Krammeria. The tincture contains leuco-anthocyanin $\rightarrow$ pelargonidin or, less probably, peonidin.

Lathyrus odoratus. The study of the genetics of the sweet-peas from the point of view of the pigments of the flowers has been undertaken in collaboration with Miss R. Scott-Moncrieff and her colleagues of the John Innes Horticultural Institution. It is not necessary, therefore, to give the results in detail at this stage but a broad summary is desirable. The most orange-red of the sweet-peas contain pelargonidin 3-monoside, always mixed with more or less pelargonidin 3:5-dimonoside. Even the variety "2LO" is now of this type, many specimens having been examined. Next in the series we find varieties with pelargonidin 3:5-dimonoside, a good example being "Colorado." This pigment may be associated with much anthoxanthin as in "Ascot." Pelargonidin 3:5-dimonoside is also associated with complex pelargonidin 3:5-dimonoside, an example being "Firefly." The red sweet-peas contain peonidin 3:5-dimonoside as chief anthocyanin accompanied by more or less cyanidin 3:5-dimonoside. For example, "Honour" is nearly free from cyanidin derivatives, "Captain Blood" has more and "Miss Hunt" a good proportion. One variety which has been examined contains nearly pure cyanidin 3:5-dimonoside. In diagnosing cyanidin 3-bioside in "Captain Blood" and pelargonidin 3-bioside in "2LO" we were probably misled by mixtures simulating pure products.

The violets, maroons and blues include pure malvidin 3:5-dimonoside and delphinidin 3:5-dimonosides with certainty. The cases diagnosed as petunidin 3:5-dimonoside are in all probability correctly so described, although the distinction between petunidin and cyanidin-delphinidin is difficult to establish. A malvidin-delphinidin mixture can, however, be differentiated with ease.

L. undulatus. Reddish violet flowers; malvidin 3:5-dimonoside.

Linum grandiflorum var. *rubrum*. Crimson-scarlet flowers; re-examination showed the anthocyanidin to be delphinidin without cyanidin admixture. The p_H of the red flowers of the annual flax is lower than that of blue flowers, and as is also the case with *Hydrangea* and *Delphinium*, the anthocyanin concentration is higher in the flowers with a redder shade; the same thing is true of violas as may be seen on inspection.

Linum perenne. Blue flowers; delphinidin 3:5-dimonoside.

Lobelia cardinalis. "Queen Victoria," scarlet flowers; pelargonidin 3-monoside, probably identical with callistephin as the anthocyanin was purified and found to have a distribution number 32.5. In every other respect it tallied with callistephin.

Lupinus polyphyllus. "Northern Fire," blood-red flowers, and "Hades," bluish-red; cyanidin 3-monoside with much anthoxanthin. "Chelsea Blue" and "Plato," blue flowers; much anthoxanthin and delphinidin 3-monoside. "Evening Tints," pale salmon flowers; pelargonidin 3-saccharide having a high distribution, actually more like pentoseglycoside than monoside. "Simpson 316," pink, contains the same anthocyanin. "Sailor Boy," violet flowers, and "Athene," reddish violet; petunidin-delphinidin 3-monoside.

Masdevillia Harryana purpurea. Bluish red flowers; cyanidin diglycoside and carotenoid. The anthocyanin is Fe^- and gives a violet coloration with aqueous sodium carbonate; on the addition of sodium hydroxide this does not become blue but is decolorised (a faint blue being more stable); the anthocyanin is of a novel type.

Meconopsis Baileyi. The anthocyanin of the blue flowers is a complex cyanidin diglycoside giving a complex cyanidin on hydrolysis.

Medinilla magnifica var. *superba*. Deep pink flowers; delphinidin diglycoside.

Mesembryanthemum. The pink flowers of the succulent leaved species common near Gibraltar contain a betanin-like anthocyanin.

Michelia fuscata. Purplish brown flowers; cyanidin 3-pentoseglycoside.

Mimulus cardinalis. Scarlet flowers; pelargonidin 3-monoside.

Morus nigra. Mulberries contain pure cyanidin 3-monoside.

Nemesia strumosa Suttoni. Bluish red flowers; cyanidin 3-saccharides, a mixture most closely approximating to the behaviour of a pentoseglycoside.

Nymphaea gigantea. Blue flowers; delphinidin complex diglycoside.

Oenothera agari. Pink petals; cyanidin 3-pentoseglycoside and a little complex anthocyanin.

O. rubricalyx. Red calyx; pure cyanidin 3-pentoseglycoside.

Opuntia. The fruits of the prickly pear contain a betanin-type anthocyanin.

