CCXXI.—*Experiments on the Synthesis of Anthocyanins. Part III.*

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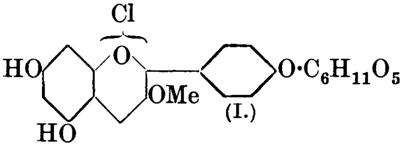
THE fact that we have investigated 4′-, 3-, and 7-glucosidoxyflavylium salts (Parts I and II), which, unlike the anthocyanins, are not derivatives of phloroglucinol, is partly due to our having attacked the problem of the synthesis of the naturally occurring pigments in stages, but partly also to the circumstance that the methods employed in the anthocyanidin syntheses have been found in some details to be inapplicable to related glucosides.

In the preparation of the latter, the condensation has hitherto been effected in dry ethereal solution and under these conditions *O*-triacetylphloroglucinaldehyde does not react satisfactorily with the appropriate ketones. In order to overcome this difficulty, it was necessary to obtain mono- or di-acetylated phloroglucinaldehydes, but all efforts to prepare the monoacetyl or monocarbomethoxy-derivatives failed. On the other hand, O-*diacetylphloroglucinaldehyde* was found to be the product when a solution of phloroglucinaldehyde in aqueous sodium hydroxide was agitated with an ethereal solution of acetic anhydride. No clear proof of the constitution of this substance can be adduced, but it must be either diacetoxysalicylaldehyde or diacetoxy-*p*-hydroxybenzaldehyde and the reaction with ferric chloride is in agreement with the former hypothesis.

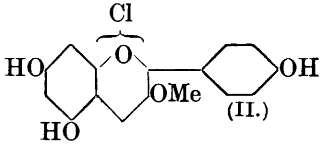
This view is supported by the observation that the substance condenses readily with ω-methoxyacetoveratrone in ethereal solution in presence of hydrogen chloride, with ultimate production of cyanidin trimethyl ether, whereas *O*-triacetylphloroglucinaldehyde cannot be employed in flavylium salt syntheses in ethereal solution. This aldehyde exhibits some tendency to condense with suitable components under the requisite conditions to glucosidoxyflavylium salts, but the yields are very unsatisfactory. It was then found that O-*monobenzoylphloroglucinaldehyde* could be prepared by the Schotten–Baumann method, and this substance not only answered our immediate purpose, but also proved to be by far the best form in which to introduce phloroglucinaldehyde in all syntheses leading to pyrylium salts of anthocyanidin type.

The results of anthocyanidin syntheses which depend in the final stages on demethylation by means of hydriodic acid are subject to unexplained variations and uniformity cannot be relied upon. Partial demethylation is a frequent occurrence, and is not only capricious in its incidence, but is also difficult to detect by analysis; in other cases, Zeisel and Herzig–Meyer determinations of the product may prove the absence of methoxyl and yet the experiment has gone awry, since the material, usually quite crystalline, does not exhibit the normal reactions. We suspect the influence of inorganic catalysts in promoting nuclear methylation by a process analogous to Claisen's transformation of phenyl allyl ether to allylphenol. In view of this uncertainty, we are engaged in the repetition of the anthocyanidin syntheses by processes not involving demethylation. Employing *O*-benzoylphloroglucinaldehyde, we have already obtained benzoylpelargonidin and cyanidin chlorides. We do not wish to imply that demethylation of methoxylated flavylium salts by means of hydriodic acid is always an unsatisfactory process, since in many cases it gives excellent results, and we are often compelled to rely upon this effective but clumsy method.

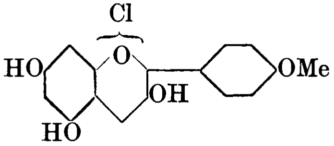
Experiments bearing on the constitution of *O*-benzoylphloroglucinaldehyde are in progress; it is either 2-benzoyloxy-4 : 6-dihydroxybenzaldehyde or 4-benzoyloxy-2 : 6-dihydroxybenzaldehyde. The benzoyloxyflavylium salts obtained with the aid of this aldehyde are stable towards acids, but are hydrolysed by alkalis, and the hydroxyflavylium salts are then recoverable by the action of acids on the product. The condensation of *O*-benzoylphloroglucinaldehyde with *p*-*O*-tetra-acetyl-β-glucosidoxy-ω-methoxyacetophenone (Part I, J., 1926, 1717) in ethereal solution with the aid of hydrogen chloride yielded an intermediate which was hydrolysed by means of methyl-alcoholic ammonia and subsequent treatment with hydrochloric acid with formation of a *pelargonidin methyl ether glucoside chloride* (I). This substance closely resembles a natural monoglucosidic anthocyanin in general character.



