369. *Experiments on the Synthesis of Anthocyanins. Part XVII. The Syntheses of Pelargonin, Peonin, and Cyanin Chlorides.*

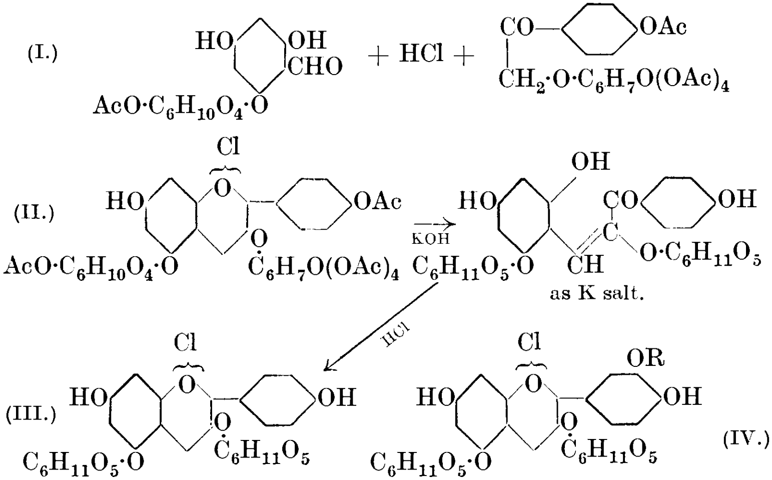
By Robert Robinson and A. R. Todd.

In the course of the syntheses of the monoglucosidic anthocyanins, namely, callistephin (perlargonidin 3-glucoside), chrysanthemin (cyanidin 3-glucoside), oxycoccicyanin (peonidin 3-glucoside), and oenin (malvidin 3-glucoside) the various "second component" intermediates were condensed with *O*-benzoylphloroglucinaldehyde, and the products hydrolysed. Methods for the preparation of these intermediates having been devised, the synthesis of any anthocyanin containing a glucose residue in position 3 became feasible and, when it was realised (Parts VI—XII) that the better known diglucosidic anthocyanins (except mecocyanin and keracyanin) are not biosides but 3 : 5-diglucosides of the anthocyanidins, it also became clear that the way to the synthesis of these pigments, the anthocyanins *par excellence*, was open.

The first case attacked was that of hirsutin chloride (Part XV), because both the required intermediates had already been prepared; the identity of the natural and the synthetic specimen confirmed the correctness of the view that anthocyanins may be 3 : 5-dimonosides. For the remainder of the anthocyanins of this type it became necessary to obtain derivatives of 2-*O*-glucosidylphloroglucinaldehyde and this proved to be a difficult problem which was only partly solved in the course of the experiments on the synthesis of pelargonenin, cyanenin, and malvenin (5-monoglucosides). Ultimately it was found that the direct glucosidation of phloroglucinaldehyde could be effected under carefully defined conditions and by condensing the 2-*O*-tetra-acetylglucosidylphloroglucinaldehyde with the intermediate for oenin a synthesis of malvin chloride was accomplished (Part XVI).

A modification of the process of glucosidation of phloroglucinaldehyde leads to the formation of 2-*O*-monoacetylglucosidylphloroglucinaldehyde (see below) and, as the method is somewhat more convenient than the older one, it was important to ascertain whether the orientation of the glucosidyl group and the stereochemical sense of the glucoside link are identical in the tetra-acetyl and monoacetyl derivatives. That this is the case has been proved by using both intermediates for the synthesis of pelargonenin chloride, a substance possessing highly characteristic properties. In confirmation we may note that the proportions in which the tetra-acetyl and monoacetyl glucosides are isolated vary with the conditions and this is easy to understand if it is merely a question of the extent of removal of acetyl groups by hydrolysis.

By condensing one or other of these acetylated glucosidylphloroglucinaldehydes with the intermediates for callistephin (Robertson and Robinson, J., 1928, 1460), chrysanthemin (Murakami, Robertson, and Robinson, J., 1931, 2666) and oxycoccicyanin (Levy and Robinson, J., 1931, 2715) we have now been able to effect the syntheses of pelargonin, cyanin, and peonin chlorides. The annexed scheme illustrating the stages in the case of pelargonin will suffice in explanation.



