218. *Experiments on the Synthesis of Anthocyanins. Part XXI. 3-β-Glucosidyldelphinidin Chloride.*

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In order to cover the field of anthocyanin synthesis it was necessary to include the delphinidin derivatives and many attempts to obtain delphinidin 3-glucoside have been made in the course of the last three years. At every stage unusual difficulties were met and although the account of our eventual success will read very much like that of any other of the syntheses, the fact is that it has cost us an altogether disproportionate expenditure of time and labour.

The first problem was the preparation of an ω-hydroxy-3 : 4 : 5-triacyloxyacetophenone. Dr. L. F. Levy found that ω-diazo-3 : 4 : 5-triacetoxyacetophenone, $(AcO)_3C_6H_2\cdot CO\cdot CHN_2$ (Bradley, Robinson, and Schwarzenbach, J., 1930, 793), could be converted into *ω-formoxy-3 : 4 : 5-triacetoxyacetophenone*, $(AcO)_3C_6H_2\cdot CO\cdot CH_2\cdot O\cdot CHO$ (I), by means of absolute formic acid, but he was unable to hydrolyse the formic ester group without affecting the rest of the molecule.

He experimented also on the direct hydrolysis of the diazo-ketone with 50% formic acid on the steam-bath, but isolated the formoxy-ketone. He then tried, without success, 15% formic acid, dilute sulphuric acid, aqueous alcohol and 60% hydrofluoric acid as the hydrolytic agents.

The work was taken up by Dr. H. S. Boyd-Barrett, who prepared *ω-diazo-3 : 4 : 5-tribenzoyloxyacetophenone* and the related *ω-acyloxy-ketones* and studied the semi-hydrolysis of these substances but without the desired outcome.

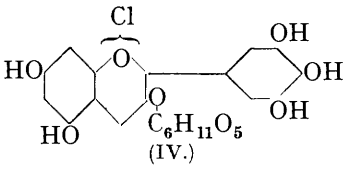
Mr. L. H. Kent also found this line unpromising and he worked with *ω-toluenesulphonyloxy-*, *ω-chloro-*, and *ω-bromo-triacetoxyacetophenones* in attempts to condense these substances with 2 : 3 : 4 : 6-tetra-acetyl glucose. Mr. Kent then returned to the original idea and discovered the conditions necessary for the hydrolysis of the ω-diazotriacetoxyacetophenone to a carbinol, $(AcO)_3C_6H_2\cdot CO\cdot CH_2\cdot OH$ (II), under the influence of 50% formic acid. But the carbinol obtained at first was not sufficiently pure and we have found that all the circumstances of this process must be narrowly observed and controlled; it is then possible to obtain consistent results. Furthermore, material isolated by the usual method, that is, by dilution of the reaction mixture with water, could not be obtained in a sufficiently high state of purity even after repeated crystallisation and the glucoside prepared from it could not be crystallised. The necessary modifications of the process are described in the experimental section.

ω-Tetra-acetyl-β-glucosidoxy-3 : 4 : 5-triacetoxyacetophenone,

$$(AcO)_3C_6H_2\cdot CO\cdot CH_2\cdot O\cdot C_6H_7O(OAc)_4 \quad \text{(III)},$$

was prepared from *ω-hydroxy-3 : 4 : 5-triacetoxyacetophenone* in the usual way. It was found to exist in two crystalline forms of m. p. 87—88° and 141—142° respectively, the higher-melting form being the more stable. The condensation of this glucoside with 2-*O*-benzoylphloroglucinaldehyde by means of hydrogen chloride in ethyl acetate solution proceeded smoothly, but much more slowly than in the cases previously described. The removal of the benzoyl and acetyl groups from the product also presented difficulties. The anthocyanin was completely destroyed by treatment with cold aqueous barium hydroxide in the absence of oxygen under conditions which permitted the isolation of delphinidin chloride.

