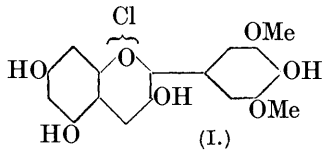
CCCLXXIV.—*Experiments on the Synthesis of the Anthocyanins. Part VIII. A Synthesis of Œnin Chloride.*

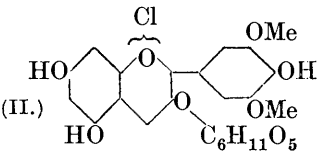
By LEOPOLD FERDINAND LEVY, THEODORE POSTERNACK, and ROBERT ROBINSON.

THE pigment of the skins of purple-black grapes has been the subject of a large number of investigations and the early history was summarised by Willstätter and Zollinger (*Annalen*, 1915, **408**, 83; 1916, **412**, 195), who isolated the substance as the crystalline chloride, $C_{23}H_{25}O_{12}Cl$, and named it œnin chloride. This was recognised as the flavylium chloride derived from a glucoside of a dimethyl ether of delphinidin and the anthocyanidin was termed œnidin. It is clear that Willstätter and Zollinger obtained these substances or at least the anthocyanin in a practically pure condition, but on account of certain small divergences, which it is now known are common with anthocyanidins derived from natural sources, it was not stated that

œnidin is identical with malvidin (Willstätter and Mieg, *Annalen*, 1915, **408**, 122). The accepted constitution of malvidin (I) was first propounded by Gatewood and Robinson (J., 1926, 1959), who based their argument on the colour reactions of the substance and of 3-*O*-methyldelphinidin chloride which they had synthesised. The first to show, indirectly, a relation between malvidin and œnidin and to give a direct proof of the position of the methoxyl groups in œnin itself were Anderson and Nabenhauer (*J. Amer. Chem. Soc.*, 1926, **48**, 2997). They converted œnin from Isabella grapes into a tetra-acetate, oxidised this by means of potassium permanganate in acetone solution, and obtained acetylsyringic acid. Almost at the same time Karrer and Widmer decomposed a number of anthocyanins, including malvin and œnin, by means of boiling 10—15% sodium or barium hydroxide and obtained syringic acid (*Helv. Chim. Acta*, 1927, **10**, 5).



(I.)



(II.)

Karrer and Widmer (*ibid.*, p. 758) consider that the cyclamin chloride which they isolated from the flowers of *Cyclamen persicum*, Mill. is probably identical with œnin, and Scott-Moncrieff (*Biochem. J.*, 1930, **24**, 767) has obtained " primulin chloride " from *Primula polyanthus*. This is not homogeneous, but the main constituent of this pigment is undoubtedly identical with œnin. The trimethyl ethers of delphinidin synthesised by Bradley, Robinson, and Schwarzenbach (J., 1930, 793) can be described as the 3-, 5-, and 7-methyl ethers of malvidin and thus their reactions on comparison with those of œnin should give a clue to the position of the glucose residue in the anthocyanin. It was at once obvious from the description of the reactions of œnin and from our own experiments on the crude pigment that the behaviour of œnin is quite different from that of the 5- and 7-methyl-malvidins but approximates closely to that of the 3-methyl ether. This finding was communicated to Miss Scott-Moncrieff, who made a direct comparison of her " primulin chloride " with the three methylmalvidins and concluded that primulin chloride is the 3-glucosidyl derivative of malvidin chloride. The synthesis of œnin chloride has been effected along the lines of the syntheses of callistephin and chrysanthemin, but many difficulties were encountered in the preparation of the required ketonic glucoside. The trouble was that the hydroxyl groups in the substance

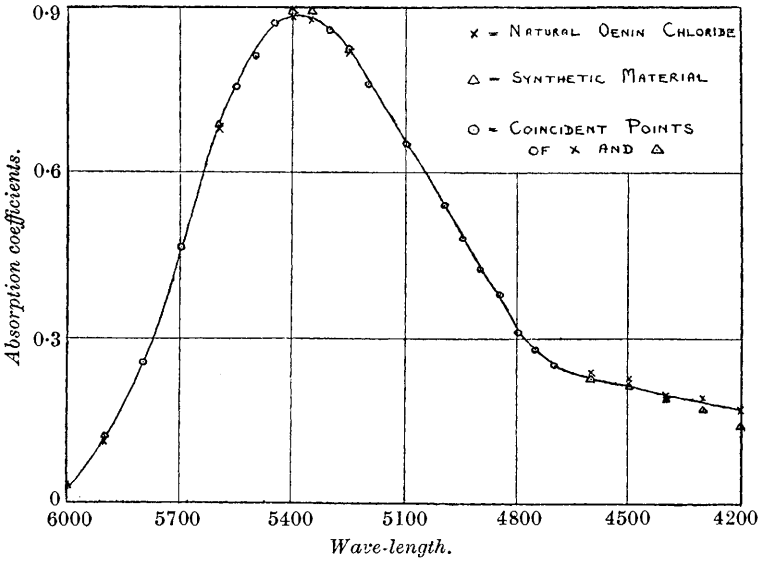
$$HO\cdot C_6H_2(MeO)_2\cdot CO\cdot CH_2\cdot OH$$

were almost equally readily acylated and the devices employed in the earlier cases proved insufficient.

