CLXXXIII. AN ANTHOCYANIN OF *OXYCOCCUS MACROCARPUS* PERS.

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HAVING effected a synthesis of idaein chloride we wished to secure a supply of the natural pigment which Willstätter and Mallison [1915] isolated from the fruits of *Vaccinium vitis idaea* Linn. (this is the English cowberry, the true cranberry with smaller fruits being *V. oxycoccus*), but the latter not being in season we secured supplies of the much larger American cranberries in the hope that these might be a source of idaein.

The first berries employed originated in Newfoundland and had a generally similar appearance to the second batch which came from the Cape Cod district.

The two were worked up separately, and the derived pigments were only combined when it was found that they had identical properties.

We have obtained the pure anthocyanin in small quantity only, but sufficient to establish its identity as a 3- β -glucosidylpeonidin chloride (I). On hydrolysis it yielded peonidin chloride (II), characterised by its analysis, habit of crystallisation and other properties, and by its colour reactions in buffered solutions.

The determination of the nature of the carbohydrate residue could not be made directly because of the paucity of material for investigation, but it happened that 3- β -glucosidylpeonidin chloride had been synthesised (Levy and Robinson) some time before the oxycoccyanin was completely purified and direct comparison has shown that the two specimens are identical.

The most probable alternative to the glucosidic configuration is the galactosidic, and we therefore synthesised 3- β -galactosidylpeonidin chloride (paper submitted to the Chemical Society) and found that this anthocyanin closely resembled the glucoside. It differed, however, in its "distribution number" being less readily extracted by amyl alcohol from a solution in 0.5 % hydrochloric acid than is the glucosidic anthocyanin.

The new anthocyanin fills a gap in the series of monoglucosides, being a methoxycallistephin and a *desmethoxyoenin*; we propose to designate it "oxycoccicyanin" following a precedent established by Nolan in naming "sambucicyanin." The alternatives are unsatisfactory, since "oxycoccin" suggests, perhaps, a derivative of carminic acid and "macrocarpin," referring simply to the size of the fruits, is not sufficiently specific.

EXPERIMENTAL.

(A) The cranberries from Newfoundland were rather smaller than those from Cape Cod; they were minced and submitted to great pressure in linen bags. We are indebted to Prof. J. C. Drummond for giving facilities and assistance at this stage. The skins were then extracted with acetic acid and the filtered extracts precipitated by the addition of ether. The syrupy material was again dissolved in acetic acid and precipitated by means of ether. The sticky red mass was then triturated with 2 % ethyl alcoholic hydrogen chloride and the solution filtered and precipitated by means of ether; this precipitate was dissolved in hot saturated aqueous picric acid and the solution kept in the ice-chest for several weeks. The crude picrate was extracted by means of boiling methyl alcohol and hydrogen chloride (to make a 1 % solution) introduced; the filtered solution was then mixed with an equal volume of ether. This precipitated impurities, including a cyanidin derivative (positive iron reaction); the filtrate from these was diluted with much ether and some light petroleum was also added. The intention had been to precipitate the chloride but, instead, the *picrate* crystallised on the sides of the vessels, which were kept for some weeks. The picrate could now be crystallised from hot ethyl alcoholic aqueous picric acid in red needles. After allowing ample time for complete separation the salt was collected, dissolved in hot methyl alcohol containing 2 % of hydrogen chloride and the chloride precipitated by means of ether. This gave a nearly pure amorphous specimen with a dark beetle-green lustre.

(B) The peelings of Cape Cod cranberries (60 lb.) were allowed to stand in 2 % methyl alcoholic hydrochloric acid for 2 to 3 weeks, using the counter flow method with the solvent. The solution was then filtered, the solvent being carefully pressed from the peelings, which were then washed with fresh solvent, the filtrate being added to the original solution. The colour was not entirely removed, but the skins were very light coloured after this treatment.

The alcoholic solution (*ca.* 11,000 cc.) was then divided into portions of 500 cc. and each portion was treated as follows: dry picric acid (10 g.) was added and the mixture was warmed to 40° and then poured into a large evaporating dish. Concentrated ammonium hydroxide (20 cc., the amount calculated almost to neutralise the acid present) was added and the mixture was thoroughly stirred and then allowed to stand in a draught chamber for 2 weeks; at the end of that time there was a crystalline residue covered with a sticky gum. The residues were collected, washed with saturated aqueous picric acid (1000 cc.), and quantities of ether, and dried (200 g.) (X).

This product was divided into 25 g. portions and each portion was boiled with absolute methyl alcohol to remove the pigment present. The combined extracts (*ca.* 1500 cc.) were filtered and dry ether (3 vols.) was added to the filtrate. A heavy precipitate formed on the addition of the ether, and this was filtered off and dried; very little anthocyanin was found in this material.

