CCXXV.—*Experiments on the Synthesis of Anthocyanins. Part I.*

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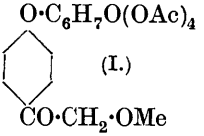
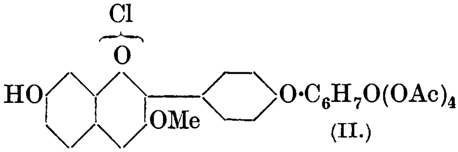
HAVING embarked in an attempt to synthesise certain of the naturally occurring anthocyanins, it became necessary for us to find answers to the following questions. (1) Is it possible to synthesise flavylium salts by the general method from suitable components, one of which is also a glucoside, without removal of the sugar residues by hydrolysis? (2) Can protective acetyl groups be detached from the product without destruction of the pyrylium nucleus and without hydrolysis of the glucoside? In studying these fundamental matters, we selected cases which were considered to be the most favourable and reduced complications to the minimum. The anthocyanidin synthesis proceeds most satisfactorily with ketones of the form R·CO·CH_2·OMe and, whilst the use of phloroglucinaldehyde is attended with difficulty, β-resorcylaldehyde reacts smoothly in most cases. Accordingly we prepared p-*tetra-acetyl*-β-*glucosidoxy*-ω-*methoxyacetophenone* (I) and attempted to condense it with β-resorcylaldehyde with the aid of hydrogen chloride. Partial removal of the sugar group occurred in formic acid solution, but normal condensation was observed in a dry ethereal medium and the *tetra-acetylglucosidoxyhydroxymethoxyflavylium chloride* (II) separated from the reaction mixture.

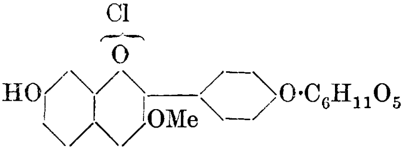


(I.)

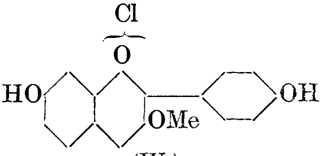


(II.)

This salt could be crystallised without change, and we were astonished to find that the acetyl-free glucoside could be obtained by the action of saturated methyl-alcoholic ammonia at 0° in accordance with Fischer's normal procedure. A potential pyrylium nucleus was preserved, probably in the form of a *pyranylamine* analogous to a pyranol, and by the subsequent action of dilute hydrochloric acid the oxonium salt was almost quantitatively regenerated. The anthocyanin-like substance (III) thus obtained closely resembles the natural pigments in many respects and, on hydrolysis with 20% hydrochloric acid, it yields an anthocyanidin



(III.)



(IV.)

(IV) which has also been produced by a direct condensation. A similar series of substances has been obtained from orcylaldehyde, and the homologue of (III) is an eosin-red salt of striking appearance.

The product of the interaction of *p*-acetoxybenzoyl chloride and ethyl sodio-$\alpha\gamma$-dimethoxyacetoacetate was hydrolysed with alcoholic potassium hydroxide. The potassium salt of *p*-hydroxy-ω-methoxyacetophenone thus conveniently isolated was converted into the glucoside (I) by treatment with tetra-acetyl-β-glucosidyl bromide * (Fischer and Armstrong, *Ber.*, 1901, **34**,2885) in aqueous acetone solution (compare Mauthner, *J. pr. Chem.*, 1918, **97**, 217).

EXPERIMENTAL.

p-*Acetoxybenzoyl Chloride*.—*p*-Hydroxybenzoic acid was quantitatively acetylated by means of acetic anhydride and one drop of sulphuric acid. The acid chloride, b. p. 145—146°/11—12 mm., m. p. 29°, was prepared by Fischer (*Ber.*, 1919, **52**, 72) with the aid of phosphorus pentachloride and we have obtained the sub-

* Often called β-bromotetra-acetylglucose, but Br here replaces OH, not H, of glucose and the name is therefore incorrect. The group $C_6H_{11}O_5\cdot$ we term *glucosido* and $C_6H_{11}O_5\cdot O\cdot$ is designated *glucosidoxy*.