Ultimately the problem was solved in the following manner.

O-Acetylsyringoyl chloride reacting with two molecular proportions of diazomethane gave a good yield of the *diazoketone*, $AcO{\cdot}C_6H_2(MeO)_2{\cdot}CO{\cdot}CHN_2$ (III), and this was changed by formic acid to a *formoxy*-derivative, $AcO{\cdot}C_6H_2(MeO)_2{\cdot}CO{\cdot}CH_2{\cdot}O{\cdot}CHO$ (IV), which could be hydrolysed by prolonged refluxing of an alcoholic solution to the ω-hydroxy-ketone, $AcO{\cdot}C_6H_2(MeO)_2{\cdot}CO{\cdot}CH_2{\cdot}OH$ (V). Alternatively (III) yields (V) directly when it is treated with 50%

FIG. 1.



formic acid. It is noteworthy that the next stage gives poor results when the acetoxy- is replaced by the benzoyloxy-group.

The tetra-acetylglucoside of (V) was, however, obtained in the usual manner and condensation with 2-*O*-benzoylphloroglucinaldehyde in ethyl acetate solution in the presence of hydrogen chloride proceeded smoothly. The product was hydrolysed by sodium hydroxide, and the anthocyanin reconstituted by the action of hydrochloric acid. It was easily purified through the picrate and finally crystallised from methyl-alcoholic hydrogen chloride. From the method of synthesis this substance must be 3-β-glucosidylmalvidin chloride (II). The salt is identical with œnin chloride and we have been able to place this beyond doubt as a result of the possession of a large specimen of œnin picrate which was very kindly

sent to us by Professor R. Parisi of the Laboratorio di Chimica agraria dell' Instituto Superiore agrario at Bologna. Professor Parisi has made a careful study of the pigments of North Italian grapes and the specimen to which we have referred was derived from a variety known as " Fogarina." After several recrystallisations of the picrate it was converted into chloride and this was crystallised in the same way as the synthetical specimen. Careful comparisons showed that the natural and the synthetic anthocyanin were identical in all respects. This is confirmed in a later paper of this series and polarimetric measurements and comparisons will be submitted in due course. We are indebted to Mrs. A. M. Robinson for kindly measuring the absorption of œnin, natural and synthetic specimens, in the visible region. It will be seen that no divergencies outside the limits of experimental error were observed.

EXPERIMENTAL.

Acetylsyringic Acid and its Anhydride.—When syringic acid is heated with acetic anhydride it yields acetylsyringic acid and also the anhydride of the latter acid; the yield of the anhydride is increased by the addition of catalysts such as pyridine or zinc chloride.

(I) Syringic acid was heated for 3 hours on the steam-bath with 4 times its weight of acetic anhydride and a few drops of pyridine. The greater part of the solvent was removed under reduced pressure, and the residue mixed with water and kept for some hours. The precipitated solid was collected and boiled with 100 times its weight of water; it melted, but was soon transformed into a hard crystalline mass. The aqueous solution, filtered hot, deposited on cooling a small quantity of acetylsyringic acid (m. p. 187°). The insoluble residue (yield 70—80%; m. p. 190—195°) was recrystallised from benzene, forming prismatic needles, m. p. 195—197°. The substance is insoluble in cold dilute alkaline solutions and sparingly soluble in cold alcohol and benzene (Found: C, 57·1; H, 5·1. $C_{22}H_{22}O_{11}$ requires C, 57·1; H, 4·8%).

Treated on the steam-bath for ½ hour with 2*N*-sodium hydroxide, the compound dissolved, and after cooling and acidification syringic acid, m. p. 202°, was recovered. Similar results were obtained by the use of a small quantity of zinc chloride as catalyst.

(II) In accordance with the prescription of Anderson and Nabenhauer (*J. Amer. Chem. Soc.*, 1926, **48**, 3000) syringic acid (15 g.) was refluxed for ½ hour with acetic anhydride (225 c.c.). The mixture was afterwards treated as above, 1500 c.c. of boiling water being used for the extraction of the crude acetylated product. Acetylsyringic acid (10 g.) and its anhydride (2·1 g.) were obtained.

(III) The best method for the acetylation of syringic acid without the formation of any anhydride is to shake a cold alkaline solution of syringic acid with a mixture of ether and acetic anhydride.

A solution of syringic acid (1 mol.) in *N*-sodium hydroxide (2·5 mols. NaOH) cooled to 0° is shaken for 5 minutes with a solution of acetic anhydride (1·5 mols.) in ether (5 vols.). The aqueous layer is acidified and pure acetylsyringic acid immediately crystallises (yield, 83%; m. p. 187°).

