267. *Experiments on the Synthesis of Anthocyanins. Part XXII. Isolation of an Anthocyanin of* Salvia patens *termed Delphin, and its Synthesis.*

By (Miss) T. M. Reynolds, Robert Robinson, and (Miss) R. Scott-Moncrieff.

Our interest in the anthocyanin pigment of the bright blue flowers of *Salvia patens* was first stimulated by a publication of Buxton and Darbishire (*J. Genetics*, 1929, **21**, 71), which contained colour plates showing that this colouring matter is blue at a lower value of p_H than any other anthocyanin so far examined. Although the possibility thus indicated of an entirely novel type did not materialise, the investigation has presented unexpected features and has enabled us to bridge a gap both in relation to the occurrence of anthocyanins in Nature and to their synthesis.

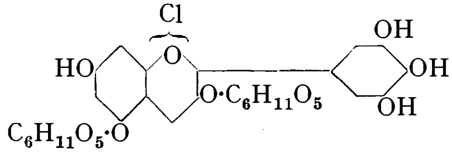
The pigment may be readily extracted by alkali-free water or by dilute aqueous or alcoholic hydrochloric acid, and fresh solutions contain an anthocyanin of the complex type either in a homogeneous form or mixed with the diglucosidic anthocyanin ultimately isolated. The distribution to *iso*amyl alcohol is at first somewhat higher than that characteristic of a diglucoside, but on keeping the acid solutions, especially the methyl-alcoholic solutions, the distribution number falls and a normal diglucoside results. The original anomalous distribution is by no means so high as that of certain well-known complex diglucosides such as monardaein, and it may be that the anthocyanin is acylated by means of an acid residue containing more hydroxyl groups (monardaein is di-*p*-coumarylpelargonin and delphinin yields 2 mols. of *p*-hydroxybenzoic acid on hydrolysis); alternatively, it may be that the anthocyanin ultimately isolated is present in the petals mixed with a small proportion of a complex diglucoside of the usual type, or that the distribution number is lowered by the presence of co-pigment (possibly responsible for the blue tone at a low p_H). This latter theory is supported by the observed rise of the distribution number as the pigment is purified.

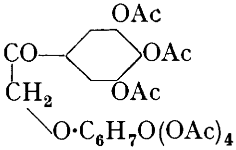
However this may be, the process of isolation brings about the hydrolysis of the complex, acylated pigment, and we were able to secure a crystalline anthocyanin which was shown to be a diglucoside of delphinidin.

Briefly, the method of isolation was in the stages: (*a*) extraction of the petals by means of methyl-alcoholic hydrogen chloride (1·0%); (*b*) precipitation of a lead salt; (*c*) solution of the lead salt in acetic acid and reprecipitation with ether; (*d*) decomposition of the lead salt by means of propyl-alcoholic–methyl-alcoholic hydrogen chloride; (*e*) precipitation of the chloride by the addition of ether to the filtrate; (*f*) hydrolysis of the complex pigment so obtained by means of cold aqueous methyl-alcoholic hydrogen chloride; (*g*) recrystallisation of the crude pigment from aqueous hydrochloric acid with and without the addition of methyl alcohol.

The substance proved, as already stated, to be a delphinidin diglucoside, and its colour reactions appeared to relate it to the cyanin type rather than to that of mecocyanin. It thus appeared that we had encountered the hitherto unknown analogue in the delphinidin series of pelargonin, cyanin, peonin, malvin, and hirsutin, and as shown below the view that the substance is actually O-3 : *5-diglucosidyldelphinidin chloride* (I) has been confirmed by synthesis.

(I.)





(II.)

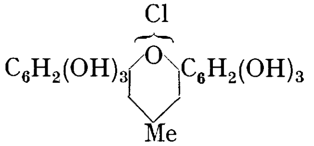
There is little doubt but that this anthocyanin will prove to be widely distributed, and qualitative tests (cf., *e.g.*, Robinson and Robinson, *Biochem. J.*, 1931, **25**, 1687) have already indicated a number of possible places of occurrence. Therefore we have carefully considered the difficult problem of nomenclature, and now propose the name *delphin* for the anthocyanin in view of its relation to delphinidin, which is the same as that of cyanin to cyanidin. A name based on *Salvia* would not be suitable, as different anthocyanin pigments have already been termed salvin, salvinin, and salvianin.

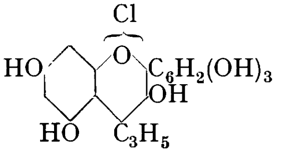
The synthesis of delphinidin 3 : 5-diglucoside, like that of delphinidin 3-glucoside (Reynolds and Robinson, this vol., p. **1039**), is surrounded by difficulties of an experimental nature. The necessary components were already known, but the conditions for the condensation, and even more for the subsequent working up of the product, were difficult to find and must be observed narrowly. The required acetophenone derivative (II) is feebly reactive in flavylium salt syntheses, and thus time is afforded for the auto-condensation of the tetra-acetylglucoside of phloroglucinaldehyde with which it is coupled. This leads to the formation of a green fluorescent xanthylium salt (probably) diglucosidic by-product, the separation of which from the required delphinidin diglucoside is exceedingly difficult, and in bad cases the attempt was perforce abandoned after fruitless efforts.

