CXCIII.—*Experiments on the Synthesis of Anthocyanins. Part V. A Synthesis of 3-β-Glucosidylpelargonidin Chloride, which is believed to be identical with Callistephin Chloride.*

By ALEXANDER ROBERTSON and ROBERT ROBINSON.

FOR reasons which have been explained in previous memoirs we were of the opinion that callistephin chloride, one of two anthocyanins isolated by Willstätter and Burdick (*Annalen*, 1916, **412**, 149) from the purple-red aster (*Callistephus chinensis*, Nees, syn. *Aster chinensis*, Linn.), must be regarded as a 3- or 7-glucoside of pelargonidin chloride and the former possibility was preferred. The 3-glucoside (III) has now been synthesised by an unambiguous method and its highly characteristic properties agree so closely with those recorded by Willstätter and Burdick for callistephin chloride that there can be no doubt that the synthetic product is identical with the natural anthocyanin, and thus for the first time an actual flower pigment has been artificially prepared.

In Part I (J., 1926, 1717) we showed in the most favourable case that the general method of the anthocyanidin syntheses of Pratt and Robinson could be applied to the preparation of glucosidoxyflavylium salts. Part II (J., 1927, 242) included a description of the synthesis of a flavylium salt 3-glucoside, but this was not a phloroglucinol derivative; and our many attempts at that stage to produce such a substance were fruitless.

It was more difficult to synthesise the 3-glucosides than the 4'- or 7-glucosides and with the methods then available it was much more difficult to obtain the phloroglucinol derivatives than the corresponding salts with a resorcinol nucleus; the combined difficulty seemed at one time insuperable.

The discovery of the valuable properties of *O*-benzoylphloroglucinaldehyde (see previous paper), with the aid of which a pelargonidin methyl ether 4'-glucoside chloride was synthesised (Part III, J., 1927, 1710), made the prospects much brighter, and after improving the technique of the employment of this substance we ultimately achieved our object.

ω-Chloro-4-hydroxyacetophenone, $HO\cdot C_6H_4\cdot CO\cdot CH_2Cl$, was converted into ω-acetoxy-4-hydroxyacetophenone,

$$HO\cdot C_6H_4\cdot CO\cdot CH_2\cdot OAc,$$

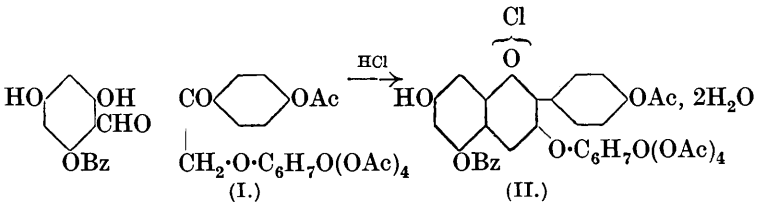
and then by means of aqueous sodium hydroxide into the sparingly soluble sodium salt of ω : 4-dihydroxyacetophenone,

$$NaO\cdot C_6H_4\cdot CO\cdot CH_2\cdot OH.$$

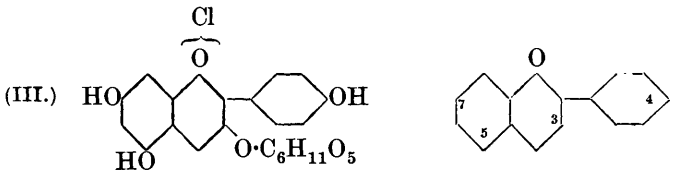
This salt served for the preparation of *carbomethoxy*, *benzoyl*, and

acetyl derivatives, of which only the last, $AcO{\cdot}C_6H_4{\cdot}CO{\cdot}CH_2{\cdot}OH$, was found to serve our purpose through all the subsequent stages.

The **O-*tetra-acetyl*-β-*glucoside*** (I) of this carbinol was obtained by condensation with *O*-tetra-acetyl-α-glucosidyl bromide in presence of silver carbonate in benzene solution at 35°. This method constitutes a considerable improvement in the methods for the preparation of such glucosides and has been used in other cases with satisfactory results. The flavylium salt synthesis by means of *O*-benzoylphloroglucinaldehyde and the glucoside (I) proceeded in ethereal solution and better in a mixture of ether and chloroform.



(I.) (II.)