ω-*Diazo*-4-*acetoxy*-3 : 5-*dimethoxyacetophenone* (III).—The method used was that described by Bradley and Robinson for the preparation of ω-diazo-4-benzyloxy-3 : 5-dimethoxyacetophenone (J., 1928, 1559); but in the present case, owing to the sparing solubility of acetylsyringoyl chloride in ether, the chloride was dissolved in benzene or chloroform.

Acetylsyringoyl chloride (17·5 g.), dissolved in the smallest possible amount of chloroform (*ca.* 30 c.c.), was gradually added to an ethereal solution of diazomethane (from 32 c.c. of nitrosomethylurethane) previously cooled to — 10°. A slow evolution of nitrogen occurred and the diazo-ketone soon crystallised. It was collected on the following day (14·0 g.; m. p. 130—132°; 1·0 g. was in addition obtained by concentration of the mother-liquor). The crude compound contained a little chlorine but was easily purified by crystallisation from benzene containing some light petroleum. The quadratic yellow tablets (m. p. 134° decomp.) are readily soluble in chloroform, sparingly soluble in cold alcohol and benzene, and very sparingly soluble in ether and light petroleum (Found : N, 10·4. $C_{12}H_{12}O_5N_2$ requires N, 10·6%). Experiments were undertaken with the object of transforming the diazo-ketone into ω-hydroxy-4-acetoxy-3 : 5-dimethoxyacetophenone directly. For this purpose, the substance was dissolved in dilute aqueous-alcoholic sulphuric acid or in aqueous alcohol, and the mixture refluxed until the evolution of nitrogen ceased. The amorphous flocculent product readily reduced Fehling's solution in the cold, but could not be crystallised.

ω : 4-*Diacetoxy*-3 : 5-*dimethoxyacetophenone*.—A mixture of ω-diazo-4-acetoxy-3 : 5-dimethoxyacetophenone (12 g.) and acetic acid (24 c.c.) was heated at 70° until the evolution of nitrogen ceased and was then boiled for a few minutes. The crystals that separated on cooling were collected and washed with ether (9·1 g.); the aqueous solution furnished a further amount (1·0 g.), and the ethereal solution contained a less pure product (m. p. 115—118°). The substance crystallised from methyl alcohol in stout prismatic needles, m. p. 123° (Found : C, 56·6; H, 5·5. $C_{14}H_{16}O_7$ requires C, 56·8; H, 5·4%).

ω : 4 - *Dihydroxy* - 3 : 5 - *dimethoxyacetophenone.*—Methyl-alcoholic potassium hydroxide (24 c.c. of 25%) was gradually added to the foregoing diacetate (6 g.) dissolved in hot methyl alcohol. The *potassium* salt separated and after the mixture had been kept at 60° for ½ hour the substance was collected and washed with alcohol and ether (yield, 5·0 g.). The microscopic prisms are rather sparingly soluble in cold water (Found : K, 15·4. $C_{10}H_{11}O_5K$ requires K, 15·6%).

The potassium salt was suspended in 5 times its weight of 2*N*-acetic acid and heated until its dissolution was complete; the phenolic *carbinol* separated on cooling in clusters of fine needles and, recrystallised from boiling water, had m. p. 93—95°. This hydrated product loses its solvent at 100—105° and then melts at 132° (Found : C, 56·8; H, 5·8. $C_{10}H_{12}O_5$ requires C, 56·6; H, 5·7%).

The substance gives a weak olive-green coloration with ferric chloride in alcoholic solution and it reduces Fehling's solution in the cold.

Attempts to acetylate this substance in position 4 were ultimately successful, but owing to the very poor yield, never more than 20—25%, we temporarily abandoned the use of 4-acetoxy-compounds and turned our attention to the benzoates. In this way a process for the preparation of an ω-hydroxy-4-acyloxy-derivative of the series was discovered. The actual application in the benzoate series failed at a later stage, but the process worked out with the benzoates succeeded with the acetates and the synthesis could be carried through to the end. An indication of the methods used in our early experiments in the preparation of the ω-hydroxy-4-acetoxydimethoxyacetophenone is given by the following examples of attempts to carry out a partial 4-acetylation of the phenolic carbinol.

(A) The potassium salt (1 g.), suspended in 5 c.c. of water, was shaken with a solution of acetic anhydride (0·6 g.) in 10 c.c. of chloroform.

(B) The potassium salt (1 g.), suspended in a solution of 0·3 g. of potassium hydroxide in 5 c.c. of water, was shaken with a solution of acetic anhydride (1·2 g.) in 10 c.c. of chloroform.

(C) The free phenol (0·8 g.) was dissolved in a solution of acetic anhydride (0·6 g.) in 10 c.c. of chloroform. The mixture was shaken with 5 c.c. of *N*-potassium hydroxide.

These variations gave similar results, but the yields did not exceed 0·2—0·25 g.; the substance crystallised from hot water in rectangular prisms, m. p. 123°; and a mixture with the diacetyl derivative (m. p. 123°) melted at 100—105°. A further description is given below.