Ultimately, however, small pure specimens were isolated, and a direct comparison and study of distribution numbers, absorption, and colour reactions show that the substance is identical with delphin chloride.

Attempts to obtain delphin from other natural sources will be made, attention being first focused on the hydrolysis of delphinin. Attempts to isolate the condensation product of the tetra-acetylglucoside of phloroglucinaldehyde in a pure state were unsuccessful, but a crystalline substance was obtained from 2-*O*-benzoylphloroglucinaldehyde under similar conditions. This substance does not appear to be analogous with the product from the glucoside; it forms green crystals soluble in acids to a red solution, and in alkalis it gives blue solutions. These reactions are anthocyanidin-like, and after hydrolytic removal of benzoyl groups a crystalline *chloride* and *picrate* were obtained. The analyses are by no means so sharp as could be wished, but point to the formula $C_{18}H_{15}O_7Cl$, and (III) and (IV) are two constitutions corresponding with this composition.

(III.)





(IV.)

These formulæ are not advanced as suggested constitutions, but merely in order to illustrate the possibilities; it does not seem to be feasible to represent the condensation in any simple fashion, *e.g.*, as leading to a xanthylium salt, and it would appear that one molecule of the phloroglucinol derivative suffers some kind of disruption, the fragments condensing with two further molecules of benzoylphloroglucinaldehyde.

EXPERIMENTAL.

Isolation of Delphin Chloride.—Owing to the large amount of anthoxanthin present, crude extracts of the flowers of *Salvia patens* give a bright green colour on the addition of sodium carbonate. The co-pigment nature of this anthoxanthin is demonstrated in the markedly blue tone of the crude extracts, both when strongly acid or in a buffered series of graded p_H, as compared with the colours obtained after purification of the anthocyanin.

The fresh flowers, from which the calyx but not necessarily the stamens were removed, were collected in bulk, dried in air at 30°, and stored in tins. About 500 g. of dried material were used in all, the purification and subsequent isolation being carried out in three separate portions.

In spite of the high concentration of pigment in the flowers, yet, owing to the necessity of employing a wasteful method of isolation, the final yield of pure crystalline diglucoside was comparatively small.

The dried flowers (180 g.) were soaked for *ca.* 12 hours and extracted with successive quantities of methyl-alcoholic hydrochloric acid (1%). Saturated aqueous lead acetate solution was added slowly and with stirring to the combined dark magenta extracts (3 l.) until the colour began to turn purple. A few drops of dilute hydrochloric acid were added in order to prevent the formation of pseudo-base, and the lead chloride was allowed to settle. 10 C.c. of the decanted liquor were set aside, the addition of lead acetate to the remainder being continued drop-wise with frequent stirring until the blue precipitate of the anthocyanin lead salt assumed a slightly green tinge due to the commencement of precipitation of the lead salt of an anthoxanthin. The 10 c.c. of solution in reserve were then added to remove the green tinge. The whole was well stirred and set aside over-night, and the greenish-blue supernatant liquor containing the greater part of the anthoxanthin and a certain amount of anthocyanin was then decanted. The dark blue precipitate was collected, washed twice with methyl alcohol, drained, and while still moist shaken occasionally during 10 hours at 30° in a large-mouthed stoppered jar with successive amounts of acetic acid (290 c.c. of glacial acid and 10 c.c. of water) until the greater part of the pigment had been extracted. (Extraction with 10% methyl-alcoholic hydrogen chloride may be substituted for that with acetic acid, but only when the amount of residual anthoxanthin is small.)

After cooling and filtering, the blue lead salt was reprecipitated by the addition of an excess of peroxide-free ether. Most of the impurities remained in the pale brown solution, which was decanted and discarded, the solid being washed with ether and converted into the chloride by solution in propyl alcohol (200 c.c.) containing 25% methyl-alcoholic hydrogen chloride (20 c.c.). After being kept for 12 hours, the lead chloride was removed, and the red pigment precipitated with ether. Solution in a less acid mixture of propyl and methyl alcohol, and later in methyl alcohol, and reprecipitation with ether was repeated until lead chloride could no longer be separated.

The pigment was finally precipitated by ether as a slightly sticky opaque mass which still retained its complex (acylated) nature. A small quantity was dissolved in the minimum of hot 0·5% aqueous hydrochloric acid, and the acid concentration of the cooled and filtered solution increased to 5%. The pigment was at once deposited as a sticky amorphous mass, and all attempts to obtain it in a crystalline form were unsuccessful.