Slightly better results were obtained with 50% methyl-alcoholic barium hydroxide, but the product was not sufficiently pure to admit of the isolation of crystalline material. The use of absolute methyl-alcoholic barium hydroxide was, however, attended with a minimum of decomposition and gave a completely acyl-free delphinidin monoglucoside (IV). This was purified through the picrate and then recrystallised several times as the chloride. The substance has been characterised by means of colour reactions, absorption spectrum and distribution number. There can be little doubt that it is a naturally occurring anthocyanin, but it has not yet been separated in a pure condition from plant material.



(IV.)

The isolation from Awobana paper of a delphinidin monoglucoside has been described by Kuroda (*Proc. Imp. Acad. Japan*, 1933, **9**, 94), and Karrer and de Meuron (*Helv. Chim. Acta*, 1933, **16**, 292) report the separation of a slightly impure delphinidin 3-monoglucoside from the mixture of pigments present in *Viola tricolor*.

Vicin from a scarlet-flowered vetch (Karrer and Widmer, *Helv. Chim. Acta*, 1927, **10**, 67, 73) was stated to consist of a rhamnoside and a monoglucoside of delphinidin, and gentianin, the pigment of flowers of *Gentiana acaulis*, was described as a *p*-hydroxycinnamoyl derivative of delphinidin monoglucoside (*idem, ibid.*). Again, Karrer and Widmer (*ibid.*, p. 9) fractionated the picrates of the mixed colouring matters of the bilberry (Heidelbeeren) and found that they consisted of a monoglucoside and a monogalactoside of delphinidin together with malvidin monoglucoside and possibly the glucoside or the galactoside of a delphinidin monomethyl ether.

In view of the fact that all monoglucosides of other anthocyanidins so far isolated have been proved to be 3-glucosides, it is probable that our delphinidin 3-glucoside is identical with one or more of the naturally occurring anthocyanins mentioned above; it seems, therefore, to be worth naming. However, it will suffice at this stage to propose the expressions myrtillin-a and myrtillin-b for the particular glucoside and galactoside, respectively, of delphinidin that occur in the skins of bilberries.

Myrtillin-a is almost certainly delphinidin 3-β-glucoside and further evidence on this point will be submitted later.

Experimental.

Substances prepared by Dr. H. S. Boyd-Barrett.—Tribenzoylgalloyl chloride, m. p. 124° after one crystallisation from carbon tetrachloride, was obtained in excellent yield by the action of boiling thionyl chloride on the acid. ω-*Diazo*-3 : 4 : 5-*benzoyloxyacetophenone* separated when a chloroform solution of the chloride (1 mol.) was added to an ethereal solution of diazomethane (2·2 mols.). It crystallised from benzene as light yellow prisms, m. p. 169° (decomp.) (yield, almost theoretical) (Found : C, 68·3; H, 3·8; N, 5·2. $C_{29}H_{18}O_7N_2$ requires C, 68·8; H, 3·6; N, 5·5%). ω-*Formoxy*-3 : 4 : 5-*tribenzoyloxyacetophenone* offered difficulty in its preparation and the application of heat is undesirable : the diazo-ketone was mixed with absolute formic acid; the product collected after 6 weeks crystallised from formic acid in colourless prismatic needles, m. p. 116—117° (Found : C, 68·5; H, 3·9. $C_{30}H_{20}O_9$ requires C, 68·7; H, 3·8%). Many unsuccessful attempts were made to hydrolyse the formoxy-group.

ω-*Acetoxy*-3 : 4 : 5-*tribenzoyloxyacetophenone*, obtained by heating the diazo-ketone with glacial acetic acid, crystallised from alcohol in pale yellow, rectangular plates, m. p. 138—140° (Found : C, 68·8; H, 4·2. $C_{31}H_{22}O_9$ requires C, 69·1; H, 4·1%).