The product (II) was hydrolysed by means of cold 8% aqueous sodium hydroxide, giving a solution of the de-benzoylated and de-acetylated glucoside in a form in which the pyran ring is probably open. On acidification, however, the pyrylium salt is regenerated and without loss of the glucose residue. The purification of the anthocyanin (III) was tedious and difficult; we followed to a large extent the methods of Willstätter and Burdick (*loc. cit.*), but also made use of the picrate. Ultimately we obtained a good specimen of crystalline, homogeneous material, $C_{21}H_{21}O_{10}Cl,2H_2O$, which gave pelargonidin chloride on hydrolysis and had all the properties of callistephin chloride.

This synthesis has a bearing on the problem of the constitution of the anthocyanins, because the recognition of callistephin as a 3-glucoside of pelargonidin debars pelargonenin, an isomeric anthocyanin obtained by Willstätter and Bolton by the step-wise hydrolysis of the diglucoside pelargonin, from being also recognised as a pelargonidin-3-glucoside. The isomerides have widely different properties (see p. 1470) and especially interesting is the fluorescence of alcoholic solutions of pelargonenin chloride and the absence of fluorescence in similar solutions of callistephin chloride. It is

evident also that the situation of the glucose residue in pelargonenin must be the situation of the diglucoside residue in pelargonin, because the alkali-colour-reactions of pelargonenin and pelargonin are stated to be very similar (Willstätter and Bolton, *Annalen*, 1915, **408**, 50; 1916, **412**, 136). Pelargonin is also fluorescent in alcoholic solution, although not so brilliantly as pelargonenin.

We are forced to the conclusion that pelargonin chloride is a 5- or 7-diglucoside of pelargonidin chloride, and this is entirely in harmony with the views previously put forward in this and the parallel series of papers dealing with anthocyanidins.

Karrer, Widmer, Helfenstein, Hürliman, Nievergelt, and Monsarrat-Thoms (*Helv. Chim. Acta*, 1927, **10**, 729) have, however, expressed the opinion that the chief anthocyanins, for example, pelargonin, cyanin, peonin, and malvin, are anthocyanidin 3-saccharides and have drawn their conclusions from the results of comparative experiments along three distinct lines, namely (*a*) the properties of malvone, (*b*) the relative yields of derivatives of benzoic acid obtained on oxidation of anthocyanidins and anthocyanins with 15% hydrogen peroxide, and (*c*) the methylation and subsequent fission of anthocyanins.

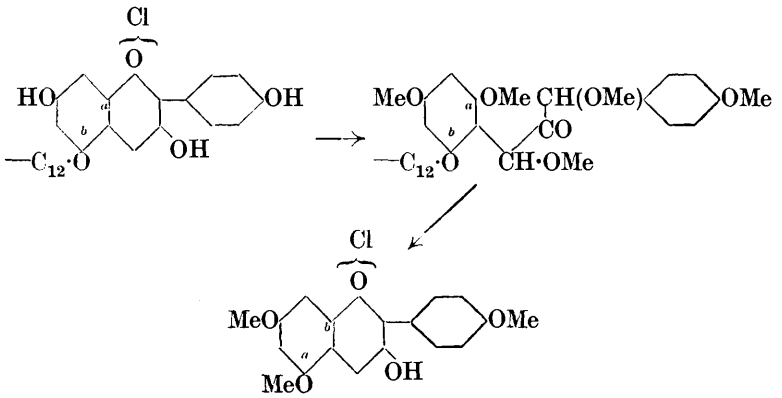
In view of the existence of the anthocyanin hirsutin (Karrer and Widmer, *Helv. Chim. Acta*, 1927, **10**, 758), which may be 3-substituted and resembles malvin in many respects, the colour-reaction evidence is not decisive in this group of delphinidin derivatives. Furthermore the valid interpretation of the oxidation experiments is by no means clear.

Consequently we need discuss only the methylation experiments to which Karrer and Widmer and their collaborators give special prominence. The anthocyanins were methylated by means of methyl sulphate and alkali and then by means of methyl iodide and silver oxide. Hydrolysis of the amorphous product was effected by rather vigorous treatment with hot methyl-alcoholic hydrochloric acid and the resulting methylated anthocyanidin was found to have one free hydroxyl group in position 3. The argument was then developed on the assumption that this position is protected from the action of the methylating agents by the sugar residues.

This is doubtless a plausible and satisfactory interpretation of these interesting observations, but there is also much to be said for an alternative hypothesis founded on the assumption that the sugar groups are attached to position 5.