The filtrate was diluted with an equal volume of light petroleum (B.P. 40–60°) and the anthocyanin extracted by means of 1 % hydrochloric acid (500 cc.). The solution was then evaporated in a vacuum at the ordinary temperature and the picrate slowly separated. This was collected, dried and dissolved in the minimum of 3 % ethyl alcoholic hydrogen chloride; the solution was kept, filtered and the chloride precipitated by the addition of ether; the last process was repeated. The anthocyanin at this stage was compared with the product of process (A) from the Newfoundland berries and found to be entirely identical in alkali-colour reactions and in distribution number, using amyl alcohol with aqueous hydrochloric acid and ethyl acetate with aqueous picric acid. The specimens were therefore combined (see below).

The pigment was completely extracted from a typical berry with formic acid (1.5 cc.), the solution was filtered and diluted to 50 cc. with absolute methyl alcohol, and the amount of pigment determined colorimetrically by comparison with a solution of 2.0 mg. of idaein in methyl alcohol (50 cc.). The two solutions were approximately identical in colour. A pound of berries contains approximately 500 berries, *i.e.* 1.0 g. of pigment.

A determination of the relative amounts of anthocyanidin, monoglucoside, and diglucoside present in the mother-liquor from the original picrate precipitate (X) was made as follows. The anthocyanidin was extracted by means of ether saturated with picric acid, the monoglucoside by carvone-picric acid, and the diglucoside by amyl alcohol-acetophenone (2 : 1 vol.) picric acid solution. In each case the solution of pigment was shaken with the extraction medium until no more colour was removed, the combined extracts were then diluted with ether (light petroleum in the case of anthocyanidin) and shaken out with 0.5 % hydrochloric acid (50 cc.). This aqueous solution was freed from picric acid by extraction with ether and then compared colorimetrically with cyanidin, 3- β -galactosidylpeonidin chloride or mecocyanin chloride. The solution was found to contain 0.03 mg. anthocyanidin, 0.21 mg. monoglucoside, and 0.07 mg. diglucoside per cc. This procedure is due to (Willstätter and Schudel [1918]).

Examination of the anthocyanin. The crude chloride (0.8 g.) was dissolved in warm 5 % ethyl alcoholic hydrogen chloride, but crystallisation did not occur on cooling, and the solution was kept in a vacuum in a vessel containing ether. The alcohol was gradually diluted with ether and a poor type of crystallisation ensued. The indefinitely crystalline crust was then triturated with 5 % alcoholic hydrogen chloride and, although a large loss resulted, an insoluble material was obtained which could be crystallised by dissolution in 0.5 % hydrochloric acid and addition of 5 % ethyl alcoholic hydrogen chloride, after

which the solution was kept in the ice-chest. A recrystallisation in the same way furnished rectangular, prismatic needles, deep-red by transmitted light under the microscope and almost black in mass. (Found in air-dried material: C, 47.9; H, 5.4; Cl, 6.5; loss at 105° in a high vacuum over phosphoric anhydride, 7.4, 7.9. $C_{22}H_{23}O_{11}Cl$, $3H_2O$ requires C, 47.8; H, 5.3; Cl, 6.4; $2H_2O$, 6.5. Found in the material dried at 105°: C, 51.0; H, 5.2; Cl, 6.6; MeO, 6.1. $C_{22}H_{23}O_{11}Cl$, H_2O requires C, 51.2; H, 4.8; Cl, 6.8; MeO, 6.0 %.)

The synthetic 3-glucosidylpeonidin chloride contains $2H_2O$, both molecules being evolved at 110° in a high vacuum. Little importance is, however, attached to this divergence, since it has been the repeated experience of workers in the anthocyanin field that the degree of hydration of the salts can be greatly affected by small concentrations of foreign substances.

Hydrolysis to the anthocyanidin. The last two alcoholic mother-liquors contained the major part of the pigment, and the alcohol was removed by distillation; the residue was then heated on the steam-bath until a test with amyl alcohol showed that the anthocyanin had all been hydrolysed. Care was taken not to heat any longer than was necessary. The anthocyanidin was converted into picrate and this was crystallised from methyl alcohol and then transformed into the chloride by means of methyl alcoholic hydrogen chloride and ether. The precipitated chloride was well dried and crystallised by dissolution in hot ethyl alcohol and addition of one-third of the volume of 12 % hydrochloric acid. It separated in prisms having a green reflex and appearing orange-brown by transmitted light under the microscope. (Found: C, 52.5; H, 4.7; loss at 105° in a high vacuum, 7.4. $C_{16}H_{13}O_6Cl$, $1.5 H_2O$ requires C, 52.8; H, 4.4; $1.5 H_2O$, 7.4. Found in anhydrous material: C, 57.2; H, 4.2; MeO, 9.4. $C_{16}H_{13}O_6Cl$ requires C, 57.1; H, 3.9; 1MeO, 9.2.)