(I.)



(II.)



(III.)

The related sugar-free flavylium salt (II) derived on hydrolysis with 15% hydrochloric acid was synthesised independently, and furthermore the isomeride (III) has been obtained for comparison. The colour reactions of these pelargonidin chloride methyl ethers with sodium carbonate differ in a characteristic manner and a consideration of them shows (*a*) that callistephin and other anthocyanins derived from pelargonidin are certainly not 4′-glucosides, callistephin being probably a 3-glucoside (the position 5 is excluded on account of the fact that alcoholic solutions of callistephin, unlike those of pelargonenin, do not exhibit fluorescence. It is probable, although not yet quite certain, that position 7 can be excluded for the same reason), and (*b*) that partial demethylation of pelargonidin chloride 3 : 4′-dimethyl ether with hydriodic acid yields the 4′-monomethyl ether (III).

EXPERIMENTAL.

O-*Diacetylphloroglucinaldehyde*.—A mixture of pure phloroglucinaldehyde (6 g.), dissolved in *N*-sodium hydroxide (54 c.c.), and ether (150 c.c.) was cooled to − 5°, and acetic anhydride (12 c.c.) gradually added with vigorous shaking. The reaction proceeded rapidly. The ethereal layer was separated, and the aqueous solution twice extracted with fresh ether. The combined ethereal solutions were mixed with water (10—15 c.c.), the ether was distilled, and the residue washed repeatedly with cold water. The oil gradually crystallised in contact with water and after a few days was ground, allowed to remain under water for 2 days longer, and then collected (5·5 g.). The solution of this product in 80% alcohol, after treatment with charcoal, deposited crystals on slow evaporation; these recrystallised from 60% alcohol (charcoal) in almost colourless, prismatic needles, m. p. 102—103°, presenting a homogeneous appearance under the microscope (Found: C, 55·4; H, 4·3. $C_{11}H_{10}O_6$ requires C, 55·5; H, 4·2%). The substance is readily soluble in ether and moderately readily soluble in cold methyl or ethyl alcohol. An alcoholic solution develops a wine-red coloration on the addition of ferric chloride. On further acetylation by means of acetic anhydride and potassium carbonate, an almost theoretical yield of *O*-triacetylphloroglucinaldehyde was obtained. The product had m. p. 156—157°, whereas Pratt and Robinson (J., 1925, **127**, 1182) give m. p. 151°. A specimen prepared from phloroglucinaldehyde, however, after repeated crystallisation from ethyl alcohol and then from methyl alcohol, sintered at 151° and had m. p. 156—157°. A mixture of the two specimens had m. p. 156—157°. Attempts to prepare monoacetylphloroglucinaldehyde by limiting the amount of acetic anhydride employed gave mixtures of the above diacetyl derivative and unchanged phloroglucinaldehyde. No definite carbomethoxy-derivatives could be obtained. Treatment of alkaline solutions of phloroglucinaldehyde at 0° with methyl chloroformate resulted in the immediate deposition of a brick-red product.

O-*Benzoylphloroglucinaldehyde*.—The phloroglucinaldehyde employed in the preparation of this substance should be obtained by the use of freshly distilled anhydrous hydrogen cyanide, since the material resulting from the modification described by Pratt and Robinson (J., 1925, **127**, 1132) is difficult to free from a colouring matter which has a deleterious effect on the yield. Phloroglucinaldehyde (5·2 g.) was added to a solution (cooled to 10°) of potassium hydroxide (1·7 g.) in water (150 c.c.). The mixture was cooled to − 5°; part of the potassium derivative then separated in glistening plates. Benzoyl chloride (2·35 g.) was then introduced in three portions with very vigorous shaking, which was continued until the odour of the chloride had almost disappeared. The mixture was stirred for ½ hour with an excess of aqueous sodium bicarbonate and the solid was then collected, washed, again triturated with aqueous sodium bicarbonate, isolated, and dried in the air (yield, 7·5 g.). The crude product was dissolved in hot alcohol (100 c.c., charcoal), and the filtered solution poured into water (400 c.c.). The emulsion quickly resolved itself into a suspension of hair-fine needles, which were isolated (5 g.) and found to be sufficiently pure for many purposes. The substance crystallises from chloroform (charcoal) in colourless, elongated prisms which become pink at 155°, darken at 185°, turn brick-red at 195°, and melt at 198—200° to a dark red liquid (Found : C, 64·8; H, 3·8. $C_{14}H_{10}O_5$ requires C, 65·1; H, 3·9%). The substance is easily soluble in ether and the simple alcohols, moderately readily soluble in hot chloroform, and very sparingly soluble in carbon tetrachloride. It givos a wine-red coloration with ferric chloride in alcoholic solution.