stance, b. p. 157°/20 mm., m. p. 30°, in 90—92% yield by the action of thionyl chloride in excess on the acid for 2 hours on the steam-bath. It was twice distilled before use.

p-*Hydroxy-ω-methoxyacetophenone*, $MeO{\cdot}CH_2{\cdot}CO{\cdot}C_6H_4{\cdot}OH$.—*p*-Acetoxybenzoyl chloride (41 g.) dissolved in a little absolute ether was gradually added to a solution and suspension of ethyl sodio-αγ-dimethoxyacetoacetate (from 4·8 g. of granulated sodium and 39·2 g. of the ester) in dry ether (150 c.c.). When obvious reaction ceased, the mixture was gently heated under reflux for 5 hours, cooled, and washed with enough water to dissolve the precipitated salt. The solution was dried by sodium sulphate, and the solvent distilled off, finally in a vacuum. The viscous, straw-coloured residue (74 g.) was dissolved in 95% alcohol (750 c.c.), and a cold solution of potassium hydroxide (20 g.) in water (50 c.c.) added. This was followed at intervals of 12 hours by the introduction of four portions of potassium hydroxide (9·7 g.) in water (20 c.c.) and 12 hours after the last addition the solution was boiled under reflux for 5 hours, concentrated to about 250 c.c., and kept in the ice-chest; the potassium salt of the hydroxyketone slowly separated in glistening plates which were washed with absolute alcohol, dried (yield 23—25 g.), and employed in this condition for the preparation of the glucoside. This derivative is moderately readily soluble in cold water. The free *ketone* crystallised from water in long prisms, which were kept in a high vacuum over sulphuric acid for 24 hours (Found: C, 63·6, 63·4; H, 6·2, 6·3. $C_9H_{10}O_3,0{\cdot}25H_2O$ requires C, 63·4; H, 6·3%). The substance was therefore dried at 100° and crystallised from benzene in fine needles, m. p. 130—131°, which were dried at 100° for 3 hours and then in a high vacuum over sulphuric acid for 12 hours (Found : C, 65·2; H, 5·8. $C_9H_{10}O_3$ requires C, 65·1; H, 6·0%). The ketone is easily soluble in alcohol and acetone, moderately soluble in ether, and sparingly soluble in light petroleum.

p-*Tetra-acetyl-β-glucosidoxy-ω-methoxyacetophenone* (I).—A solution of tetra-acetyl-β-glucosidyl bromide (8 g.) (Fischer, *Ber.*, 1916, **49**, 584) in acetone (20 c.c.) was gradually added to one of potassium *p*-ω-methoxyacetylphenoxide (4 g.) in water (20 c.c.) and acetone (40 c.c.) maintained below 18° and continuously shaken. After 20 minutes, water (10 c.c.) and acetone (about 20 c.c.) were added so as to give a homogeneous solution and 5 hours later the greater part of the acetone was removed by exposure to a vacuum at room temperature. The residual oil was repeatedly washed with cold water and on stirring became a thick paste which partly crystallised over-night in contact with water in the ice-chest. The product crystallised from a cooled solution in 85% methyl alcohol (charcoal) in short, colourless, prismatic needles (2·2—2·5 g. or 22—26%). This result constitutes an improvement on most of those recorded by Mauthner (*loc. cit.*), who employed sodium derivatives of such phenols as *p*-hydroxyacetophenone. The *glucoside* was crystallised until its m. p., 125—126°, was constant (Found : C, 55·3 ; H, 5·8. $C_{23}H_{28}O_{12}$ requires C, 55·6 ; H, 5·7%). The substance is moderately readily soluble in cold absolute methyl or ethyl alcohol and much less readily soluble in the cold 80—85% alcohols. It is readily soluble in acetic acid and formic acid, and 250 c.c. of ether dissolve about 1·0 g. of the derivative. The success of the condensation to a flavylium salt depends on this circumstance.