This complex pigment resembled the normal diglucoside in its colour reactions, but it was much more readily soluble in water, alcohol, and dilute hydrochloric acid; it had the high distribution ratio of a complex anthocyanin, this value being reduced to that of a diglycoside after treatment while hot with a slight excess of 20% aqueous sodium hydroxide and re-acidification.

The normal diglycoside was obtained from the complex pigment by hydrolysis in cold acid solution. About 0·5 g. of the amorphous pigment was dissolved in methyl alcohol (20 c.c.), and an equal volume of hydrochloric acid (*d* 1·16) added. After 24 hours a considerable amount of an amorphous or semi-crystalline dark bluish-purple pigment had separated, and this had a distribution number that was almost zero (*iso*amyl alcohol). It was very sparingly soluble in cold aqueous acids, alcohols, and water, and on being gently heated with alcohol or water, a colourless pseudo-base was easily formed. After dissolution in boiling 0·5% aqueous hydrochloric acid, however, crystallisation occurred very slowly. It was effected by solution in the minimum of boiling water containing a trace of hydrochloric acid, filtration after keeping overnight, acidification with concentrated hydrochloric acid drop by drop until a slight precipitate formed, filtration after 10 hours and final increase of the acid concentration to 3%, and keeping for 48 hours in the ice-chest. This process was repeated several times until the clusters of short, flat, leaf-like crystals had a striking bronze reflex, were quite homogeneous under the microscope, and with sodium carbonate gave a pure blue reaction fading to a clear green. (In the case of impure specimens the solutions faded to a brownish-green.) The pigment was finally collected, washed with 5% aqueous hydrochloric acid, and dried in the air at room temperature. The yield was about 0·25 g., but further amounts were obtained on working up the less pure fractions. The specimens (A) and (B) were isolated in this way.

Another specimen (C) was obtained by adding an equal volume of 3·0% methyl-alcoholic hydrogen chloride to a concentrated cooled solution of the pigment in 0·5% hydrochloric acid; the diglucoside then crystallised in single short flat crystals, which after drying in air formed a bronze powder containing the same number of molecules of water of crystallisation as the cluster crystal forms previously described.

The solubility of the pigment was found to decrease as the purification proceeded. The fact that during some of the later crystallisations the mother-liquors remained unusually pigmented is attributed, on the strength of the rise in the distribution number, to the presence of small amounts of monoglycosidic anthocyanin, presumably formed by semi-hydrolysis of a part of the diglycoside during the dissolutions in hot dilute acid. Any traces of monoglycoside adhering to the crystals of the diglycoside were easily removed on washing with cold 5% hydrochloric acid.

The specimen (A) was analysed (Found: C, 45·0; H, 5·2; Cl, 5·4; loss in high vacuum at 105° over phosphoric anhydride, 9·9, 9·3; MeO, 0·0. $C_{27}H_{31}O_{17}Cl,3{\cdot}0H_2O,0{\cdot}1HCl$ requires C, 45·0; H, 5·1; Cl, 5·4; $3H_2O$, 7·5%. Found, in material dried in high vacuum at 105°: C, 49·7; H, 4·7; Cl, 4·7. $C_{27}H_{30}O_{17},0{\cdot}9HCl$ requires C, 49·2; H, 4·7; Cl, 4·7; loss from $C_{27}H_{31}O_{17}Cl,3H_2O,0{\cdot}1HCl$, 8·6%). Thus, if our interpretation is correct, two frequently encountered phenomena are together exhibited in this case. In the first place, co-ordinated hydrogen chloride is present in the salt in small proportion, and in the second place the salt loses a portion of its hydrogen chloride on drying at 105° in a vacuum. The properties of *delphin chloride* are described below (p. 1241).

Hydrolysis of Delphin Chloride.—The crystalline glucosidal pigment was hydrolysed by means of an excess of boiling 10% hydrochloric acid during two minutes. The anthocyanidin separated as an apparently amorphous, nearly black precipitate, and showed all the qualitative reactions of delphinidin chloride. It was almost completely extracted from acid aqueous solution by means of amyl alcohol, and the organic layer shaken with sodium acetate became violet blue. Washing with 1% hydrochloric acid extracted a little of the pigment from the amyl-alcoholic solution.

The pigment was not extracted from a solution in 1% hydrochloric acid by means either of a mixture of anisole, ethyl *iso*amyl ether, and picric acid under the conditions prescribed by Robinson and Robinson (*loc. cit.*), or of *cyclo*hexanol–toluene (1 : 5).

On the addition of aqueous sodium hydroxide (10%) to a dilute solution, the pigment was at once destroyed. The air-dried anthocyanidin chloride lost 13·8% on being dried at 105° in a vacuum over phosphoric anhydride. In a quantitative experiment the air-dried delphin chloride (30·2 mg.) was dried at 105° in a vacuum over phosphoric anhydride (loss, 9·6%) and hydrolysed, giving delphinidin chloride (air dried, 17·4 mg.) (A) and glucose (11·63 mg., by Bertrand's method). Calculated as anhydrous materials this gives delphinidin chloride, 54·9%, and glucose, 42·6% ($C_{27}H_{31}O_{17}Cl$ requires $C_{15}H_{11}O_7Cl$, 51·1; $2C_6H_{12}O_6$, 54·2%).