Peonidin chloride synthesised by Murakami and Robinson [1928] was crystallised in the same way and with the same results ($1.5 H_2O$), but this is the first time that the prism-crystals have been obtained with peonidin from natural sources. The very small amount of the pure peonidin chloride that remained was employed for a study of the colour reactions in buffered solutions of graded p_H . There was only sufficient for twelve tests and the solutions numbered (2)–(13), inclusive in the memoir of Robertson and Robinson [1929], were employed; the results with these twelve solutions agreed completely with the description there given for peonidin.

Properties of oxycoccicyanin chloride and comparisons with 3-β-glucosidylpeonidin chloride. The anthocyanin is rather readily soluble in 0.5 % hydrochloric acid; much less readily soluble in 5 % hydrochloric acid to solutions which become very much more blue-red on dilution with alcohol. Its solution in aqueous sodium carbonate is rich violet in colour and there is no change on the addition of sodium hydroxide, except that the colour soon fades becoming greenish yellow.

The *absorption* of light in the visible region was identical with that of a specimen of 3-β-glucosidylpeonidin (see Fig. 1). The slight divergence from

3- β -galactosidylpeonidin is not significant and probably means that the galactoside was exceptionally pure.

Fig. 1. $\odot$ 3- β -Glucosidylpeonidin chloride (natural).
 $\triangle$ 3- β -Glucosidylpeonidin chloride (synthetic).
 $\square$ 3- β -Galactosidylpeonidin chloride.
 $\times$ Coincident points.

The *distribution number* was determined using 0.5 % hydrochloric acid (50 cc.) and amyl alcohol (50 cc.). Using 2.432 mg. of the anthocyanin, 26.6 % passed into the alcoholic layer; a second shaking extracted 28.5 % of the material remaining in the aqueous solution. Using 4.907 mg. the corresponding figures were 21.4 % and 22.3 %. These figures did not agree as sharply as we anticipated either with the synthetic glucoside or synthetic galactoside.

However, we had previously tested the crude anthocyanin against glucosidylpeonidin and found identity, so that we were inclined to suspect that the conditions were in some way not quite standard. Using exactly the same conditions, that is the same actual samples of 0.5 % hydrochloric acid and amyl alcohol, we found that with synthetic 3- β -glucosidylpeonidin chloride (5.017 mg.) the distribution numbers for the first and second shakings were 21.9 and 22.6 respectively.

Colour reactions in buffered solutions. The solutions employed were those specified by Robertson and Robinson [1929], and oxycoccicyanin chloride, 3- β -glucosidylpeonidin chloride and 3- β -galactosidylpeonidin chloride gave identical results, except that a few notes on very slight and probably non-significant divergences of the galactoside were made. (2) salmon pink, rapidly fading; (3) similar, fading more rapidly; (4) a little bluer, also fading;

(5) cherry red, much more stable; (6) bluer, more intense, more stable; (7) similar but brownish tinge; (8) a shade bluer; (9) similar, dull red-violet; (11) sharp change to deep violet; (13) the same; (450 cc.), the same but rapidly fading to smoky colour and then yellow. After 1 hour: (2), (3), (4) and (5) formed a decreasing faded series; (6) faded, but intensity between (2) and (3), the galactoside a little the more stable; (7), (8) faded brown-violet; (9) bluer and more intense; (11), (13) violet, little changed; (450 cc.), yellow. Hydrochloric acid added to (4) and (5) restored the colour quantitatively. After 24 hours the solutions in the three series were identical: (2) pink; (3) nearly colourless; (6) colourless; (7), (8), (9) gradual increase to smoky violet; (11), (13) good violet; (450 cc.) yellow.

The identical colour reactions of chrysanthemin chloride and idaein chloride (synthetic specimens) have also been observed by the standard method. (1) salmon red; (2) a little bluer; (3) a little bluer, fading most rapid here; (4) cherry red of greater tinctorial intensity; (5) deeper, fades more slowly; (6) still deeper; (7) duller and browner; (8) still browner; (9) brown-red; (10) the same; (11) the same; (12) the same; in the series (9)–(12) we thought idaein had a little the greater intensity; (13) brown-violet; (14) reddish violet; (15) violet, (13)–(15) lose the blue tinge on keeping; (16) much bluer violet; (17) pure blue fading to greenish blue and then yellow. After 24 hours the 2 series were identical: (1) salmon red; (2) weak bluish red with small precipitate; (3), (4) nearly colourless with violet precipitate; (5) weak violet and precipitate; (6) violet, no precipitate; (7), (8), (9), (10), (11) increasing series to violet-red; (12), (13) a little weaker colour than (11); (14) much weaker violet-red; (15), (16) weak yellow; (17) more intense yellow.

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SUMMARY.

The fruits of *Oxycoccus macrocarpus* Pers. have been found to contain a pigment which has been isolated as the chloride and identified as a 3-glucoside of peonidin by analysis and comparison with a synthetic specimen.

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