7 : 4′-*Dihydroxy*-3-*methoxyflavylium Chloride* (IV).—A slow stream of hydrogen chloride was passed for ½ hour into a solution of ω-methoxy-*p*-hydroxyacetophenone (1·7 g.) and β-resorcylaldehyde (1·4 g.) in formic acid (8 c.c.) and next day a portion of the condensation product had separated in small, dark red plates. The salt (3 g.) was collected after complete precipitation by ether. It was recrystallised by solution in hot water and addition of 20% hydrochloric acid so as to bring the acid concentration to 4—5%. The salt is very sparingly soluble in cold or hot dilute hydrochloric acid (even in 0·5%), giving orange or red solutions according to the concentration, and crystallises in dark, chocolate-brown, prismatic needles which exhibit a brilliant green reflex (Found in material dried in a high vacuum over sulphuric acid for 48 hours : C, 59·7 ; H, 4·8. $C_{16}H_{13}O_4Cl,H_2O$ requires C, 59·5 ; H, 4·7%). The solution in aqueous sodium carbonate has a bright bluish-crimson colour and becomes brownish-red and then yellow in a few minutes. A dilute alcoholic solution is yellow in thick layers, pink in thin layers, and exhibits a green fluorescence. On the addition of sodium carbonate, the solution becomes bluish-violet, pure blue in thin layers, and is stable. With aqueous or alcoholic sodium hydroxide this salt gives only a transitory intense coloration and a yellow solution is obtained. Lime water added to an alcoholic solution gives an unstable red coloration. Pseudo-base formation and complete extraction from an acid aqueous solution by *iso*amyl alcohol were observed. The colour-base, obtained by the addition of sodium acetate to an aqueous solution of the salt, is a blackish-violet precipitate. It is insoluble in benzene, but dissolves in alcohol to an intense bluish-crimson solution. The yellow solution in concentrated sulphuric acid exhibits an intense green fluorescence. The *ferrichloride*, which is freely soluble in acetic acid, was obtained by adding a concentrated solution of ferric chloride in hydrochloric acid to a hot concentrated solution of the oxonium chloride in acetic acid. On cooling, the derivative

separated in dark red, elongated prisms, m. p. 179—180°, exhibiting a dark green reflex. The *picrate* separated from aqueous alcoholic picric acid in long, dull red prisms, m. p. 250—252° after darkening at about 220°. It exhibits a brilliant golden-green reflex.

4′-Tetra-acetyl-β-glucosidoxy-7-hydroxy-3-methoxyflavylium Chloride (II).—A cooled solution of *p*-tetra-acetyl-β-glucosidoxy-ω-methoxyacetophenone (1 g.) and β-resorcylaldehyde (3 g.) in dry ether (250 c.c.) was saturated with hydrogen chloride and kept for 48 hours. The pyrylium salt, which separated in feathery clusters of orange, prismatic needles, was washed with ether and dried in a vacuum over sulphuric acid for 7 days; the m. p. was then 219—220° with darkening at 200° (Found: C, 55·2; H, 5·2; Cl, 5·4. $C_{30}H_{31}O_{13}Cl,H_2O$ requires C, 55·2; H, 5·0; Cl, 5·4%). The salt could be recrystallised by solution in a little hot methyl alcohol to which hot 2% aqueous hydrochloric acid was added so as to bring the acid concentration to 0·5%. The clusters of orange-red, elongated, rectangular prisms were collected and became more orange (less red) when dried in a vacuum over sulphuric acid (Found: C, 54·5; H, 4·8. $C_{30}H_{31}O_{13}Cl,1{\cdot}5H_2O$ requires C, 54·4; H, 4·1%). This specimen also darkened at 200° and had m. p. 218—220°. The substance is sparingly soluble in cold 0·5% hydrochloric acid, but readily soluble in the simple alcohols to reddish-orange solutions. The colour reactions are almost identical with those of the deacetylated derivative described below. The *picrate* crystallised from hot alcoholic picric acid in bright scarlet, prismatic needles, m. p. 225—226° (decomp.), which exhibit a faint green reflex and are orange by transmitted light. The salt does not appear to be stable in alcoholic solution in the absence of picric acid.