In a second hydrolysis, air-dried delphin chloride (27·3 mg.) gave delphinidin chloride (14·1 mg., and 12·4 mg. after drying at 105° in a vacuum over phosphoric anhydride) and glucose (11·12 mg., by Bertrand's method). Thus the yields are delphinidin chloride (dried), 45·4, and glucose, 40·8% ($C_{27}H_{31}O_{17}Cl,3H_2O$ requires $C_{15}H_{11}O_7Cl$, 47·4; $2C_6H_{12}O_6$, 50·3%). The high values for anthocyanidin and low values for sugar have been previously observed, and in Willstätter's researches a value for glucose 7—12% below the theoretical was frequently recorded (cf. Willstätter and Mieg, *Annalen*, 1914, **408**, 131; Willstätter and Bolton, *ibid.*, p. 54; Willstätter and Weil, *Annalen*, 1916, **412**, 246; Willstätter and Burdick, *ibid.*, p. 224; Willstätter and Bolton, *ibid.*, p. 132). On the other hand, Willstätter and Nolan (*Annalen*, 1914, **408**, 12), in the analysis of cyanin by hydrolysis, obtained good values in the determination of sugar; the figure for cyanidin chloride was too high. The dried delphinidin chloride from the second hydrolysis gave analytical figures indicating loss of hydrogen chloride, so the specimen (A) was crystallised by solution in 0·5% aqueous hydrochloric acid and increase of the acid concentration to 10%. The anthocyanidin chloride crystallised in small, dense aggregates, and was dried in the air for 24 hours (Found : C, 44·9; H, 4·7; Cl, 8·5. $C_{15}H_{11}O_7Cl,3{\cdot}5H_2O$ requires C, 45·1; H, 4·5; Cl, 8·8%). A delphinidin chloride *hydrate* of this composition has not previously been observed, but the behaviour of the substance is known to be dependent on the conditions of crystallisation. Willstätter and Weil (*Annalen*, 1916, **412**, 178) described four hydrates, and among them one with $1{\cdot}5H_2O$ crystallising from 20% or more concentrated acid, and another with $4H_2O$ crystallising from 5% hydrochloric acid. Our preparation evidently represents an intermediate stage.

The colour reactions of this sample in buffered solutions of graded p_H were compared with those exhibited by a specimen of synthetic delphinidin chloride (Bradley, Robinson, and Schwarzenbach, J., 1930, **793**), and no divergences were noted at the time of mixing or on keeping the solutions.

The above observations, taken together, establish the identity of the anthocyanidin chloride with delphinidin chloride. The characterisation of the sugar could not be so satisfactorily accomplished owing to the small amount available, but the following experiment indicates that it is probably glucose and certainly not galactose, which is the only likely alternative.

We compared the behaviour of the sugar obtained on hydrolysis of the natural and synthetic diglycosides in Sörensen and Hangaard's orcinol–sulphuric acid colour reaction under conditions similar to those adopted by Bell and Robinson (this vol., p. 816). The specimens (2·2 mg. N and S) were separately dissolved in 0·1% sulphuric acid, colorimetrically adjusted to equal tinctorial intensity in 30 c.c., mixed with concentrated sulphuric acid (10 c.c.), and heated on the steam-bath for 75 minutes. The delphinidin was then removed by two extractions with *iso*amyl alcohol (in the case of N, the second extraction removed 5·3% of the anthocyanidin, and with S the corresponding value was 5·7%; in both cases the aqueous solution retained a very pale colour perceptible only when looking through 10 cm. or longer), and the aqueous solutions were washed with pure benzene; their volumes were then adjusted by the addition of a few drops of sulphuric acid of the appropriate concentration to N. The actual experiment was carried out as described by Bell and Robinson but with double the volumes throughout. The ratios of the colours observed were the following, S being the more intense where a slight difference was noted. After 7 minutes, 1·0; 17 minutes, 0·97; 35 minutes, 0·95; 65 minutes, 0·98. The colour change was from a smoky brownish-yellow to deep orange-brown, and the colours were identical throughout; a blank showed that the greater part of the coloration observed could be attributed to the sugar in the solution.