The synthetic colouring matters were proved to be identical with natural anthocyanins. Cyanin chloride used for this purpose was derived from cornflowers (Willstätter and Everest, *Annalen*, 1913, **401**, 189) and from red dahlias (Willstätter and Mallison, *Annalen*, 1915, **408**, 147), and peonin from deep violet-red peonies (Willstätter and Nolan, *Annalen*, 1915, **408**, 136). We are greatly indebted to Geheimrat Professor R. Willstätter and to Professor Nolan for specimens of these anthocyanins.

Karrer and Widmer (*Helv. Chim. Acta*, 1927, **10**, 729) have shown that monardin is a diglucoside of pelargonidin chloride and that it is identical with pelargonin (Willstätter and Bolton, *Annalen*, 1915, **408**, 42) and salvinin (Willstätter and Bolton, *Annalen*, 1916, **412**, 113); they consider, however, that punicin may be a different anthocyanin, although it is isomeric with the others and has very similar properties. We have not been able to test the latter point by direct comparison with our synthetic product, but owing to the courtesy of Professor P. Karrer, to whom we express our thanks, we have been able to examine specimens of monardin, salvinin, and pelargonin chlorides. Unfortunately the pelargonin was not quite pure, but the monardin and salvinin tallied in all respects with the synthetical product. In connexion with a survey of natural anthocyanins, Mrs. G. M. Robinson has observed the frequent occurrence of malvidin derivatives in flowers of pelargoniums. The only observed divergence of the natural pelargonin from the synthetic product could be explained in this way because it concerned the colour of the solutions. Therefore the specimen (of natural pelargonin) was hydrolysed and the presence of a trace of malvidin in the substance was detected. The aqueous solution (0·5% HCl) of the anthocyanidin was repeatedly extracted with *cyclo*hexanol—toluene (1 : 5) until the pelargonidin was removed; the residual bluish-red solution then gave the reactions for malvidin (compare Robinson and Robinson, *Biochem. J.*, 1931, **25**, 1687).

Since the divergence is explicable and since Karrer and Widmer have already shown that pelargonin and monardin are identical, we describe our synthetic substance as pelargonin because the anthocyanin was first described under that name.

Further comparisons of the natural and the synthetic pigments mentioned in this communication will be submitted in due course.

EXPERIMENTAL.

Acetylated Glucosides of Phloroglucinaldehyde.—It has been found that Me_2CO may replace MeCN as the solvent of the acetoglucosidisation of phloroglucinaldehyde (this vol., p. 2301). The yield of cryst. monoglucoside is slightly higher (1·6 g. from 5 g.) and less of the diglucoside is produced. The relative proportion of needle-shaped and prismatic crystals varies in different expts., but in general the needle form predominates under the new conditions. The more sparingly sol. glucoside occurring as prisms was previously shown to be 2-*O*-tetra-acetyl-β-glucosidylphloroglucinaldehyde. The needle form appears to be 2-*O*-monoacetyl-β-glucosidylphloroglucinaldehyde (I), 3AcO groups being removed by hydrolysis [Found: C, 50·2; H, 5·2. $C_{15}H_{18}O_{10}$ (monoacetyl) requires C, 50·3; H, 5·0%. $C_{21}H_{24}O_{13}$ (tetra-acetyl) requires C, 52·1; H, 5·0%].

The compound crystallises from 70% MeOH in colourless needles, m. p. 109—110°; it undergoes decomp. gradually on prolonged heating at 100°. Under the conditions already prescribed for 2-*O*-tetra-acetylglucosidylphloroglucinaldehyde (this vol., p. 2302) it condenses with ω : 4-dihydroxyacetophenone to give pelargonenin chloride; similarly, both glucosides give rise to one and the same pelargonin chloride on condensation with ω-*O*-tetra-acetyl-β-glucosidoxy-4-acetoxyacetophenone, followed by de-acetylation. Moreover this monoacetylated glucoside has a specific rotation $[\alpha]_{5461}^{21^\circ} = -49·32^\circ$ in $CHCl_3$ ($c = 1·460$) indicating that, like the tetra-acetylated derivative ($[\alpha]_{5461}^{19^\circ} = -47·84^\circ$), it is a β-glucoside.

If these conclusions are valid it is to be expected that the uncrystallisable portion of the monoglucosidic fraction from the acetoglucosidisation of phloroglucinaldehyde should consist mainly of other partly acetylated 2-β-glucosides. This was the case, as the gum gave rise to pelargonenin chloride when condensed with ω : 4-dihydroxyacetophenone.