ω-*Diazo*-4-*benzoyloxy*-3 : 5-*dimethoxyacetophenone.*—The conditions

for the preparation of benzoylsyringoyl chloride were not detailed by Heap and Robinson (J., 1929, 71) and we operated as follows :

Benzoylsyringic acid (16 g.) was suspended in benzene, phosphorus pentachloride (1 mol.) gradually added, and the mixture kept at 0° for 5—6 hours. It was then worked up after the addition of water and 12 g., m. p. 125° (7° higher than recorded), were obtained. The chloride (6 g.) yielded the *diazo-ketone* (5 g.) in the usual manner; it crystallised from benzene–light petroleum in four-sided plates, m. p. 168—172° (decomp.) (Found : C, 62·5; H, 4·5; N, 8·5. $C_{17}H_{14}O_5N_2$ requires C, 62·6; H, 4·2; N, 8·6%).

4-*Benzoyloxy*-ω-*formoxy*-3 : 5-*dimethoxyacetophenone*.—On the addition of the diazo-ketone (4·0 g.) to absolute formic acid (8 c.c.) evolution of nitrogen occurred in the cold and soon ceased. The mixture was heated, then cooled; the *formyl* derivative, which crystallised, was washed with ether (yield 3·1 g., and 0·4 g. was obtained from the mother-liquor) (Found : C, 62·9; H, 4·7; CH_3O, 17·9. $C_{18}H_{16}O_7$ requires C, 62·8; H, 4·7; CH_3O, 18·0%).

ω-*Hydroxy*-4-*benzoyloxy*-3 : 5-*dimethoxyacetophenone*.—(I) The diazo-ketone (1 g.) was refluxed with *N*-sulphuric acid (7·5 c.c.) and sufficient ethyl alcohol (10 c.c. of 96%) to keep the substance in solution until the evolution of nitrogen ceased (about 5 minutes). The liquid was cooled, and diluted with water, and the product crystallised from 50% alcohol. The microscopic, stellate, prismatic needles had m. p. 173—175° (yield, 0·6 g.). This substance is insoluble in dilute alkaline solutions, gives no ferric chloride reaction, and reduces Fehling's solution.

(II) 4-Benzoyloxy-ω-formoxy-3 : 5-dimethoxyacetophenone (2·0 g.) was refluxed for 15 hours with water and alcohol just sufficient to keep it in solution. The product (yield, 1·8 g.) had m. p. 175—177° alone or mixed with the substance prepared by method I.

(III) Probably the best method is the following : The ω-diazo-ketone (2 g.) was added to 50% formic acid (50 c.c.), and the mixture heated on a water-bath. A brisk evolution of nitrogen occurred and the reaction was completed after 5 minutes. On cooling and dilution with water the product was precipitated (yield, 1·8 g.; m. p. 168—172°). After two crystallisations from aqueous alcohol the substance melted at 175—177° (the mixed m. p. with the ω-hydroxy-ketone gave no depression). The substance is very sparingly soluble in water, moderately easily soluble in alcohol and benzene, easily in acetone and chloroform, and insoluble in ether and light petroleum (Found : C, 64·2; H, 5·0; CH_3O, 19·9. $C_{17}H_{16}O_6$ requires C, 64·5; H, 5·1; CH_3O, 19·6%).

4-*Benzoyloxy*-ω-*acetoxy*-3 : 5-*dimethoxyacetophenone*.—(A) The ω-diazo-ketone (1 g.) and glacial acetic acid (5 c.c.) were heated on a

water-bath. A brisk evolution of nitrogen occurred and the reaction was complete after 5 minutes. On cooling and dilution with water the *ω-acetoxy-ketone* was precipitated; it crystallised from aqueous alcohol in pale brown clusters of prisms, m. p. 143° (Found : C, 63·6; H, 5·1. $C_{19}H_{18}O_7$ requires C, 63·7; H, 5·0%). The substance quickly reduced Fehling's solution on heating.

(B) The ω-hydroxy-ketone (1 g.) was dissolved in acetic anhydride (10 c.c.), and a drop of pyridine added. After being heated on a steam-bath for 2 hours, the mixture was cooled and mixed with water. The solid, crystallised from dilute alcohol, melted at 143° (mixed m. p. not depressed).

ω : 4 - *Dibenzoyloxy* - 3 : 5 - *dimethoxyacetophenone*.—ω - Hydroxy-4-benzoyloxy-3 : 5-dimethoxyacetophenone (0·75 g.) was dissolved in pyridine (10 c.c.), benzoyl chloride (0·4 g.) added, and the mixture shaken and allowed to remain. The product solidified when repeatedly washed with cold water and crystallised from benzene–light petroleum in microscopic needles, m. p. 128° (Found : C, 68·0; H, 5·0. $C_{24}H_{20}O_7$ requires C, 68·6; H, 4·8%). The substance is moderately easily soluble in alcohol, easily in acetone and chloroform, and insoluble in ether and light petroleum.