7 : 3′ : 4′ : 5′-*Tetrahydroxy*-3 : 5-*di*-β-*glucosidoxyflavylium Chloride* (*Synthesis of Delphin Chloride*).—A solution of ω-tetra-acetyl-β-glucosidoxy-3 : 4 : 5-triacetoxyacetophenone (3·8 g.) (Reynolds and Robinson, *loc. cit.*) and 2-*O*-tetra-acetyl-β-glucosidylphloroglucinaldehyde (2·4 g.) (Robinson and Todd, J., 1932, 2490) in pure, dry ethyl acetate (50 c.c.) was cooled to 0° and saturated with dry hydrogen chloride. After keeping for 6 days at 0°, the solution was diluted with dry ether, and the bright red precipitate was washed with ether, collected, and dried (3 g.). It gave a pure blue solution with an intense green fluorescence in aqueous sodium carbonate; the blue colour faded slowly to a brownish-yellow, but the fluorescence was very stable.

A methyl-alcoholic solution of barium hydroxide (70 c.c. of 6%) was added to a solution of this acetylated material (3 g.) in 0·5% methyl-alcoholic hydrogen chloride (15 c.c.) in an atmosphere of dry hydrogen; a bluish-green precipitate formed, and the mixture was stirred for 4 hours by means of a current of pure hydrogen. Methyl-alcoholic sulphuric acid (21 c.c.; previously titrated) was added, the barium sulphate was removed by centrifuging, and the flavylium salt (0·8 g.) precipitated by the addition of dry ether. This material had a diglucosidic distribution (0·5% hydrochloric acid and *iso*amyl alcohol); it gave a pure blue solution with an intense green fluorescence in aqueous sodium carbonate, and its dilute aqueous solution (pseudo-base) had a dull yellow colour.

This deacetylated material (0·8 g.) was dissolved in hot 1% aqueous hydrochloric acid (10 c.c.), and concentrated hydrochloric acid (2 c.c.) was added to the cooled solution. The sticky precipitate which separated over-night was isolated, and the mother-liquor was diluted with ethyl-alcoholic hydrogen chloride (6·5 c.c. of 5%), inducing crystallisation on keeping for a few hours. The solid (0·2 g.) was collected after 24 hours, and ethyl-alcoholic hydrogen chloride (13 c.c. of 5%) and ether (*ca.* 5 c.c.) were added to the filtrate, giving a second crop of crystals (*ca.* 75 mg.). The mother-liquor yielded only amorphous material on further treatment. The two crystalline fractions, both of which gave a pure blue solution with only a faint green fluorescence in aqueous sodium carbonate, were combined and dissolved in hot 1% hydrochloric acid (75—80 c.c.); the addition of ethyl-alcoholic hydrogen chloride (20 c.c. of 15%) to the cooled solution caused crystallisation to commence after about 30 minutes, and the solid was isolated (0·15 g.) after 24 hours. This material had a more marked fluorescence in sodium carbonate solution than before recrystallisation. Approximately 30 mg. were recrystallised in the same way, but the crystals (10 mg.) were collected after 12 hours and then exhibited no fluorescence in sodium carbonate solution. The remainder of the material was then recrystallised, and collected after 3 hours' keeping (40 mg.). These two fractions were combined (total 80 mg.) with a corresponding fraction (30 mg.) from the condensation described below and recrystallised in the same way (using 55 c.c. of 1% hydrochloric acid and 15 c.c. of 20% ethyl-alcoholic hydrogen chloride) (yield, 55 mg.) (Specimen I) (Found, in material dried in vacuum over phosphoric anhydride at room temperature: C, 44·2; H, 4·9; Cl, 5·3; loss in high vacuum at 105° over phosphoric anhydride, 10·7, 11·5. $C_{27}H_{31}O_{17}Cl,4H_2O,0{\cdot}1HCl$ requires C, 43·9; H, 5·3; Cl, 5·3; $4H_2O$, 9·8%. Found, in material dried in high vacuum at 105°: C, 49·5; H, 4·5; Cl, 5·9. $C_{27}H_{31}O_{17}Cl,0{\cdot}1HCl$ requires C, 48·9; H, 4·7; Cl, 5·9%). The high loss on drying indicated that water may have been removed from the molecule itself ($4{\cdot}5H_2O$ from $C_{27}H_{31}O_{17}Cl,4H_2O,0{\cdot}1HCl$ requires 11·0. $C_{27}H_{31}O_{17}Cl,0{\cdot}1HCl - 0{\cdot}5H_2O$ requires C, 49·3; H, 4·6; Cl, 5·9%). Alcohol and ether were added to the mother-liquors of both preparations, and the material precipitated was fractionated in a similar manner and finally recrystallised from methyl-alcoholic hydrogen chloride (*ca.* 3%) (Specimen II, 30 mg.).

Both specimens gave a pure blue, fading slowly through green to an almost colourless, solution in aqueous sodium carbonate, and their dilute aqueous solutions were colourless. The former contained a trace of monoglucoside, as shown by its distribution to *iso*amyl alcohol.