4′-β-Glucosidoxy-7-hydroxy-3-methoxyflavylium Chloride (III).—When a solution of the above-described tetra-acetyl derivative in concentrated aqueous ammonia was kept for 12 hours and then acidified with hydrochloric acid, a red solution giving anthocyanin reactions was obtained. We therefore adopted the method of Fischer and Bergmann (*Ber.*, 1917, **50**, 1065) under the following conditions. A solution of the acetylated glucosidic flavylium salt (1·5 g.) in dry methyl alcohol (150 c.c.) was cooled to 0°, slowly saturated with ammonia, and kept at 0° for 14—15 hours. The ammonia and methyl alcohol were then cautiously removed in a vacuum at about 18° until the volume of the almost colourless solution was 10—12 c.c. On the addition of 4% aqueous hydrochloric acid, the flavylium chloride glucoside was deposited as a bright red precipitate consisting of extremely slender needles. The material was washed with 2% hydrochloric acid and dried in a vacuum over sulphuric acid (yield, 1·1 g.). The glucoside crystallised from 2% hydrochloric acid in red, microscopic needles which matted together and acquired a fine golden lustre when dried. A specimen was powdered and further dried in a high vacuum over sulphuric acid for 24 hours (Found: C, 55·5; H, 5·0; Cl, 7·5. $C_{22}H_{23}O_9Cl,0{\cdot}5H_2O$ requires C, 55·5; H, 5·0; Cl, 7·5%). The salt is much more readily soluble in aqueous hydrochloric acid than its tetra-acetyl derivative; it is easily soluble in hot 2% hydrochloric acid, but crystallises almost completely on standing. The dried specimens are hygroscopic and when heated char at about 300°. The orange solution in water rapidly becomes colourless on dilution and the colour is fully restored by the addition of hydrochloric acid. Sodium acetate added to an acid aqueous solution of the glucoside gives a bright bluish-crimson solution which becomes yellow in the presence of alkalis, even sodium carbonate. The salt is partly extracted by *iso*amyl alcohol from a solution in 0·5% hydrochloric acid and, on the addition of sodium carbonate and shaking, the aqueous layer becomes red and the alcoholic layer reddish-violet. The eosin-pink alcoholic solutions exhibit a green fluorescence. The *picrate* crystallised from 80% alcohol containing a little picric acid in masses of hair-fine, scarlet needles, m. p. 199—220° (decomp.), clustering like blades of grass on a stem. The pseudo-base is readily formed in an alcoholic solution, since the latter is almost colourless in the absence of picric acid, addition of which restores the colour. The hydrolysis of the glucosido-flavylium salt is of especial interest, since the phenomena observed are closely similar to those occurring in the course of the hydrolysis of natural anthocyanins. The process was effected by boiling 15% hydrochloric acid during 5 minutes, and the sugar-free salt crystallised from the hot solution. The substance was recrystallised by addition of 20% hydrochloric acid to a solution in hot water, and separated in brownish-red needles exhibiting a brilliant green reflex and showing all the reactions and properties characteristic of 7 : 4′-dihydroxy-3-methoxyflavylium chloride.

7 : 4′-*Dihydroxy*-3-*methoxy*-5-*methylflavylium Salts* (formula after IV).—The chloride was prepared like its lower homologue and by substituting orcylaldehyde (1·5 g.) for β-resorcylaldehyde (yield, 1·5 g.). This salt is sparingly soluble in boiling 1% aqueous hydrochloric acid and was recrystallised by solution in hot water and addition of hot 15% hydrochloric acid so as to bring the acid concentration to 2—3%. The cooled solution deposited dark crimson, plank-shaped, rectangular prisms exhibiting a green reflex and having a strong tendency to form twinned clusters. Aqueous acid solutions are orange to red and on the addition of sodium carbonate give bluish-crimson colorations. The colour reactions

with alkalis resemble those of the lower homologue, but the stability of the colorations is a little increased. When lime water is added to an alcoholic solution, a pure blue coloration is produced. The formation of a colour base and pseudo-base were observed. The fluorescence of an acid alcoholic solution is yellowish-green. The *ferrichloride* was obtained by dissolving the chloride (1 g.) in a hot mixture (75 c.c.) of acetic acid with an equal volume of concentrated hydroferrichloric acid. On cooling, the derivative separated in dark red, prismatic needles exhibiting a green reflex and was recrystallised in a similar form from acetic acid (Found: C, 42·5; H, 3·4. $C_{17}H_{15}O_4Cl_4Fe$ requires C, 42·4; H, 3·1%). This salt decomposes, without melting, at 220—222° after softening at about 150°.