4′-β-*Glucosidoxy*-5 : 7-*dihydroxy*-3-*methoxyflavylium Chloride* (I).—A cold solution of *O*-benzoylphloroglucinaldehyde (0·6 g.) and *p*-*O*-tetra-acetyl-β-glucosidoxy-ω-methoxyacetophenone (0·5 g.) in dry ether (100 c.c.) was slowly saturated with hydrogen chloride; it gradually assumed a deep orange-red colour and then exhibited a strong yellowish-green fluorescence. A part of the product crystallised, and after 4 days the remainder was precipitated by the addition of ether (100 c.c.). The isolated salt (0·5—0·7 g.) dissolved in alcohol to an intensely fluorescent solution, but it could not be recrystallised, because pseudo-base formation occurred in solutions containing less than 5—6% of hydrochloric acid, and the use of more concentrated acid solutions caused hydrolysis. A solution of the crude product (2·7 g.), from several experiments, in methyl alcohol (200 c.c.) was saturated at 0° with ammonia and allowed to remain at 0° for 24 hours. The ammonia and methyl alcohol having been removed in a vacuum in the cold, the residue crystallised from 75% methyl alcohol containing 3% of hydrogen chloride in masses of microscopic, prismatic needles (1·2 g.) (deposition slow). The salt was then dissolved in 4% aqueous hydrochloric acid at 60—70° and separated on cooling in red, prismatic needles which showed a weak golden reflex only after being dried in a vacuum (Found in air-dried material: C, 47·7; H, 5·7. Found in material dried over soda–lime in a vacuum for 24 hours: C, 53·1; H, 5·3. $C_{22}H_{23}O_{10}Cl,4H_2O$ requires C, 47·6; H, 5·7%. $C_{22}H_{23}O_{10}Cl,H_2O$ requires C, 52·8; H, 5·0%. The monohydrate is very hygroscopic). When heated in a capillary tube, the salt evolves steam above 100°, sinters at 125°, and darkens and melts at 130—131°. The glucoside is easily soluble in cold 0·5—2% hydrochloric acid and in cold 50% methyl alcohol containing 4% of hydrogen chloride; it is easily soluble in hot aqueous hydrochloric acid (up to 20%).

The aqueous acid solutions are not completely extracted on being shaken with *iso*amyl alcohol. The alcoholic hydrochloric acid solutions are deep red, dilute aqueous solutions are orange-red, and in all cases the solutions are not fluorescent. The sodium carbonate reaction is dark wine-red, becoming dirty brown, whilst the addition of sodium acetate to an acid solution produces a more stable, intense dark red coloration. The *picrate* separated from a hot 80% alcoholic solution of the salt containing an excess of picric acid in compact, deep red, spherical masses of microscopic crystals. It darkens at 125°, is almost black at 170°, and melts with further decomposition at 186—188°.

5 : 7 : 4′-*Trihydroxy*-3-*methoxyflavylium Chloride* (II).—(A). A slow stream of hydrogen chloride was passed through a solution of *p*-hydroxy-ω-methoxyacetophenone (Part I, *loc. cit.*) (1·6 g.) and *O*-triacetylphloroglucinaldehyde (2·8 g.) in anhydrous formic acid (10 c.c.) for 4 hours, and the mixture kept for 36 hours. The salt precipitated by ether was collected (3 g.) and hydrolysed by boiling aqueous alcoholic hydrochloric acid during 5 minutes; on cooling, a red, amorphous material was deposited. This was extracted by boiling water, and 20% hydrochloric acid was added to the filtrate until a faint turbidity appeared. On cooling, the salt separated in stellate aggregates of dark red, prismatic needles. Recrystallisation from 3—4% hydrochloric acid gave freely twinning, elongated, nearly rectangular, microscopic prisms which were dark red in mass and crimson by transmitted light under the microscope (Found in material dried over soda–lime for 12 hours: C, 56·6; H, 4·6. $C_{10}H_{15}O_5Cl,H_2O$ requires C, 56·7; H, 4·4%). When crystallised from a mixture of hydrochloric acid and ethyl alcohol, highly characteristic, boat-shaped, microscopic plates are obtained. These have a reddish-brown colour and a greenish reflex, but this is lost on exposure to air. This salt is sparingly soluble in cold dilute hydrochloric acid (down to 0·5%) and is only moderately readily soluble in hot hydrochloric acid; very dilute solutions have a brownish orange-red colour, hot concentrated aqueous solutions and alcoholic solutions are crimson, and all solutions are non-fluorescent. Aqueous sodium carbonate dissolves the salt, giving a stable, intense, reddish-violet solution, more bluish-violet in thin layers. Willstätter and Burdick (*Annalen*, 1916, **412**, 162) state that " Die säure Losung des Callistephins schlägt mit Soda und sogar mit Alkalilauge nur in Rotviolett bis Violettrot um, während Pelargonenin schon mit Soda violett blau wird." In this connexion, it is interesting to note that 3 : 7 : 4′-trihydroxyflavylium chloride gives a definitely bluer alkali colour-reaction than the 3 : 5 : 7-trihydroxymethoxyflavylium chloride. In alcoholic solution, for