Origanum vulgare. The dried leaves contain leuco-anthocyanin $\rightarrow$ cyanidin.

Orychophragnus violaceus. Violet-blue flowers; cyanidin 3 : 5-dimonoside.

Oxalis rosea. Reddish mauve flowers; malvidin 3 : 5-dimonoside.

Paeonia officinalis. "Delavaye," maroon flowers contain a mixture of peonidin 3 : 5-dimonoside and cyanidin 3-monoside [cf. Willstätter and Nolan, 1915].

Passiflora racemosa. Brownish pink petals; pelargonidin 3-monoside; red-violet stamens, cyanidin 3-monoside.

Pentapterygium serpens. Brownish red flowers; cyanidin 3-monoside.

Pentas carnea. Pale mauve flowers; pigment behaved abnormally. *P. coccinea*, scarlet flowers; cyanidin 3-bioside, with no abnormality.

Petracum erodinum. Deep mauve flowers; malvidin 3 : 5-dimonoside.

Petunia hybrida. "Silver Lilac," violet flowers; malvidin 3 : 5-dimonoside. The anthocyanidin is Fe^- and contains no trace of petunidin [Willstätter and Burdick, 1917].

Phaseolus multiflorus. The scarlet runner "Scarlet Emperor" contains pure pelargonidin 3-bioside in its flowers.

Phlox divaricata var. *Laphami*. Violet-blue flowers; delphinidin 3 : 5-dimonoside mixed with a trace of a second anthocyanin.

Pinus excelsa. The young cones obtained from Sweden contain cyanidin 3-monoside and also leuco-anthocyanin $\rightarrow$ cyanidin.

P. sylvestris and other pines have leuco-anthocyanin $\rightarrow$ cyanidin in the cones.

Platytheca galioides. Violet-blue flowers; complex malvidin diglycoside. The reactions were much obscured by the presence of substances which caused the precipitation of the anthocyanin in alkaline solution.

Plumbago rosea. Bright pink flowers; complex peonidin 3-saccharide.

Polygala myrtifolia. Rosy magenta flowers; cyanidin 3 : 5-dimonoside.

Polygonum amplexicaule. Dull red flowers; cyanidin pentoseglycoside.

Pontideria. A blue-violet flowered species; delphinidin 3 : 5-dimonoside.

Potentilla argyrophylla var. *astrosanguinea*. Scarlet flowers; cyanidin 3-monoside.

Primula mollis. Red-violet flowers; pure malvidin 3 : 5-dimonoside. *P. malacoides*, "The Prince," violet-rose; malvidin 3 : 5-dimonoside; other varieties such as "The Dawn," pink, and "Dainty," pinkish mauve, and "Princes Mary," mauve, contain malvidin 3 : 5-dimonoside with more anthoxanthin. *P. obconica*, "Giant Crimson"; delphinidin 3 : 5-dimonoside. *P. officinalis*, reddish brown flowers; hirsutidin or, less probably, malvidin pentoseglycoside with much co-pigment. *P. sinensis*, "Reading Ruby," or "Giant Terracotta," contains malvidin 3-monoside, not dimonoside as formerly stated. The very large amount of co-pigment holds the pigment in the aqueous layer so that the distribution appears diglycosidic; it is monoglycosidic after purification. The leaves of *P. sinensis* contain a leuco-anthocyanin.

Prunus persica. The pink petals and sepals contain an anthocyanin yielding a complex cyanidin on hydrolysis and some leuco-anthocyanin $\rightarrow$ cyanidin. The leaf-curl contains much anthoxanthin and cyanidin 3-monoside. The leaves of *P. pissardii* remain green if not exposed to light (compare Part I) but contain leuco-anthocyanin $\rightarrow$ cyanidin.

Pterocarpus marsupium. The kino contains leuco-anthocyanin $\rightarrow$ cyanidin and a little delphinidin.

Pyrus malus Niedzwetzkiana. The fruit develops red stains when injured and the uncoloured parts contain leuco-anthocyanin $\rightarrow$ cyanidin. We neither suggest nor reject the view that the stains arise from transformation of the leuco-anthocyanin.

Ranunculus asiaticus. Orange flowers; cyanidin 3-bioside with much anthoxanthin and carotenoid; a salmon-pink-flowered kind was the same but free from carotenoid. A scarlet garden hybrid *Ranunculus* contained cyanidin 3-bioside, carotenoid and anthoxanthin; a deep rosy pink hybrid contained cyanidin 3-bioside and anthoxanthin, exactly the same difference as in the *R. asiaticus* varieties.

Rehmannia angulata. Deep bluish pink flowers; cyanidin 3-pentoseglycoside.