4-*Benzoyloxy* - ω - O - *tetra-acetyl* - β - *glucosidoxy*-3 : 5-*dimethoxyacetophenone*.—ω - Hydroxy - 4 - benzoyloxy-3 : 5 - dimethoxyacetophenone (1·13 g.; dried at 120° for 5 hours) was dissolved in dry benzene at 40° (30 c.c.), *O*-tetra-acetyl-α-glucosidyl bromide (2 g.) added, followed by dry silver oxide (3·2 g.), the mixture was well shaken for 45 minutes at 40° and refluxed for 15 minutes, and the liquid filtered. The cooled filtrate on dilution with light petroleum (300 c.c.) furnished a gum, which was washed with hot and then with cold water. A solution of it in methyl alcohol (8 c.c.) was cooled and diluted with water; the gum was then reprecipitated but in a less sticky condition. The treatment was continued until the substance was obtained as a colourless solid. The *tetra-acetyl-glucoside* crystallised from 70% aqueous methyl alcohol in colourless leaflets, m. p. 80—90° (Found : C, 57·9, 57·8; H, 5·4, 5·0. $C_{31}H_{34}O_{15}$ requires C, 57·6; H, 5·3%). The yield, however, was only 0·1 g., and several attempts to improve it were unsuccessful. In tests, the glucoside condensed with *O*-benzoylphloroglucinaldehyde in ethereal solution saturated with hydrogen chloride to give a pyrylium salt. This substance on hydrolysis with cold 8% sodium hydroxide solution and subsequent acidification with 7% hydrochloric acid gave a solution of an anthocyanin chloride with the distribution number of a monoglucoside. This was doubtless the first synthesis of œnin.

ω-*Formoxy*-4-*acetoxy*-3 : 5-*dimethoxyacetophenone* (IV).—The ω-diazo-4-acetoxy-3 : 5-dimethoxyacetophenone (4 g.) was added to

anhydrous formic acid (8 c.c.); instant evolution of nitrogen occurred and the substance dissolved. The mixture was heated on a steam-bath for 10 minutes, a blue colour appearing. On cooling, the *ω-formoxy-4-acetoxy-ketone* crystallised, m. p. 148—150°. After two crystallisations from benzene–light petroleum the ketone was obtained in long, colourless, prismatic needles, m. p. 152·5° (Found : C, 55·8; H, 5·2; CH_3O, 22·5. $C_{13}H_{14}O_7$ requires C, 55·3; H, 5·0; CH_3O, 22·0%). The mother-liquor on dilution with dry ether gave a further quantity (total yield, 3·1 g.) and the blue colour of the solution changed to yellow. Addition of formic acid or hydrochloric acid reproduced the blue colour. The substance is very sparingly soluble in water, ether, and light petroleum.

ω-Hydroxy-4-acetoxy-3 : 5-dimethoxyacetophenone (V).—The first preparation of this substance is mentioned on p. 2706.

(A) The ω-diazo-4-acetoxy-ketone (9 g.) was heated with 50% formic acid (50 c.c.) on the steam-bath for 10 minutes. On cooling and dilution with water (300 c.c.), a cream-coloured precipitate was obtained and after two crystallisations from hot water the substance occurred as small flat needles, m. p. 119—120° after drying at 100° to remove water of crystallisation (yield, 8 g.) (Found : C, 56·6; H, 5·4; CH_3O, 23·9. $C_{12}H_{14}O_6$ requires C, 56·7; H, 5·5; CH_3O, 24·4%).

(B) ω-Formoxy-4-acetoxy-3 : 5-dimethoxyacetophenone (2 g.) was refluxed with sufficient 50% alcohol to keep it in solution. After 4 hours the alcohol was removed by distillation; the residue crystallised from hot water in small flat needles, m. p. 105° after drying in a vacuum and 121—122° after drying at 100°. The m. p. of a mixture with the product from (A) was not depressed. The substance is sparingly soluble in water and soluble in the usual organic solvents; its aqueous solution quickly reduces Fehling's solution in the cold.

ω-Benzoyloxy-4-acetoxy-3 : 5-dimethoxyacetophenone.—(A) The ω-diazo-4-acetoxy-ketone (0·57 g.) and benzoic acid (0·6 g.) were well ground together and heated in a wax-bath at 110—120° for about an hour; effervescence of nitrogen then ceased. The orange-coloured molten mixture was cooled, and the solid well ground and extracted with boiling water. The *ω-benzoyloxy-4-acetoxy-ketone* crystallised from 95% alcohol in rectangular plates, m. p. 158—159° (Found : C, 63·4; H, 4·9; CH_3O, 17·4. $C_{19}H_{18}O_7$ requires C, 63·7; H, 5·0; CH_3O, 17·3%).

(B) The ω-hydroxy-4-acetoxy-ketone (0·5 g.) was dissolved in pyridine (10 c.c.), benzoyl chloride (1 c.c.) added with shaking, and the mixture kept for 1 hour; water was then added. The product was crystallised twice from alcohol and obtained in needles, m. p. 158—159° (mixed m. p. with substance from A, no depression).

The substance is very sparingly soluble in water, but soluble in the usual organic solvents.