7-Hydroxy-3-O-tetra-acetyl-β-glucosidoxy-5-O-monoacetyl-β-glucosidoxy-4′-acetoxyflavylium Chloride (II).—A solution of 2-*O*-monoacetyl-β-glucosidylphloroglucinaldehyde (0·6 g.) and ω-*O*-tetra-acetyl-β-glucosidoxy-4-acetoxyacetophenone (1·2 g.) (Part V; Robertson and Robinson, *loc. cit.*) in dry EtOAc (25 c.c.) was saturated at 0° with dry HCl. The liquid slowly acquired a deep orange-red colour and a green fluorescence, and after keeping for 60 hrs. in the ice-chest the *flavylium* salt was precipitated by addition of dry Et_2O (300 c.c.). The scarlet-red powder was washed with Et_2O and dried (1·08 g.) (Found: C, 51·1; H, 5·1; Cl, 4·0. $C_{39}H_{43}O_{21}Cl,2H_2O$ requires C, 51·0; H, 5·1; Cl, 3·9%).

Pelargonin Chloride [*7 : 4′-Dihydroxy-3 : 5 di-(β-glucosidoxy)flavylium Chloride*] (III).—A solution of the above flavylium salt (1·03 g.) in 10% NaOH aq. (9 c.c.) was kept at room temp. in an atmosphere of H_2 during 90 mins. The dark greenish-brown solution was acidified with conc. HCl (3·9 c.c.), and the deep red-orange solution warmed at 50° on the water-bath for a few moments. On cooling, the 2% acid solution thus obtained set to a mass of scarlet-red needles, which were collected after being kept over-night in the ice-chest. The product, recryst. from H_2O–MeOH–HCl by the method of Willstätter and Bolton (*loc. cit.*) for natural pelargonin chloride, formed magnificent slender scarlet-red needles (0·35 g.) (Found in air-dried material: C, 46·3; H, 5·8; Cl, 5·3; loss at 110° in a high vac., 8·0, 8·4. $C_{27}H_{31}O_{15}Cl,4H_2O$ requires C, 46·1; H, 5·6; Cl, 5·1; $4H_2O$, 10·25%. Found in dried material: C, 50·5; H, 5·2; Cl, 5·8. $C_{27}H_{31}O_{15}Cl$ requires C, 51·4; H, 4·9; Cl, 5·6%. $C_{27}H_{31}O_{15}Cl,4H_2O$, losing 8·2% as H_2O, requires C, 50·4; H, 5·1; Cl, 5·5%).

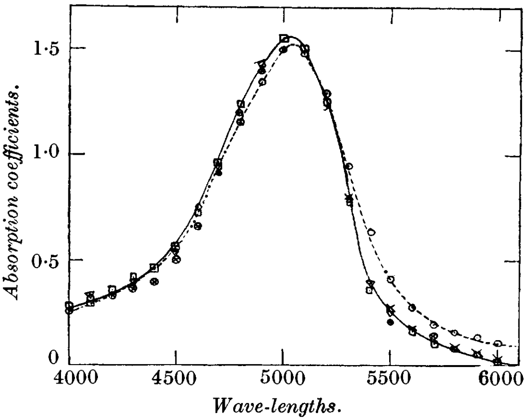
The m. p. of pelargonin chloride is given in the literature as 180°. The synthetic material, heated moderately slowly, sinters at 178° and melts at about 187°. Under the same conditions monardin and salvinin melted at 185—186°. These m. p.'s have, however, little significance, as the temp. at which decomp. and fusion occur is dependent on the rate of heating, and in the case of synthetic pelargonin chloride lies between 180° and 200°. It therefore appears that punicin chloride (Karrer and Widmer, *Helv. Chim. Acta*, 1927, **10**, 78), which sinters at 180° and melts at 193°, may be identical with pelargonin chloride, since all other properties of the two anthocyanins are identical.

The absorption coefficients for $0{\cdot}154N/10^4$-solutions of synthetic pelargonin chloride, natural pelargonin chloride, monardin, and salvinin chlorides in 0·1% MeOH–HCl were determined over the range 4000—6000 Å. The results are shown in Fig. 1 and it will be seen that synthetic pelargonin, monardin, and salvinin chlorides agree very closely, but natural pelargonin chloride (dotted curve) shows a slight divergence from the others, the maximum being displaced to a slightly lower value and a higher wave-length, causing a displacement of the curve in the direction of increasing wave-length. The absorption of malvin chloride (compare this vol., p. 2304) is such that a small percentage of it in the pelargonin would produce the observed result.