Substances prepared by Mr. L. H. Kent.—ω-Diazo-3 : 4 : 5-triacetoxyacetophenone was decomposed by means of *p*-toluenesulphonic acid in acetone solution at 40°, and the solvent removed. The residue could with difficulty be induced to solidify on trituration with several changes of water, and then crystallised from light petroleum–acetone (5 : 1) in colourless needles, m. p. 134° (yield, 90%) (Found : C, 54·5; H, 4·3; S, 7·0. $C_{21}H_{20}O_{10}S$ requires C, 54·3; H, 4·3; S, 6·9%). This substance is ω-p-*toluenesulphonoxy*-3 : 4 : 5-*triacetoxyacetophenone*; the 3 : 4 : 5-tribenzoyloxy-analogue forms colourless needles, m. p. 148°. The required carbinol could not be obtained from these sulphonic esters either by the usual methods of hydrolysis or by means of benzylamine. The triacetoxy-diazo-ketone was also decomposed by picric acid, but there is some doubt as to the nature of the product, m. p. 212—215°, which could be crystallised from acetic acid.

It is remarkable that ω-diazo-3 : 4 : 5-triacetoxyacetophenone does not react readily with

hydrogen bromide in chloroform solution, but in acetone liberation of nitrogen occurs at 0°. The solution was evaporated when gas was no longer evolved; the residue crystallised from alcohol as light lemon-yellow rhombs, m. p. 123° (mixed with diazo-ketone, m. p. 103°) (yield, 95%) (Found: C, 45·3; H, 3·3; Br, 21·0. $C_{14}H_{13}O_7Br$ requires C, 45·0; H, 3·5; Br, 21·3%). *ω-Bromo-3 : 4 : 5-triacetoxyacetophenone* is readily soluble in acetone, chloroform, benzene, and acetonitrile, sparingly soluble in ether, and very sparingly soluble in light petroleum. The *ω-chloro*-analogue forms colourless rhombs, m. p. 114·5°, from alcohol, in which it is more sparingly soluble than the bromo-derivative (Found: C, 51·2; H, 3·9; Cl, 10·2. $C_{14}H_{13}O_7Cl$ requires C, 51·1; H, 3·9; Cl, 10·5%). Many attempts to condense these *ω-p*-toluenesulphonoxy- and ω-halogenated-acetophenones with 2 : 3 : 4 : 6-tetra-acetyl glucose were made in order to obtain the required glucosides, but although indications of the desired reactions were obtained, the general scheme proved to be unsatisfactory and was abandoned in favour of a renewed attack on the lines described below.

ω-Formoxy-3 : 4 : 5-triacetoxyacetophenone.—When pure formic acid (5 c.c.) was added to ω-diazo-3 : 4 : 5-triacetoxyacetophenone (1 g.), the solid dissolved rapidly in the cold with vigorous evolution of nitrogen; the reaction was completed by heating at 55° for 10 minutes. Addition of water (50 c.c.) precipitated a crystallising oil (0·7 g.). After three crystallisations from benzene, the product had m. p. 99—100° (Found: C, 53·3; H, 4·2. $C_{15}H_{14}O_9$ requires C, 53·3; H, 4·2%) (Dr. Levy precipitated the substance from the reaction mixture with dry ether and crystallised it from light petroleum in prismatic needles, m. p. 99—100°. Found: C, 53·5; H, 4·5%). Boiling with aqueous alcohol for several hours did not effect the hydrolysis of this substance, although a similar procedure worked well in the case of the intermediate required for oenin (compare J., 1931, 2707). Other methods were tried but were equally unsuccessful.

ω-Hydroxy-3 : 4 : 5-triacetoxyacetophenone (II).—Both the yield and the purity of the product are adversely affected by using larger quantities than those given below.

Freshly distilled formic acid (25 c.c. of 50%) was added to ω-diazo-3 : 4 : 5-triacetoxyacetophenone (5 g.), and the mixture heated at 55—60° for 10 minutes with stirring. The initially rapid evolution of nitrogen almost ceased and the colourless or slightly yellow solution obtained was diluted with water (25 c.c.), cooled, and filtered through charcoal, the precipitate being discarded. The filtrates from three batches were combined, diluted with water (about 300 c.c.), and neutralised with solid potassium bicarbonate in the presence of chloroform. The neutral solution was extracted thrice with chloroform and the combined extracts were washed once with potassium bicarbonate solution and once with water, dried over sodium sulphate, and evaporated to a small volume (25—30 c.c.) under diminished pressure. The addition of light petroleum (2—3 vols.) precipitated an oil, which crystallised rapidly (12·5 g.) and was sufficiently pure for the preparation of the glucoside; recrystallised from benzene, it formed colourless needles, m. p. 87—88° (Found: C, 54·5; H, 4·6; Ac, 42·6. $C_{14}H_{14}O_8$ requires C, 54·2; H, 4·5; 3Ac, 41·6%).