example, the former, like pelargonenin, gives a pure blue coloration (compare Willstätter and Bolton, *Annalen*, 1916, **412**, 136; Pratt and Robinson, J., 1923, **123**, 193). *iso*Amyl alcohol extracts the salt completely from an aqueous acid solution. Pseudo-base formation was observed.

(B). Characteristic, twinning, rectangular prisms separated in the course of 2 minutes when the above-described glucosidoxydihydroxymethoxyflavylium chloride was boiled in 15% aqueous hydrochloric acid solution. The substance was recrystallised and identified by its reactions, solubility relations, and crystalline form with the pelargonidin chloride 3-methyl ether prepared as in (A) above. The filtrate from this hydrolysis was extracted with *iso*amyl alcohol in order to remove the last traces of flavylium chloride and with ether to remove the *iso*amyl alcohol; it was then found to reduce Fehling's solution.

3 : 5 : 7-*Trihydroxy-4'-methoxyflavylium Chloride* (III).—A solution of anisoylcarbinol (0·6 g.) (Tiffeneau, *Compt. rend.*, 1910, **150**, 1182) and *O*-triacetylphloroglucinaldehyde (1 g.) in formic acid (15 c.c.) was saturated with hydrogen chloride and after 36 hours the product was precipitated by ether and collected. It was dissolved in alcohol (200 c.c.) and concentrated hydrochloric acid (20 c.c.), the solution boiled for 5 minutes, the greater part of the alcohol removed by distillation, and the salt precipitated by the addition of concentrated hydrochloric acid. Separation from accompanying red impurities was effected by extraction with boiling 0·5% hydrochloric acid, the acid concentration of the filtrates was increased to 8—10%, and finally the substance was crystallised from 2% hydrochloric acid, separating in crimson, microscopic, rhombic prisms (Found in material dried over sodalime for 12 hours: C, 56·4; H, 4·6. $C_{16}H_{13}O_5Cl,H_2O$ requires C, 56·7; H, 4·4%). The yield, as in the similar synthesis of peonidin, was poor and we have since found that *O*-benzoylphloroglucinaldehyde gives much better results than the triacetate in condensations with benzoylcarbinol derivatives. In mass this salt has a dark brown colour and exhibits a weak bluish-green reflex. When it is crystallised from aqueous-alcoholic hydrochloric acid (about 5%), highly characteristic, regular, diamond-shaped plates are obtained. The salt is very sparingly soluble in cold aqueous hydrochloric acid and not readily soluble even in boiling 2% hydrochloric acid. Concentrated aqueous solutions are crimson, very dilute solutions are brownish orange-red; alcoholic solutions are bluish eosin-red. The reaction with sodium carbonate is characteristic, the solutions, both alcoholic and aqueous, being strongly dichroic, red in thick layers and bluish-purple in thin layers. Under

some conditions of illumination, the liquid appears green and violet. The specimen of incompletely demethylated pelargonidin chloride dimethyl ether described as crystallising in diamond-shaped plates (Pratt and Robinson, J., 1925, **127**, 1188) has been re-examined and undoubtedly contains a large proportion of the monomethyl ether now described. The crystal forms are identical and the colour reactions with sodium carbonate are closely similar. The dichroism observed in both cases was identical, but the specimen from the demethylation gave a slightly more bluish-purple colour in thin layers and probably contains a little pelargonidin chloride.

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