Other Comparisons of Natural and Synthetic Specimens of Pelargonin Chloride (Monardin Chloride).—The superficial characters and all the ordinary reactions of the specimens were completely identical; this applies to the appearance in mass and cryst. form under the microscope, the colour in various solvents, and the fluorescence in methyl-, ethyl-, and butyl-alcoholic solution. The prep. of the basic chloride under the conditions prescribed by Willstätter and Bolton (*loc. cit.*) succeeded and the dark violet needles with metallic lustre had the properties recorded for the pelargonin derivative. Orange-red aq. acid solutions of pelargonin become bluish-red on the addition of tannin, and this phenomenon was exhibited to the same degree by the two specimens (synth. and monardin).

The colour reactions, pseudo-base formation, and stability in alkaline solutions were investigated in a range of buffered solutions under the conditions prescribed by Robertson and Robinson (*Biochem. J.*, 1929, **23**, 35).

FIG. 1.



× *Pelargonin chloride (synthetic).* ⊙ *Pelargonin chloride (natural).*
▽ *Monardin chloride.* ⊗ *Salvinin chloride.* ⊡ *Coincident points.*

The natural and the synthetic specimen gave identical results. (1) Weak rose, fading; (3) bluer, colourless in 30 secs.; (5) bluish-red, colourless in 3 mins.; (7) more intensely coloured, red-violet on the addition of 10 c.c. of acetone; (9) permanganate, blue-violet with acetone; (11) reddish-violet, pure blue with acetone ; (13) the same; (15) violet, fading quickly and nearly colourless in 30 secs. After 2 hrs.: (1) faint pink; (3), (5), (7) colourless; (9) pale reddish-violet; (11) pale violet; (13) pale slate; (15) nearly colourless. Solutions to which acetone was added : (7) pale reddish-violet; (9) pale violet; (11), (13) blue. On addition of conc. HCl, recovery of the colour occurred in (1)—(11), some loss was apparent in (13), and no recovery occurred in (15).

The distribution number (*n*-BuOH) was determined by the method detailed in Part XV with the following modification (adopted also for cyanin and peonin). The pigment (4·00 mg.) was dissolved in 30 c.c. of the special 0·5% hydrochloric acid and shaken with 20 c.c. of the special BuOH. 25 C.c. of the aq. layer were then shaken with 25 c.c. of the special BuOH and the distribution was determined.

Monardin chloride and the synthetic specimen gave exactly the same value, namely, 20·0.

The above procedure has the advantage that errors due to the presence of traces of monoglucosides are minimised. The distribution (amyl alcohol) is very low, but more of the colour passes into the org. layer on saturation of the aq. solution with salt (comparison—identity).

ω-O-Tetra-acetyl-β-glucosidoxy-4-acetoxy-3-methoxyacetophenone.—The following improved method of prep. of this intermediate (Levy and Robinson, *loc. cit.*) has been employed.

Dry Ag_2O (9 g.) was added to a solution of ω-hydroxy-4-acetoxy-3-methoxyacetophenone (3·7 g., dried at 100° in vac.) and *O*-tetra-acetyl-α-glucosidyl bromide (11·9 g.) in dry C_6H_6 (25 c.c.) at 40°. After being shaken for 30 mins., the mixture was boiled on the steam-bath for a few mins. and filtered. On addition of light petroleum to the filtrate the product was pptd. as a pale yellow syrup, which was washed with hot, and then with cold, H_2O and dissolved in hot 70% MeOH. On cooling, the tetra-acetylglucoside separated as long, colourless, rectangular plates (1·9 g.), m. p. 103°. Levy and Robinson (*loc. cit.*) give m. p. 74—76° (decomp.), but the material described was probably partly de-acetylated (Found: C, 53·9; H, 5·3. Calc. for $C_{25}H_{30}O_{14}$: C, 54·2; H, 5·4%).