This *ω-hydroxy-3 : 4 : 5-triacetoxyacetophenone* is readily soluble in acetone, chloroform, ether, ethyl acetate, hot benzene, and hot alcohol, slightly soluble in water, and insoluble in light petroleum. It reduces Fehling's solution in the cold, is insoluble in aqueous sodium carbonate, and gives no colour with ferric chloride in alcoholic solution. When heated for 5 minutes in a boiling water-bath with acetic anhydride (1·5 c.c.) and pyridine (0·5 c.c.), ω-hydroxy-3 : 4 : 5-triacetoxyacetophenone (0·3 g.) afforded ω : 3 : 4 : 5-tetra-acetoxyacetophenone (m. p. 124—125° after recrystallisation from alcohol) in quantitative yield. No depression in m. p. was noted when this product was mixed with a specimen prepared directly from ω-diazo-3 : 4 : 5-triacetoxyacetophenone (Bradley, Robinson, and Schwarzenbach, *loc. cit.*).

ω-Tetra-acetyl-β-d-glucosidoxy-3 : 4 : 5-triacetoxyacetophenone (III).—Freshly prepared silver carbonate (25 g.) was added to a solution of ω-hydroxy-3 : 4 : 5-triacetoxyacetophenone (10 g.) and tetra-acetyl-α-glucosidyl bromide (20 g.) in pure dry benzene (120 c.c.) at 40°, and the mixture was shaken vigorously and kept at 50—60° for 30 minutes. The product was filtered through charcoal, and light petroleum was added to the clear colourless filtrate, giving a syrup, which was washed twice with hot water, dissolved in methyl alcohol, and reprecipitated by the addition of water. The amorphous product was dissolved in hot 50% acetone, from which it crystallised in colourless needles (7 g.), m. p. 78—80°.

The *glucoside* was recrystallised from methyl alcohol and when first prepared had a constant m. p. 87—88° (Found: C, 52·3; H, 5·1; Ac, 48·9.* $C_{28}H_{32}O_{17}$ requires C, 52·5; H, 5·0; 7Ac,

* Kindly carried out by Dr. H. Roth of Heidelberg, using the Kuhn–Roth micro-method (CrO_3 oxidation and estimation of $C_2H_4O_2$ formed). Derivatives of polyhydric phenols frequently give somewhat high results.

47·0%). Later a second form, m. p. 141—142°, was obtained and it was then found that the low-melting form always passed into the high-melting form when its solution was seeded with the latter or was boiled for several minutes to remove all traces of solid before cooling. The reverse process could not be brought about and no intermediate m. p.'s were observed. Both forms had the same rotation, $[\alpha]^{15°}_{Hg\ green} = -26·0°$ ($c = 2·000$), in chloroform.

The same phenomenon has been observed by Mr. J. Resuggan in the case of ω-tetra-acetyl-β-*d*-glucosidoxy-3 : 4-diacetoxyacetophenone, which was described by Murakami, Robertson, and Robinson (J., 1931, 2668), m. p. 105—105·5°, and has now been obtained in a second form, m. p. 147°.

3-β-*Glucosidyldelphinidin Picrate.*—A solution of ω-tetra-acetyl-β-*d*-glucosidoxy-3 : 4 : 5-triacetoxyacetophenone (5 g.) and 2-*O*-benzoylphloroglucinaldehyde (Léon, Robinson, and Seshadri, J., 1931, 2682) (4 g.) in pure dry ethyl acetate (170 c.c.) was cooled to 0° and saturated with dry hydrogen chloride. A considerable proportion of the benzoylphloroglucinaldehyde crystallised, but dissolved again slowly; after being kept for 6 days in the refrigerator, the deep red solution was diluted with dry ether, and the dark red precipitate collected and washed with ether (5·5 g.).