2-O-*Tetra-acetyl-*β*-glucosidyl-*4-O-*benzoylphloroglucinaldehyde* (?).—A solution of 2-*O*-tetra-acetyl-β-glucosidylphloroglucinaldehyde (2·3 g.) and benzoyl chloride (0·7 g.) in pyridine (60 c.c.) was heated to 35°, kept at room temperature during 30 minutes, and then poured into dilute aqueous hydrochloric acid; the precipitated oil solidified rapidly (2·4 g.). It could not be crystallised, but it separated in an amorphous form, m. p. 72—74°, from aqueous methyl alcohol. The analyses of this material were not sufficiently sharp; it yielded cyanin (colour tests and distribution) when condensed with ω-tetra-acetyl-β-glucosidoxy-3 : 4-diacetoxyacetophenone and then hydrolysed in the usual way.

7-*Benzoyloxy-*3′ : 4′ : 5′-*triacetoxy-*3 : 5-*di*(*tetra-acetyl-*β*-glucosidoxy*)*flavylium Chloride* (?).—A solution of ω-tetra-acetyl-β-glucosidoxy-3 : 4 : 5-triacetoxyacetophenone (1·6 g.) and 2-*O*-tetra-acetyl-β-glucosidyl-4-*O*-benzoylphloroglucinaldehyde (1·5 g.) in pure, dry ethyl acetate (40 c.c.) was cooled to 0° and saturated with dry hydrogen chloride. After 7 days at 0°, the condensation product (1·9 g.) was precipitated with dry ether. It gave a pure blue coloration and a green fluorescence with aqueous sodium carbonate.

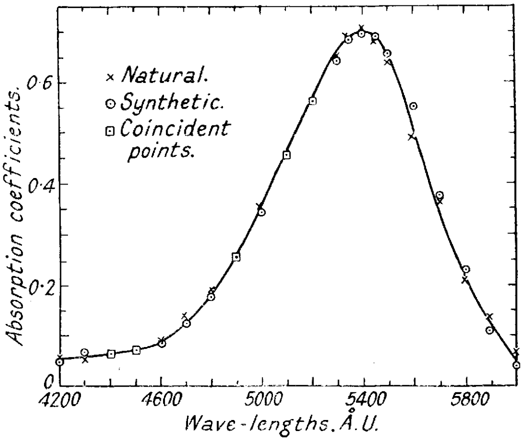
This material was hydrolysed with methyl-alcoholic barium hydroxide as above; the product (0·5 g.) resembled that from the previous condensation and was fractionally crystallised in a similar manner (30 mg. as mentioned above).

Comparison of Natural and Synthetic Anthocyanins.—The three specimens of the natural pigment (A, B, and C, see p. 1238) and specimens I and II of the synthetic material exhibited the same behaviour towards reagents and in all usually observed properties except as indicated below.

The absorption curves of all five were plotted (1·2 mg. in 100 c.c. of 0·1% methyl-alcoholic hydrogen chloride). It appeared from the results that the natural specimen A might prove to be identical with the synthetic specimen II if it were freed from the trace of monoglucoside which appeared to be present. Solutions of these two specimens in 0·5% hydrochloric acid (previously equilibrated with *n*-butyl alcohol) were washed with *n*-butyl alcohol and used for the determination of absorption curves, distribution ratios, and colour reactions in a range of buffered solutions. The absorption curves agreed closely, but the distribution ratios were different, as were also the colour reactions, those of the natural pigment becoming blue at a lower p_H than those of the synthetic substance.

Both specimens were, therefore, recrystallised; the synthetic anthocyanin (10 mg.) was dissolved in warm 0·5% methyl-alcoholic hydrogen chloride (10 c.c.), and concentrated hydrochloric acid (1 c.c.) added to the cooled solution; recrystallisation commenced almost immediately and the solid (3·55 mg.) was collected after 14 hours. The natural anthocyanin (20 mg.) was recrystallised in the same way with a similar result.

The absorption curve of the recrystallised natural specimen agreed with that of Specimen II before recrystallisation (see fig.). The distribution ratios (0·5% hydrochloric acid and *n*-butyl alcohol) were determined as described by Robinson and Todd (J., 1932, 2492), 3·55 mg. being used in each case, and were found to be 11·7 for the synthetic and 12·0 for the natural specimen. The 0·5% hydrochloric acid solutions (saturated with butyl alcohol) remaining from these determinations were used for the comparison in buffered solutions. 1 C.c. was added to 10 c.c. of each buffered solution. The colours given by the two recrystallised anthocyanins were identical, and agreed with those of the synthetic specimen II before recrystallisation (for numbers see Robertson and Robinson, *Biochem. J.*, 1929, **23**, 35); (1) Rose-pink, fading slowly : (3) same, fading to very pale pink in less than 1 min.; (5) bluish-pink, fading to colourless in about 30 secs.; (7) reddish-violet; (9) blue-violet; (11) same; (13) violet-blue; (15) pure blue; (17) dull blue, fading to colourless and then becoming cream in a few seconds. Blue-violet with aqueous sodium acetate; pure blue with aqueous sodium carbonate. After 1 hour (1), (3), (5) unchanged; (7) colourless; (9) faded somewhat and slightly bluer; (11), (13) unchanged; (15) faded to very pale blue; (17) unchanged. After 18 hours, (1) somewhat paler; (3) slightly pink; (5), (7), colourless; (9) almost colourless, slaty tinge; (11) slaty blue; (13) tinge of blue; (15) slightly yellowish; (17) unchanged.