7-Hydroxy-3 : 5-di-(O-tetra-acetyl-β-glucosidoxy)-4′-acetoxy-3-methoxyflavylium Chloride.—A solution of 2-*O*-tetra-acetyl-β-glucosidylphloroglucinaldehyde (0·25 g.) and ω-*O*-tetra-acetyl-β-glucosidoxy-4-acetoxy-3-methoxyacetophenone (0·4 g.) in dry EtOAc (5 c.c.) was saturated with dry HCl at 0°. The liquid, which gradually acquired a deep red-violet colour, was kept at 0° for 60 hrs., dry Et_2O then added, and the carmine-red ppt. of flavylium salt collected, washed with Et_2O, and dried (0·39 g.) [Found: C, 50·9; H, 5·5; Cl, 2·7; MeO, 3·6. $C_{46}H_{51}O_{25}Cl$ (fully acetylated) requires C, 53·2; H, 4·9; Cl, 3·4; MeO, 3·0%, indicating partial deacetylation. $C_{42}H_{47}O_{23}Cl,2H_2O$ (hepta-acetate) requires C, 50·9; H, 5·2; Cl, 3·6; MeO, 3·1%].

Peonin Chloride (*7 : 4′-Dihydroxy-3 : 5-di-β-glucosidoxy-3′-methoxyflavylium Chloride*) (IV, R = Me).—The above flavylium salt (0·35 g.) was de-acetylated by keeping a solution in 10% NaOH aq. (3 c.c.) in H_2 for 90 mins. The brownish liquid thus obtained was acidified with sufficient conc. HCl (1·3 c.c.) to give a 2% acid solution, warmed at 50° for a few secs. to ensure complete oxonium salt formation, and allowed to cool. The acid conc. was increased to 6% in the cold, and the dark red-violet solution set aside in the ice-chest. During 36 hrs. the peonin chloride separated as a mass of small reddish-brown needles (0·08 g., *i.e.*, 36%). It was recrystallised by dissolution in hot 0·5% HCl aq. (5 c.c.) and increase of the acid conc. to 6% in the cold by addition of the requisite amount of 20% HCl aq.; the product separated as a mass of small reddish-violet needles with a green lustre (Found in air-dried specimen: C, 44·6; H, 5·9; Cl, 4·8; MeO, 4·6; loss at 110° in a high vac., 11·6. $C_{28}H_{33}O_{16}Cl,5H_2O$ requires C, 44·7; H, 5·7; Cl, 4·7; MeO, 4·1; $5H_2O$, 11·9%. Found in anhyd. material: C, 50·4; H, 5·2; Cl, 5·7. $C_{28}H_{33}O_{16}Cl$ requires C, 50·9; H, 5·0; Cl, 5·4%).

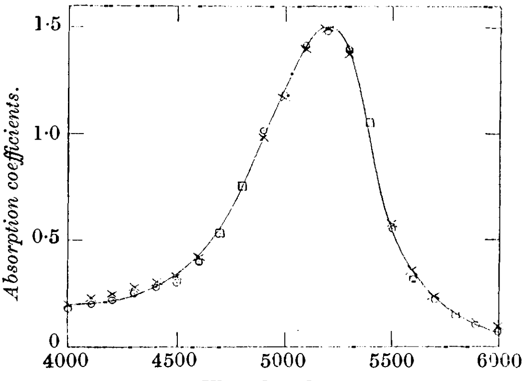
Synthetic peonin chloride decomposes at 165—167°.

The absorption coefficients for natural and synthetic peonin chlorides in $0{\cdot}164N/10^4$-solutions in 0·1% MeOH–HCl were measured over the range 4000—6000 Å. No divergences outside the range of experimental error were detected (Fig. 2).

Other Comparisons of Natural and Synthetic Peonin Chlorides.—The properties of peonin chloride described by Willstätter and Nolan (*loc. cit.*) were confirmed and found to be characteristic of the synthetic specimen. The appearances of the crystals and the habits of crystn. were directly compared and found to be identical. The colour of acid solutions, change on addition of alcohol, and the alkali-colour reactions were all identical for both specimens. The colour reactions in buffered solutions are very interesting in comparison with those of cyanin because, contrary to expectation, peonin begins to give blue tones at a lower p_H than does cyanin; in the more alkaline solutions cyanin gives a greener, purer blue coloration than peonin. Natural and synthetic peonin give the same results.