Crude benzoylacetylglucosidyldelphinidin chloride (2·5 g.) was dissolved in 0·5% methyl-alcoholic hydrogen chloride (15 c.c.) in a flask fitted with a dropping-funnel and with an inlet and an outlet tube; the latter was attached to a wash-bottle containing alkaline pyrogallol; the dropping-funnel also was fitted with inlet and outlet tubes. Air was removed from the apparatus by means of a stream of oxygen-free, dry hydrogen (alkaline pyrogallol; calcium chloride) and a methyl-alcoholic solution of anhydrous barium hydroxide (70 c.c., approx. 6%) was placed in the dropping-funnel (rendered air-free in the same way) and then run into the flask. The solution immediately became deep violet-blue and a blue precipitate separated; the appearance of the mixture remained unchanged throughout the experiment. The contents of the flask were stirred for 6 hours by means of a current of hydrogen and were then acidified by the addition of air-free methyl-alcoholic sulphuric acid (30 c.c.; previously titrated against the methyl-alcoholic barium hydroxide). The deep red solution was freed from barium sulphate by centrifuging and glucosidyldelphinidin chloride was precipitated by the addition of dry ether, collected, and washed with ether. The crude product was dissolved in hot 0·1% aqueous hydrochloric acid (40 c.c.), filtered, and hot 1% aqueous picric acid (100 c.c.) added. The well-crystallised *picrate* which separated was dried, triturated with ethereal picric acid until free from delphinidin picrate, and then recrystallised once from 0·5% aqueous picric acid. A specimen was recrystallised thrice from 0·5% aqueous picric acid and then washed well with ether (Found : loss in a high vacuum at 105°, 6·1. Found in dried material : C, 47·1; H, 3·1; N, 6·3. $C_{27}H_{23}O_{19}N_3,2·5H_2O$ requires H_2O, 6·1%. $C_{27}H_{23}O_{19}N_3$ requires C, 46·8; H, 3·3; N, 6·1%).

3-β-*Glucosidyldelphinidin Chloride* (IV).—The purified 3-β-glucosidyldelphinidin picrate from the above experiment was decomposed by the addition of cold 1% methyl-alcoholic hydrogen chloride (40 c.c.). Part of the chloride separated and the remainder was precipitated by the addition of ether. It was thrice recrystallised by dissolving it in hot 0·5% aqueous hydrochloric acid, filtering, and adding sufficient 25% ethyl-alcoholic hydrogen chloride to the cooled solution to bring the concentration of acid to 5%. Deep purple, microscopic crystals separated over-night. The solubility decreased markedly with increasing purity and 300 c.c. of hot aqueous 0·5% hydrochloric acid were required for the final recrystallisation (yield of pure material, 0·6 g.). The hot aqueous solutions of picrate and chloride were filtered each time through fine filter-paper, as it was difficult to remove the last traces of barium sulphate; this fact rendered valueless the figures for intermediate yields (Found in material dried in a vacuum over phosphoric oxide at room temperature : C, 46·4; H, 4·6; Cl, 11·7. $C_{21}H_{21}O_{12}Cl,H_2O,0·8HCl$ requires C, 46·1; H, 4·4; Cl, 11·5%. Found : loss on drying in a high vacuum at 105°, 6·2%. Found in dried material : C, 49·8; H, 4·4; Cl, 10·5. $C_{21}H_{21}O_{12}Cl + C_{21}H_{20}O_{11}Cl_2$ requires C, 49·4; H, 4·1; Cl, 10·4%). Crystallisation with attached hydrogen chloride is a common property of anthocyanins and is the cause of much difficulty in interpreting the analyses. In the present case some of the hydrogen chloride is " fixed " on drying, probably by Cl replacing OH. The total loss was 6·2%, of which 2·0% was found to be HCl. Therefore the loss due to H_2O was 4·2% and $1H_2O$ requires only 3·3%. This supports the theory of replacement of hydroxyl by chlorine on drying.