Rheum officinale. The drug *Rhei radix* contains leuco-anthocyanin $\rightarrow$ cyanidin. *R. rhaponticum*, green leaves contain leuco-anthocyanin $\rightarrow$ cyanidin.

Rhododendron Griersonianum. Vermilion and *R. euchaetes*, scarlet, both contained pure cyanidin 3-monoside. This has been previously recorded for cultivated varieties but these are species from N.W. Yunnan.

Richardia Elliottiana. The yellow petals contain leuco-anthocyanin $\rightarrow$ cyanidin.

Rochea ciliata. Rosy pink flowers; pelargonidin 3-pentoseglycoside and some cyanidin derivative. The reactions were much obscured by tannin.

Rosa. The orange-red polyantha roses such as "Gloria Mundi" and "Prince of Orange" contain pelargonidin 3 : 5-dimonoside in a pure condition. On one plant some of the flowers were pink and some orange; the pink contained cyanin and the orange pelargonidin dimonoside. "Veilchen Blau" appeared to develop less blue-red flowers in this dry season (1934) than normally. The bluer petals were separated and found to contain complex cyanidin 3 : 5-dimonoside with some cyanin. The redder petals contained less of the complex pigment.

Salpiglossis. Dark crimson hybrid; complex diglycoside of petunidin (or cyanidin-delphinidin).

S. sinnata. Brown flowers; complex diglycoside of petunidin together with carotenoid.

Sambucus racemosa. The red berries contain cyanidin 3 : 5-dimonoside.

Scabiosa. "Azure Fairy," lavender-blue flowers; delphinidin diglycoside. "Black Prince," crimson flowers; cyanidin 3-pentoseglycoside. "Fire King," bluish red flowers; pelargonidin 3-pentoseglycoside.

Seaweeds. We have not observed normal anthocyanins in seaweeds, but *Ceramium rubrum* Ag., *Griffithsia setacea* Ag., and *Plocamium coccineum* Syngb. contained leuco-compounds yielding pigments soluble in dilute acids and somewhat resembling betanin in reactions.

Senecio speciosus. Bright reddish mauve flowers; cyanidin diglycoside with a carbohydrate or other residue on hydroxyl in positions 3' or 4', the anthocyanin being Fe^- .

The cineraria, "Crimson Star," brownish bluish red; pelargonidin 3-bioside as previously reported for an unnamed variety.

Sutton's "Superb Crimson" cineraria was carefully studied and the anthocyanin is based on cyanidin only; its Fe^- property and diglycosidic nature again suggests substitution at 3' or 4'.

Strelitzia reginae. The orange petals contain no anthocyanin; the blue pistils contain delphinidin diglycoside, a little acylated pigment is present.

Streptocarpus Dunnii. Brownish pink flowers, cyanidin 3-pentoseglycoside. Dull salmon-pink flowers of another variety contained the same anthocyanin and much yellow pigment.

Symphylum caucasicum. Blue flowers; delphinidin diglycoside.

Syncarpia laurifolia. The sawdust contains leuco-anthocyanin $\rightarrow$ cyanidin with some delphinidin.

Torenia Fourniers Grandiflora. Blue and violet flowers; cyanidin 3 : 5-dimonoside.

Tritoma speciosa. Orange-salmon flowers; pelargonidin 3-pentoseglycoside.

Tropaeolum majus. "Yellow Gleam" and "Golden Gleam" contain no anthocyanin. The double red-scarlet "Hermann Gnashoff" is coloured by pelargonidin 3-bioside.

Verbena. A range of hybrids has been examined, but the variety containing cyanidin dimonoside previously noted was not among them. Sutton's "Bedding Scarlet," "Firefly" and "Giant Pink" contained pelargonidin 3-monoside. "Purple White Eye" and "Bedding Blue" contain the same anthocyanin, the difference in colour, very large at a glance, is diminished on crushing the flowers and is due to local chemical or physical conditions to a large extent. The anthocyanin is a delphinidin saccharide with a high monoglycosidic type of distribution. It may be a complex diglycoside but certainly not of the usual type. The colour reactions fit in better with the 3-monoglycoside hypothesis. A series from an Oxford garden was arranged in order, most blue to most red. The violets and purples were all of the delphinidin type; there was no cyanidin, and the greatest surprise was the absence of pelargonidin 3-monoside. The rose-coloured varieties contained pelargonidin 3 : 5-dimonoside.

Viola. "La Grandeur," red-violet, complex delphinidin pentoseglycoside as anticipated but "Maggie Mott," blue, appears to be diglycosidic. "Archie Grant" seems to contain delphinidin pentoseglycoside free from acyl groups.