(1) Pink, fading; (3) bluish-red, colourless in 30 secs.; (5) more intense, fading more slowly; (7) reddish-violet; (9) intense violet; (11) blue-violet; (13) blue, violet in thick layers; (15) blue, fading. After 2 hrs.: (1) faint pink; (3), (5) colourless; (7) weak violet; (9) violet; (11) blue; (13) greenish-blue; (15) yellow. On addition of HCl aq. : recovery except in (15), some loss in (13). The distribution number (n-BuOH, method above) was 17·3 for the synthetic salt and 17·1 for the natural product. The co-pigmenting effect of tannin on the aq. acid solution was observed (no divergence) and the addition of $FeCl_3$ to MeOH solutions of the specimens gave the same orange coloration.

FIG. 2.



× *Peonin chloride* (*synthetic*). ⊙ *Peonin chloride* (*natural*).
⊡ *Coincident points.*

7-Hydroxy-3 : 5-di-(O-tetra-acetyl-β-glucosidoxy)-3′ : 4′-diacetoxyflavylium Chloride.—A solution of 2-*O*-tetra-acetyl-β-glucosidylphloroglucinaldehyde (1 g.) and ω-*O*-tetra-acetyl-β-glucosidoxy-3 : 4-diacetoxyacetophenone (Murakami, Robertson, and Robinson, *loc. cit.*) (1·5 g.) in dry EtOAc (25 c.c.) was saturated with dry HCl at 0°. The red solution was kept in the ice-chest for 60 hrs., the colour becoming intense red-violet. The crude *flavylium* salt was pptd. as a scarlet-red powder (1·68 g.) by the addition of anhyd. Et_2O, and was collected, washed, and dried [Found: C, 50·9; H, 5·0; Cl, 3·0. $C_{47}H_{51}O_{26}Cl,2H_2O$ (deca-acetate) requires C, 51·1; H, 5·0; Cl, 3·2%].

Cyanin Chloride (IV, R = H).—The above acetylated compound (1·65 g.) was dissolved in 10% NaOH aq. (14 c.c.) and kept for 90 mins. at room temp. in H_2. On acidifying the brownish-green solution with conc. HCl (6·03 c.c.) and warming the 2% acid solution so obtained to 50° to complete the formation of the oxonium salt, a deep reddish-violet solution was obtained. After cooling, the acid concn. was increased to 6%, and the filtered solution set aside in a partially evacuated desiccator. During 48 hrs., cyanin chloride separated as small brownish-violet prisms with a bronze lustre (0·06 g.). A further quantity separated from the mother-liquor in the course of a few days (Found in air-dried material: C, 45·7; H, 5·7; Cl, 6·1. $C_{27}H_{31}O_{16}Cl,3H_2O$ requires C, 46·2; H, 5·3; Cl, 5·1%. These figures indicate the presence of adsorbed HCl in the air-dried substance. $C_{27}H_{31}O_{16}Cl,3H_2O$ + 0·2 mol. adsorbed HCl requires C, 45·7; H, 5·3; Cl, 6·0%. On drying for a short time at 110° in high vac., only part of the H_2O of crystn. is removed. Found: C, 49·0; H, 4·8; Cl, 5·5; loss on drying, 6·1. $C_{27}H_{31}O_{16}Cl,0{\cdot}75H_2O$ requires C, 49·1; H, 4·9; Cl, 5·4; loss on drying, 6·7%). Cyanin chloride obtained from the cornflower crystallises as a trihydrate (Willstätter and Everest, *loc. cit.*) and the stable hydrate $C_{27}H_{31}O_{16}Cl,0{\cdot}75H_2O$ has also been observed in the case of the natural material.

This phenomenon was, indeed, the cause of the assignment of an incorrect composition to the pigment in the early stages of Willstätter's researches.

Attempts to work up the crude cyanin chloride after hydrolysis by pptn. from the acid solution by addition of AcOH, followed by excess of Et_2O, and repeated treatment of the residue with org. solvents were partly successful in increasing the yield. Synthetic cyanin chloride had m. p. 205° (decomp.) (natural pigment, m. p. 203—204°; *loc. cit.*).