ω - O - *Tetra - acetyl - β - glucosidoxy - 4 - acetoxy - 3 : 5 - dimethoxyacetophenone.*—Dry silver oxide (30 g.) was added to a solution of ω-hydroxy-4-acetoxy-3 : 5-dimethoxyacetophenone (10 g.) and *O*-tetra-acetyl-α-glucosidyl bromide (22·3 g.) in dry benzene (60 c.c.) at 40°. When the mixture was shaken, the temperature immediately rose to 73°. Agitation was continued for 30 minutes and finally the mixture was warmed on a water-bath before filtration. The filtrate was cooled and mixed with light petroleum (400 c.c.), precipitating a pale straw-coloured syrup, which was washed with hot and then with cold water, dissolved in warm methyl alcohol (10 c.c.), and reprecipitated by water. After this process had been repeated five times a crystalline substance, m. p. 65—75°, was obtained. It was dried (13·4 g. or 57·5%) and crystallised from dry ether, forming plank-shaped needles, m. p. 83—85° (decomp.) (Found : C, 53·3; H, 5·6; CH_3O, 11·3. $C_{26}H_{32}O_{15}$ requires C, 53·4; H, 5·5; CH_3O, 10·6%. $C_{24}H_{30}O_{14}$ requires C, 53·1; H, 5·5; CH_3O, 11·43%). Hence it seems that this glucoside may have lost one acetyl group.

7 - *Hydroxy* - 5 - *benzoyloxy* - 3 - O - *tetra-acetyl*-β-*glucosidoxy*-4′-*acetoxy*-3′ : 5′-*dimethoxyflavylium Chloride.*—Dry hydrogen chloride was passed into a solution of ω-*O*-tetra-acetyl-β-glucosidoxy-4-acetoxy-3 : 5-dimethoxyacetophenone (5·6 g.) and *O*-benzoylphloroglucinaldehyde (5·0 g.) in dry ethyl acetate (75 c.c.; distilled over phosphoric oxide) cooled to 15° and protected from access of moisture. A small quantity of a yellow crystalline substance separated in the course of 2 hours. After 72 hours the deep red mixture was submitted to filtration, and the filtrate diluted with dry ether (500 c.c.); a dark red precipitate was then obtained. This was collected, washed with dry ether, and air-dried (yield, 4·1 g.) (Found : C, 54·4; H, 4·9; Cl, 4·2. $C_{40}H_{39}O_{18}Cl,2H_2O$ requires C, 54·6; H, 4·9; Cl, 4·1%).

The salt is readily soluble in alcohol to a red solution, and this becomes purple on the addition of sodium hydroxide. Dilute sodium carbonate solution gives a violet coloration much more slowly than the concentrated reagent, probably indicating a slow hydrolysis of the 4′-acetoxyl group. This hydrolysis is a necessary preliminary to the exhibition of a strong alkali colour-reaction.

3-β-*Glucosidylmalvidin Picrate.*—The crude benzoylpenta-acetylglucosidylmalvidin chloride (5·25 g.) was finely powdered and added to 8% sodium hydroxide solution (105 c.c.) cooled to 10°. Air was excluded from the apparatus by nitrogen. The solid quickly dissolved to a dark reddish-brown, almost black solution. After remaining for 3 hours at room temperature, the solution was acidified with 7% hydrochloric acid (189 c.c.) so that the concentration of the

hydrogen chloride was brought to 2%. The liquid, which immediately assumed a deep red colour, was heated to 60° in order to complete the regeneration of the oxonium salt; a precipitate, probably malvidin chloride, was removed. Saturated aqueous picric acid (200 c.c.) was added to the cooled filtrate; 3-β-glucosidylmalvidin picrate then separated as a reddish mass. This was collected after 24 hours (yield, 3·5 g.). The picrate was powdered and washed with an ethereal picric acid solution in order to remove traces of malvidin picrate. The crude picrate (6·5 g.) was crystallised by dissolving it in alcohol (300 c.c.) and adding an equal volume of hot aqueous picric acid. On cooling, long slender red needles with a greenish reflex were obtained (Found: C, 44·1; H, 4·4; N, 5·2. $C_{29}H_{27}O_{19}N_3,4H_2O$ requires C, 43·8; H, 4·5; N, 5·3%). The substance decomposed at 202°.

Other specimens of this picrate have been obtained and these contained 1·5 H_2O, probably as the result of a variation in the conditions of exposure to the air, or perhaps it is a matter of the degree of purity of the samples.