All the specimens, natural and synthetic, were very sparingly soluble (or insoluble) in cold water, alcohol, and dilute acids. Solution occurs readily in hot dilute hydrochloric acid, and crystallisation from such solutions is always slow and sometimes fails altogether. The pseudobase is formed on heating with water or a simple alcohol, and the natural and synthetic specimens were decolorised at the same rates, and the colour was recovered on acidification to the same extent. All specimens dissolved in aqueous sodium carbonate to a bright, pure blue solution, fading slowly to a clear green; this occurred quickly with sodium hydroxide.

Sodium acetate gives a violet-blue, and ferric chloride added to an aqueous solution develops a bluish-violet coloration. In alcoholic solution the iron reaction is violet-blue.

The addition of lead acetate to a solution of the synthetic salt afforded a royal-blue precipitate, the formation of which enabled us to isolate the natural pigment. Addition of picric acid to an acid solution gives no precipitate.

The solubilities in 1% methyl-alcoholic hydrogen chloride were compared, but were only approximately identical (the particular specimens employed had not been fully purified as described above). After seven washings with the methyl-alcoholic acid (the solvent being left in contact with the solid for several days in each case), the difference in concentration was only just perceptible. The natural pigment was then centrifuged and added to the solution from the synthetic pigment and *vice versa*; in neither case was any further solution observed (colorimetric comparison). The solutions were then mixed, and the chlorides of malvin, cyanin, and mecocyanin added to separate portions; in all three cases a considerable increase of the intensity of the colour of the solution occurred.

A saturated solution of the natural pigment in hot 2% methyl-alcoholic hydrogen chloride was cooled, divided, and one portion dusted with the synthetic pigment. Crystallisation occurred first in the inoculated solution, and apparently to some extent (although not wholly) by increase of the size of the visible particles introduced. The habit of crystallisation of the specimens is the same, but we have not seen crystals of the synthetic material that are as well defined as some of those of the natural pigment. This is a matter under the influence of traces of impurities, and with substances of different origin some divergence in the shape and size of crystals is tolerable. On the other hand, rapid crystallisation of natural and synthetic specimens afforded material having exactly the same appearance in solution, in mass, and under the microscope.

Condensation of 2-O-*Tetra-acetyl-β-glucosidylphloroglucinaldehyde under the Influence of Hydrogen Chloride.*—A solution of the aldehyde (1 g.) in pure, dry ethyl acetate (50 c.c.) was cooled to 0° and saturated with dry hydrogen chloride. After 6 days at 0° addition of dry ether to the brown solution gave an orange-yellow solid (0·6 g.). This was dissolved in 0·1% methyl-alcoholic hydrogen chloride (5 c.c.) and hydrolysed with methyl-alcoholic barium hydroxide in an atmosphere of hydrogen in the usual way. The orange-yellow, amorphous product was readily soluble in water and dilute acids to lemon-yellow solutions with diglucosidic distributions to amyl and butyl alcohols. It gave an orange solution with an intense green fluorescence in aqueous sodium carbonate, and it was readily hydrolysed to a sugar-free (as shown by its high distribution to amyl alcohol) compound which gave yellow acid and neutral solutions, and a light pinkish-brown coloration with no fluorescence in aqueous sodium carbonate. It is doubtless this substance that adhered to our synthetic anthocyanin so tenaciously.

Auto-condensation Product from 2-O-*Benzoylphloroglucinaldehyde.*—A solution of 2-*O*-benzoylphloroglucinaldehyde (1 g.) in pure, dry ethyl acetate (50 c.c.) was cooled to 0°, saturated with dry hydrogen chloride and kept at 0°. The solution became dark red over-night, and crystals began to separate after 2 days; these were collected (0·4 g.) after 5 days, and dry ether was added to the filtrate, precipitating a brown amorphous powder (0·2 g.). The crystals appeared to be a mixture of green and purple substances, but the latter proved to be the starting material coloured by traces of the condensation product and was removed by extraction with ether (residue, 0·3 g.). In another preparation, 0·4 g. of 2-*O*-benzoylphloroglucinaldehyde yielded 0·2 g. of the green condensation product.