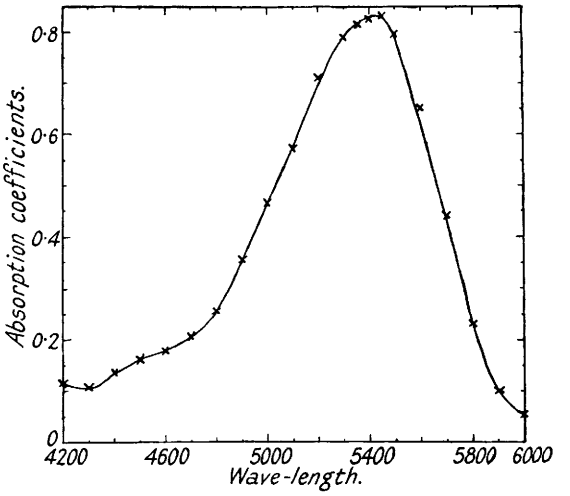
The pure 3-*glucosidyldelphinidin chloride* was only very slightly soluble in 1% methyl-alcoholic hydrogen chloride and practically insoluble in ethyl-alcoholic hydrogen chloride.

The distribution number (*iso*amyl alcohol and equilibrated 0·5% hydrochloric acid) was determined by the standard method. The weights indicated are those of pigment in 50 c.c. of the mixed solvents : 4·13 mg., 9·7; 4·5 mg., 9·3; 5·05 mg., 8·7; 5·51 mg., 8·2; 6·0 mg., 7·7.

These results show a concentration effect, but the graph log C_W/log C_{AA} is not a straight line. It would appear that, over the range examined, the degree of association is increasing in the aqueous layer and that some triple molecules are formed.

The absorption spectrum was examined in a cell of 20 mm., a solution of the delphinidin glucoside (1·18 mg.) in 0·1% methyl-alcoholic hydrogen chloride (100 c.c.) being used.

The colour reactions in solutions of graded p_H were the following (Robertson and Robinson, *Biochem. J.*, 1929, **23**, 35) : the solution in faintly acid methyl alcohol (5 mg. in 20 c.c.) was considerably bluer than an equivalent one of chrysanthemin chloride, (*a*) 0·5% hydrochloric acid, rather bluish salmon-red, bluer than chrysanthemin, (1) cherry-red, fading; (3) bluer cherry-red, fading more rapidly; (5) reddish-violet; (7) browner reddish-violet; (9) still browner; (11) reddish-mauve, no great change; (13) rich bluish-violet, fading rapidly to slate, smoky grey, yellow; (15), (16), the same, colour changes increasing rapidity; (17) blue, fades at once to nearly colourless and then brownish-yellow.



Chrysanthemin is much redder in the whole series but (17), fairly stable blue.

After 1 hour : (1) faded a little; (3) much faded, redder than (5); (5) faded violet; (7) and (9) brownish-violet; (11) faded brown; (13), (15), (16), (17), brown-yellow.

After 24 hours : (1), (3), (5) as after 1 hour; (7) pale dull violet; (9) brownish-violet; (11), (13), (15), (16), (17) pale brownish-yellow. (1) had about one-third of the intensity of (*a*) which was unchanged. Addition of concentrated hydrochloric acid gave about 75% recovery of colouring matter in (1), (3), (5), (7), and (9); (11) very faint pink; (13)—(17), no recovery.

When the methyl-alcoholic solution was added to saturated aqueous sodium carbonate, a blue colour was produced; this quickly changed to dirty green and then yellow. Saturated sodium acetate gave a very blue shade of violet.

When a solution at p_H 4·6 was heated, rapid full decoloration occurred; addition of hydrochloric acid caused full regeneration of the colour. But with decoloration at p_H 11·0, the destruction of pigment was rapid; acidification immediately after full decoloration (loss of blue shade in brownish-violet) gave only a trace of recovered anthocyanin.

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