3-β-*Glucosidylmalvidin Chloride* (*Œnin Chloride*) (II).—The crude picrate was crystallised once, dried, and dissolved in warm 4% methyl-alcoholic hydrogen chloride; the chloride was then precipitated by the addition of ether. This material became beautifully crystalline when rubbed with cold 6% methyl-alcoholic hydrogen chloride and could then be crystallised from the warm solvent in very friable hexagonal plates violet by transmitted light and exhibiting a wonderful yellow metallic glance; in mass the substance was brown-bronze. This specimen gave rather poor analyses, but the natural product crystallises in the same form and distribution-ratio experiments proved that the material crystallised from hot 6% methyl-alcoholic hydrochloric acid contains about 2% of malvidin. The best way to obtain the pure chloride is to dissolve the substance in very dilute methyl-alcoholic hydrogen chloride, increase the concentration of acid, and almost at once precipitate the chloride with ether. The amorphous solid can then be dissolved in 1% methyl-alcoholic hydrogen chloride, the solution filtered, and the concentration increased to 4—5% in the cold. The chloride then crystallises with a remarkable sheen and is perfectly pure and free from anthocyanidin (Found in air-dried material: C, 47·4; H, 5·4; Cl, 6·3; MeO, 10·3. Found in material dried at 110° in a high vacuum: C, 52·1; H, 4·8; Cl, 6·5; MeO, 11·2. $C_{23}H_{25}O_{12}Cl,3H_2O$ requires C, 47·1; H, 5·3; Cl, 6·1; MeO, 10·6%. $C_{23}H_{25}O_{12}Cl$ requires C, 51·9; H, 4·7; Cl, 6·6; MeO, 11·7%). Crystallisation by Willstätter and Zollinger's method (*loc. cit.*, p. 93) gives a mixture of prismatic forms and the substance has a *green* lustre. Slow

crystallisation from dilute methyl-alcoholic hydrogen chloride produces hard dense balls like bronze filings; the substance gives a violet-blue smear on paper or porcelain.

The number of comparisons that we have made between synthetic and natural specimens is considerable. At first we prepared œnin chloride from purple-black grapes of uncertain origin and Miss R. Scott-Moncrieff kindly sent us both œnin and primulin, which is identical with œnin. Using only 1·5 mg./50 c.c., we found the distribution number of all these specimens to be about 17·5. The primulin contained anthocyanidin and gave 21·3, 17·5 in successive shakings; œnin chloride (natural) gave 17·8, and the synthetic specimen gave 17·4. This was an apparent anomaly, since Willstätter and Zollinger (*loc. cit.*) give the distribution number 10·4 for œnin chloride and Scott-Moncrieff (*loc. cit.*) gives 8·6 for primulin chloride (doubtless this is partly due to the less completely methylated delphinidin glucoside which primulin contains). The explanation of this discrepancy is given in the following communication, and under the standard conditions œnin specimens, natural or synthetic, exhibit the same behaviour in the distribution test. These specimens of natural pigments (including primulin) all crystallised in the highly characteristic manner which we have described. The opportunity to carry the matter a stage further arose when Professor Parisi, to whom we are deeply indebted, sent us a fine specimen of partly purified œnin picrate from " Fogarina " grapes. It was found that crystallisation of œnin picrate from aqueous picric acid is not a good process of purification, since the separation of the salt is complete on each occasion; it is necessary to lose something.

(A) The natural " œnin " picrate (2·2 g.) was dissolved in boiling alcohol (150 c.c.), and saturated aqueous picric acid (75 c.c.) added; the crystals (X) were collected soon after their formation (further separation occurs on keeping, but it was not desirable to add this material to the first crop) and the mother-liquor was diluted with cold saturated picric acid and kept in the ice-chest, thus giving a second fraction (Y). (X) (1·35 g.) gave a weaker ferric chloride reaction than did (Y) (0·5 g.), and the latter was much darker in colour. (X) was then crystallised from a mixture of alcohol (70 c.c.) and saturated aqueous picric acid (35 c.c.), the orange-red hair-like crystals being allowed to separate completely. The latter process was repeated with the same quantities and the bundles of scarlet needles were collected, washed with ether, and dried at 100° (0·87 g.) (Found : C, 46·4; H, 4·1; N, 5·6; MeO, 6·8. $C_{29}H_{27}O_{19}N_3,1{\cdot}5H_2O$ requires C, 46·5; H, 4·1; N, 5·6; MeO, 8·3%. Found in material dried at 110° in a high vacuum : C, 47·9; H, 3·9; N, 5·8. $C_{29}H_{27}O_{19}N_3$ requires C, 48·1; H, 3·7; N, 5·8%). The methoxyl

determinations, as might be expected, gave low results with the picrate, but the derived chloride gave satisfactory figures. The conversion was carried out in the manner already described and with precisely the same results (Found in air-dried material: C, 47·6; H, 5·2; Cl, 6·2; MeO, 10·2, 10·1. $C_{23}H_{25}O_{12}Cl,3H_2O$ requires C, 47·4; H, 5·3; Cl, 6·1; 2MeO, 10·6%. Found in material dried at 110° in a high vacuum: C, 52·0; H, 4·8; Cl, 6·5; MeO, 11·3. $C_{23}H_{25}O_{12}Cl$ requires C, 51·9; H, 4·7; Cl, 6·6; 2MeO, 11·7%).

(B) "Œnin" chloride (0·4 g.) from "Fogarina" grapes (without having been purified through the picrate) was dissolved in 0·2*N*-aqueous sodium hydroxide (100 c.c.), and a stream of air passed for 10 minutes. The greenish-brown liquid was just acidified, concentrated hydrochloric acid (5 c.c.) then added, and the solution heated on the steam-bath until no further colour change occurred (a few minutes). The picrate was precipitated and crystallised from saturated aqueous picric acid (30 c.c.) mixed with alcohol (20 c.c.). The substance separated in orange-red slender needles (0·09 g.) (Found in material dried at 100°: C, 46·5; H, 4·0; N, 5·6. Found in material dried at 110° in a vacuum: C, 48·0; H, 3·7; N, 5·8%). This process gives a product which is quite pure; it is expeditious but wasteful.