4′-*Tetra-acetyl-β-glucosidoxy-7-hydroxy-3-methoxy-5-methylflavylium Chloride.*—This salt was first obtained by condensation of the appropriate components in formic acid solution, but the yield was unsatisfactory. The substance crystallised from hot 0·5% hydrochloric acid in fine, scarlet needles, which were dried in a high vacuum over sulphuric acid for 24 hours (Found: C, 56·1; H, 5·4; Cl, 5·3. $C_{31}H_{33}O_{13}Cl,H_2O$ requires C, 55·9; H, 5·2; Cl, 5·4%). A better method of preparation is the following. A solution of orcylaldehyde (3 g.) and *p*-tetra-acetyl-β-glucosidoxy-ω-methoxyacetophenone (1 g.) in dry ether (300 c.c.) was saturated at 10—12° with hydrogen chloride and then kept for 48 hours. The oxonium salt, which crystallised from the solution in clusters of irregular, scarlet prisms (yield, 1·1 g.), was recrystallised from 0·5% hydrochloric acid and identified by careful comparison with the analysed specimen. The *picrate* crystallises from ethyl alcohol containing picric acid in clusters of bright red, thick, blunt, prismatic needles, m. p. 222—223° (decomp.) after darkening and softening at 218°.

4′-β-*Glucosidoxy-7-hydroxy-3-methoxy-5-methylflavylium Chloride* (formula after III).—The tetra-acetyl derivative (1·5 g.) was deacetylated as described in the case of the lower homologue, and the volume of the methyl-alcoholic solution reduced to 15 c.c. Warm 3% hydrochloric acid (30 c.c.) was then added and on cooling, the glucoside chloride was deposited in very beautiful, eosin-red, oblong plates with a brilliant, golden-green reflex (yield, 0·9 g.). On recrystallisation from 2% hydrochloric acid the salt was again obtained in this characteristic form and was then dried in a high vacuum over sulphuric acid for 24 hours (Found: C, 54·3; H, 5·9; Cl, 6·8. $C_{23}H_{25}O_9Cl,1{\cdot}5H_2O$ requires C, 54·4; H, 5·5; Cl, 7·0%). The salt is sparingly soluble in cold 2% hydrochloric acid and is readily soluble in hot 2% and 4% hydrochloric acid. When an acid aqueous solution is shaken with *iso*amyl alcohol, most of the salt remains in the dark orange-red, watery layer; the alcoholic layer becomes orange. At lower concentrations the aqueous layer is orange and the alcoholic layer pink. Addition of sodium carbonate to the mixture produces, after shaking, a reddish-violet or, if the amount of salt present is small, a yellow aqueous layer. A similar colour is produced by sodium acetate and is doubtless due to the formation of the colour-base. Sodium acetate or sodium carbonate when added to an acid aqueous solution gives an intense crimson coloration, unstable in the presence of alkalis. Lime water added to an alcoholic solution gives a reddish-violet coloration which becomes pale bluish-green on standing. The salt is sparingly soluble in alcohol to a pink solution exhibiting a green fluorescence. The *picrate* crystallised from 80% ethyl alcohol containing picric acid in glistening, garnet masses of irregular plates with jagged edges, m. p. 202—203° (decomp.). The glucosidoflavylium chloride (0·2 g.) was added to 20% hydrochloric acid (20 c.c.) and, on boiling, a clear solution was obtained from which the sugar-free salt rapidly crystallised. After 15 minutes, the liquid was cooled, the solid collected and recrystallised from dilute hydrochloric acid in short dark red rods with a green reflex. The substance possessed all the properties of the above-described 4′ : 7-dihydroxy-3-methoxy-5-methylflavylium chloride.

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