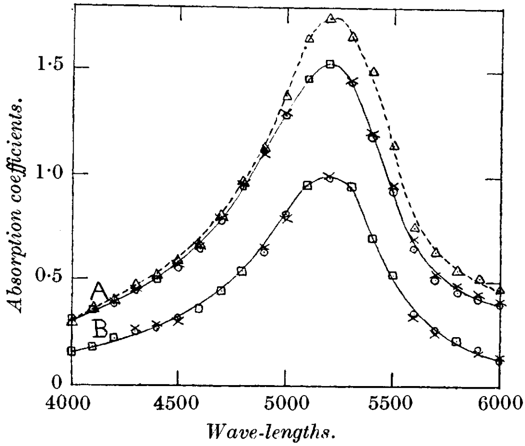
Cyanin Chloride from the Cornflower.—The crude pigment kindly supplied to us by Professor Willstätter (1 g.) was dissolved in hot H_2O (35 c.c.), and an equal vol. of 4% EtOH–HCl added. On cooling and keeping over-night, the product separated as a mass of brownish crystals with a greenish-bronze lustre. This material, however, contained a considerable quantity of a monoglucoside and the substance (2 g.) was dissolved in 0·5% HCl aq. (70 c.c.) which had previously been shaken with *n*-BuOH; it was then shaken twice with BuOH (50 c.c.) saturated with 0·5% HCl aq., and finally with *iso*amyl alcohol (60 c.c.). The aq. layer was warmed, and mixed with an equal vol. of 4% EtOH–HCl; on cooling, cyanin chloride separated in a pure condition as small brownish crystals with a greenish-bronze lustre (1·4 g.). This material had a very low distribution number (amyl alcohol) and was taken as a standard specimen of the natural pigment.

Absorption of Natural and Synthetic Cyanin Chlorides (Fig. 3).—In section (A) of the diagram are the data for synthetic cyanin chloride and for the natural substance from dahlias (broken curve) and from cornflowers, the concn. in each case being $0{\cdot}126N/10^4$ in 0·1% MeOH–HCl. The data for the synthetic material and cornflower cyanin fall on the same curve, any minor discrepancies being well within experimental error. The curve for dahlia cyanin, however, suggests that the specimen was contaminated with monoglucoside giving the effect of greater absorption. That this is indeed the case has been shown in the following way.

Cyanin chloride (1 mg. *ex* dahlia) was dissolved in 0·5% HCl aq. (20 c.c.) previously saturated with *n*-BuOH. A similar solution of synthetic cyanin chloride was prepared and both solutions were shaken twice with BuOH (20 c.c. each time) previously saturated with 0·5% HCl aq., and finally with amyl alcohol (20 c.c.). Each aq. layer (10 c.c.) was diluted to 50 c.c. with 0·1% MeOH–HCl, the solutions matched colorimetrically, and their absorption coefficients determined in the usual way. The results are shown in curve (B)—both sets of data lie on the same curve, showing that after removal of monoglucosidic material cyanin chloride *ex* dahlias is identical with the synthetic specimen.

Other Comparisons of Natural and Synthetic Cyanin Chlorides.—Recrystn. under identical conditions furnished in each case rhombic plates, violet by transmitted light under the microscope. All the superficial characters of the specimens were identical and they furnished solutions (aq. and alc.) having the same colour and behaviour under all conditions. All the ordinary reactions, *e.g.*, the iron reaction and the colours with NaOAc and Na_2CO_3, were observed and in addition the co-pigment phenomena with tannin and quercitrin were the same in the two cases.

FIG. 3.



A. × *Cyanin chloride (synthetic).*
⊙ *Cyanin chloride (cornflower).*
△ *Cyanin chloride (dahlia).*
⊡ *Coincident points.*

B. × *Cyanin chloride (synthetic, washed).*
⊙ *Cyanin chloride (dahlia, washed).*
⊡ *Coincident points.*

The colour reactions in buffered solutions were the following :—(1) bluish-rose, fading quickly; (3) bluer and colourless in 30 secs.; (5) permanganate, fading less rapidly; (7) more intense and more stable; (9), (11) reddish-violet; (13) violet; (15) blue. After 2 hrs.: (1) pink; (3), (5) colourless; (7) faint pink; (9) reddish-violet, fairly intense; (11) paler reddish-violet, (13) nearly colourless; (15) yellow. Recovery on addition of HCl in (1)—(11), (13) some loss; (15) no recovery at first, some colour on keeping or heating. The distribution number (*n*-BuOH) was 14·8 for both specimens.

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THE DYSON PERRINS LABORATORY,
UNIVERSITY OF OXFORD.

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