Careful comparison in detail showed that our synthetic œnin chloride and the material of natural origin were identical in every respect. Pure œnin chloride is not entirely unchanged on the addition of ferric chloride to an alcoholic solution; there is a definite darkening and dulling of the colour, and a change towards violet.

Willstätter and Zollinger (*loc. cit.*) observed the effect of addition of tannin to œnin solutions; they become bluer and the tinctorial intensity is increased. The synthetic and the natural œnin chloride exhibited this interesting phenomenon to precisely the same extent (colorimeter). We have directly compared solubilities, crystal form and appearance, rate of pseudo-base formation and recovery of oxonium salt on acidification, and numerous reactions and properties and have found no divergencies whatsoever between the behaviour of the two specimens.

The *picrate* was prepared from the *synthetic* chloride and dried at 100° (Found: C, 46·5; H, 4·0; N, 5·6. Found in material dried at 110° in a vacuum: C, 48·0; H, 3·7; N, 5·8%); this removed the only discrepancy which we noted in the course of our work, since the earlier specimen contained $4H_2O$.

More exact distribution number determinations appear in the following communication.

Comparison of Colour Reactions in Buffered Solutions of Graded p_H. —The method was that of Robertson and Robinson (*Biochem. J.*,

1929, **23**, 35) and the p_H of the numbered solutions and other details are recorded in the memoir cited. The natural and the synthetic œnin chloride gave identical results at every stage. (1) Pink; (2) bluer, rapidly fading; (3) cherry-red, fading; (4) deeper and bluer and fades more slowly; (5) still deeper red-violet and fades still more slowly; (6) similar; (7) duller and bluer violet; (8) duller violet, tendency to slate-colour; (9) bluish-violet; (10) richer bluish-violet; (11) still more intense violet, blue in thin layers; (12)—(16) the same as (11). After $1\frac{1}{2}$ hours: (1) rose; (2) very faint pink; (3), (4), (5) almost colourless; (6) pale dull violet; (7) a little deeper colour; (8) similar; (9) dull bluish-violet; (10)—(15) somewhat faded but still rich bluish-violet; (16) slate. After 24 hours: (1) rose; (2), (3), (4) decreasing to faint pink; (5), (6), (7) colourless; (8) weak grey; (9) more intense bluish-grey; (10)—(14) bluish-violet; (15) very pale violet-blue; (16) yellow. In summer the colours are more faded; (14), (15) being very pale after 24 hours.

Examination of the More Soluble Fraction of the Picrate from "Fogarina" Grapes.—The picrate (Y, above) was converted into the chloride and this gave a very intense ferric chloride reaction (bluish-violet) in alcoholic or aqueous solution. The salt was dissolved in 0·5% hydrochloric acid, an equal volume of concentrated hydrochloric acid added, and the solution boiled for 30 seconds; the anthocyanidin, which separated as a black powder in the ice-chest, was collected, washed, and dried at 100° (Found: Cl, 8·4, 8·6; MeO, 9·2, 9·2%). The methoxyl content, if it is all due to malvidin, gives the percentage of that anhydrous anthocyanidin present as 54·5. Direct estimation was made by treatment of a very dilute solution with sodium hydroxide and subsequent acidification. Under standard conditions the recovery of delphinidin was less than 2%, and that of malvidin about 80%. The result was to show that this anthocyanidin specimen contained about 45% of malvidin.

The remainder may then be petunidin (like delphinidin, this is destroyed in the sodium hydroxide treatment), approximately 20%, and delphinidin or other substances.

Extraction with successive quantities of a reagent that easily takes up malvidin, has a certain solvent action on petunidin, but does not extract delphinidin from dilute acid solutions confirmed the view that petunidin is present in the mixture. The reagent is a mixture of anisole (4 vols.) and ethyl amyl ether (1 vol.) containing picric acid (5 g. in 100 c.c.). The anthocyanidin (3·0 mg.) was dissolved in 0·5% hydrochloric acid (100 c.c.) and shaken with 5 successive volumes (50 c.c. each) of the reagent. All the malvidin was found in the first two extracts, which were deep brown-orange; the last three extracts formed a series in which the colour gradually

decreased; ultimately delphinidin was left in the solution, and was not extracted. This was demonstrated on a smaller scale by working rapidly with larger volumes of the reagent; it was necessary to take into account the fact that delphinidin is gradually destroyed in the aqueous solution.

The figures we have given are provisional, since, for example, the petunidin content is very indirectly derived; it depends on the accuracy of the methoxyl determination and of the estimation of malvidin as well as on the assumption that the methoxyl groups occur wholly in the anthocyanidins present. Nevertheless the indications are that delphinidin 3′-methyl ester is present together with malvidin and delphinidin.

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