Vitis heterophylla humulifolia. The berries (at first colourless) change in colour from green to red to purple to purplish blue to turquoise-blue to yellow. The colourless and the green berries contain leuco-anthocyanin $\rightarrow$ cyanidin. This is definitely not pseudo-base as it requires vigorous treatment with hydrochloric acid in order to produce the anthocyanidin: the anthocyanin reaches a maximum in the purplish blue berries and it consists of malvidin 3 : 5-dimonoside.

Zea Mays var. *amylacea*. Dark blue and bright red cobs were procured from N.W. Arizona. The blue pigment appears as a fine powder in the starch protected by a cellulose sheath; the red cob contains the pigment more evenly distributed in all the structures. The blue pigment is easily soluble in 1 % hydrochloric acid and is cyanidin 3-monoside (evidently in neutral form or in colloidal combination). The red pigment is insoluble in 1 % hydrochloric acid but can be extracted by means of alcoholic hydrogen chloride; it is a complex derivative of pelargonidin.

Zinnia. "Fire King," bright pink-orange flowers; carotenoid and cyanidin 3 : 5-dimonoside.

The following experiments were made by Mr E. A. H. Roberts in collaboration with the authors.

The relation between co-pigment effects and distribution properties.

If an anthocyanin (m mg.) is distributed between V ml. each of equilibrated isoamyl alcohol and 0.5 % hydrochloric acid, D being the distribution number (% passing to the isoamyl alcohol), then if the anthocyanin is associated to double molecules in the aqueous layer, remaining unassociated in the isoamyl alcohol, we have $mD^2 + kVD - 100kV = 0$, in which m and D are variables under standard experimental conditions. The condition laid down is satisfied if the curve $\log C_w / \log C_{AA}$ is linear with a slope of 2 and this is the case for oenin chloride and chrysanthemin chloride [Levy and Robinson, 1931]. Knowing D for some particular value of m , well inside the region of agreement with the formula, we can find the concentration at which the double molecules break up in the aqueous solution to a significant extent. We now estimate that this is about 2 mg. in 25 ml. of 0.5 % hydrochloric acid for oenin chloride and about double this concentration for both chrysanthemin and oxycoccicyanin chlorides; callistephin at such concentrations shows no association. More dilute solutions contain a considerable number of single molecules; more concentrated solutions contain double molecules and association to triple molecules is naturally a possibility, but has not been observed within the limits of our experiments.

The following supplementary determinations of D were made in the case of chrysanthemin: m , 1.32, D , 25.8; 2.1, 22.9; 2.9, 20.7; 3.6, 19.2; 5.6, 16.2.

Distribution numbers in the presence of papaverine or narcotine were determined by the standard method using 0.5 % hydrochloric acid containing the alkaloid (0.438 g. in 250 ml.). After the separation of the layers the solutions were made up to the same composition of solvents as usual, but they were also each saturated with papaverine or narcotine hydrochloride and compared at about the same concentration of pigment. Otherwise the co-pigment effect obscured the colorimetry. The results for oenin chloride and papaverine hydrochloride were: m , 1.53, D , 10.65; 2.02, 10.9; 2.96, 10.6; 3.3, 10.2; 4.56, 9.6; 5.7, 8.7. For chrysanthemin chloride and papaverine hydrochloride: m , 1.44, D , 17.9; 1.78, 16.7; 2.4, 15.0; 3.17, 14.1; 4.57, 13.7; 6.3, 13.7; 7.47, 12.3; 11.44, 12.5.

The effect of adding narcotine was relatively small (for oenin: m , 2.36, D , 10.2; 3.0, 18.2; 3.45, 18.0; 5.9, 14.0) and when the curves $\log C_w / \log C_{AA}$ are plotted it is found that over a considerable range the slope, with papaverine added, approximates to 1. Evidently the papaverine has broken up the double molecules probably by combining with the pigment, and as the alkaloid is in great excess this gives the effect of single molecules.

The slopes in the cases in which narcotine was introduced are approximately 2 and at no point so low as 1.

The effect of these addenda on the absorption spectra is shown in Fig. 1 for chrysanthemin (0.66 mg. in 25 ml. of 0.5 % hydrochloric acid), alone and with

Fig. 1. I, chrysanthemin chloride; II, chrysanthemin chloride with narcotine; III, chrysanthemin chloride with papaverine.

the addition of papaverine hydrochloride (0.052 g.). The addition of narcotine (0.16 g.) produces a much smaller effect. Similar results were obtained with oenin chloride, the shift being in the same direction but smaller in magnitude; in this case the addition of papaverine slightly raised the position of the maximum.

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