This substance was practically insoluble in water or aqueous acids, but dissolved readily in methyl-alcoholic hydrogen chloride, giving a deep reddish-violet solution; it gave a violet-blue and a pure blue solution respectively in aqueous sodium carbonate and sodium hydroxide. It was not readily recrystallised, and was accordingly hydrolysed by means of sodium hydroxide (0·2 g. in 5 c.c. of ethyl alcohol and 5 c.c. of 10% aqueous sodium hydroxide) in an atmosphere of hydrogen. The solid dissolved slowly to a stable, dark blue solution, and after 4 hours at room temperature concentrated hydrochloric acid (3 c.c.) was added. The deep violet-red solution was heated to 50—60° to complete the formation of the chloride; it was then cooled and a further 10 c.c. of concentrated hydrochloric acid added. Crystals began to separate immediately, and were collected after 12 hours; these tended to become sticky in air, but were quite stable after drying in a vacuum; they were dark greyish-violet with a green reflex.

This salt was appreciably soluble in cold water and dilute hydrochloric acid, and readily soluble in the hot solvents to bluish-red solutions, brownish-red when concentrated. The aqueous solution is bluer in tone than the acid solution. The bluish-violet solution in aqueous sodium carbonate is stable for several hours, but the pure blue solution in aqueous sodium hydroxide fades to light violet after 4 hours. The pure blue ammoniacal solution fades through violet to a pale plum colour in about 4 hours; it gave a light reddish-violet coloration with aqueous sodium acetate. Its distribution to *iso*amyl alcohol was 100, the alcoholic solution being violet-red, and violet-blue in thin layers; with sodium acetate the alcoholic layer became a bluer red, pure blue in thin layers; with sodium carbonate the colour changed to blue and passed into the aqueous layer. Its distribution to ethereal picric acid was 100. It gave no reaction with ferric chloride.

This material was recrystallised by solution in hot 1% aqueous hydrochloric acid and addition of concentrated hydrochloric acid to the cooled solution (Found: C, 50·2; H, 4·8; Cl, 9·1. $C_{18}H_{15}O_7Cl,3H_2O,0{\cdot}1HCl$ requires C, 49·6; H, 4·8; Cl, 9·0%). It was noticed that after several weeks the initially deep red mother-liquors became yellow, and a brown solid separated which gave a pinkish-yellow solution in aqueous sodium carbonate and appeared to be allied to the brown solid obtained in the original condensation. If a larger volume of concentrated hydrochloric acid was added in the recrystallisation process, so that almost the whole of the solid was precipitated, the mother-liquors were yellow, and also a solution of the substance in 1% hydrochloric acid became yellow when refluxed for 4 hours.

A further quantity (0·2 g.) of the original condensation product was hydrolysed with sodium hydroxide in an atmosphere of hydrogen as before. The resultant solution was acidified with concentrated hydrochloric acid (2 c.c.), warmed slightly, and immediately mixed with an equal volume of hot 1% aqueous picric acid. A small amount of solid separated on cooling, and the addition of a further 2 vols. of 1% aqueous picric acid gave a voluminous, beautifully crystalline, deep purple *picrate*. This was recrystallised twice from 50% methyl alcohol (Found : loss in high vacuum at 105°, 5·6, 6·9. Found, in dried material : C, 50·7; H, 2·9; N, 7·7. $C_{24}H_{17}O_{14}N_3$ requires C, 50·4; H, 3·1; N, 7·3%). A crystalline *chloride* was obtained by dissolving this picrate in methyl-alcoholic hydrogen chloride and adding ether; this was analysed without recrystallisation and found to contain some inorganic matter which has been allowed for in the results (Found : C, 53·3; H, 4·8; Cl, 8·2; loss in high vacuum at 105°, 7·0. $C_{18}H_{15}O_7Cl,1{\cdot}5H_2O$ requires C, 53·3; H, 4·4; Cl, 8·6; H_2O, 6·7; loss of $1{\cdot}5H_2O + 0{\cdot}1HCl$ requires 7·7. Found in material dried in high vacuum at 105° : C, 57·4; H, 4·5; Cl, 7·9. $C_{18}H_{14}O_7,0{\cdot}9HCl$ requires C, 57·7; H, 4·0; Cl, 8·3%). The reactions of this material agreed in all respects with those of the specimen described above.

The brown amorphous condensation product (0·2 g.) was hydrolysed with sodium hydroxide and precipitated from aqueous acid solutions in the same way as an almost black, probably amorphous, solid (Found : C, 51·9; H, 4·1; Cl, 13·5; loss in high vacuum at 105°, 8·7; Cl, after drying, 14·3%). It gave yellow solutions in dilute hydrochloric acid, and pinkish-yellow solutions in sodium carbonate, reactions which suggest a xanthylium salt constitution.

The authors are indebted to Mr. J. Resuggan for assistance in the preparation of material in connexion with the synthetic work. They also thank the Department of Scientific and Industrial Research for a Research Grant (R. S.-M.) and the Royal Commissioners for the Exhibition of 1851 for an Overseas Studentship (T. M. R.).

THE DYSON PERRINS LABORATORY, OXFORD UNIVERSITY.
THE JOHN INNES HORTICULTURAL INSTITUTION.

[*Received, July 6th*, 1934.]