Under the conditions of the experiments it is highly improbable that the pyran ring could remain intact and when it is broken the three hydroxyls of the phloroglucinol nucleus should be methylated. The recovery of anthocyanidin would then be difficult. Again,

Karrer and his co-workers did not observe *C*-methylation of the phloroglucinol nucleus, and this would certainly have occurred if the pyran ring opened at an early stage. A recent example of this phenomenon is found in the work of Asahina, Shinoda, and Inubuse (*J. Pharm. Soc. Japan*, 1927, 133) on sakuranetin : methyl sulphate and alkali effect the introduction of two or three methyl groups in the phloroglucinol nucleus of the flavanone. The only difficulty met with in explaining Karrer's results by the 5-glucoside structures is that the substances produced by the ring fission of the anthocyanins should be of the form R·CH:C(OH)·CO·R′, and Malkin and Robinson have shown (J., 1925, **127**, 369) that such enols are readily converted into their methyl ethers. The hydroxyl group here considered is the one in position 3, and we must therefore assume that the three carbon atoms connecting the aromatic nuclei take some form, such as ·CH(OMe)·CO·CH(OMe)·, in which the central carbon atom does not bear a methoxyl group. If the possibility of this be granted, then Karrer's results are not in conflict with our hypothesis that the sugar is in position 5. The annexed scheme illustrates a possible course of the reactions.



Summarising, we may say that a consideration of the properties of callistephin and of the reactions of synthetical hydroxyflavylium salts leads to the conclusion that pelargonin (pelargonenin), peonin, and cyanin are 5- or 7-saccharides : Karrer's important experiments are consistent only with the hypothesis that these anthocyanins are 3- or 5-saccharides. It seems, therefore, that the weight of evidence favours the 5-saccharide configuration and in that case the close correspondence in intimate structural detail between the anthoxanthins and anthocyanins breaks down, at least in these instances (compare Atree and Perkin, J., 1927, 234).

EXPERIMENTAL.

ω-Chloro-4-hydroxyacetophenone.—The following modification of Tutin's method (J., 1910, **97**, 2503) was adopted in order to avoid the intense irritation of the eyes and skin involved in working with ethereal solutions of the crude ketone.

Chloroacetyl chloride (12 g.) was added in one portion to a mixture of anisole (10 g.), carbon disulphide (50 c.c.), and anhydrous aluminium chloride (30 g.), previously heated under reflux on the steam-bath. The solvent was removed by distillation, and the residue heated for 4 hours on the steam-bath and then decomposed by ice and dilute hydrochloric acid. The solid product was dissolved in the minimum volume of methyl alcohol and added in a thin stream to 10% sodium carbonate solution (300 c.c.); the mixture was agitated for an hour, treated with charcoal, and acidified with hydrochloric acid. The ketone was then crystallised from 80% alcohol (yield, 7—8 g.; m. p. 148°).

ω : 4-Diacetoxyacetophenone.—This substance was obtained by Nolan, Pratt, and Robinson (J., 1926, 1968) by acetylation of ω-acetoxy-4-hydroxyacetophenone; it may be more conveniently prepared by heating a mixture of ω-chloro-4-hydroxyacetophenone (6 g.), anhydrous potassium acetate (3 g.), and acetic anhydride (30 c.c.) on the steam-bath for 2 hours (yield, 6·5 g.). It crystallised from light petroleum in elongated, pointed prisms and from benzene in diamond-shaped plates, m. p. 98° (as previously stated, *loc. cit.*).

ω-Acetoxy-4-hydroxyacetophenone.—The m. p. of this derivative (Nolan, Pratt, and Robinson, *loc. cit.*) has been raised 6°. The best conditions for its preparation are the following: A mixture of ω-chloro-4-hydroxyacetophenone (35 g.), anhydrous potassium acetate (30 g.), acetic acid (10 c.c.), and absolute ethyl alcohol (250 c.c.) was refluxed for 1 hour, and the greater part of the solvent then removed by distillation. Water (600 c.c.) was added, and the solid collected (32 g.) after the whole had been kept for 12 hours in the ice-chest.

The derivative crystallises from warm water (charcoal) in long, colourless, prismatic needles, m. p. 133°. It is soluble in dilute aqueous sodium carbonate.

ω : 4-Dihydroxyacetophenone.—ω-Acetoxy-4-hydroxyacetophenone (32 g.) was added to 16% aqueous sodium hydroxide (150 c.c.), and a clear solution resulted after 15 minutes' heating on the steam-bath. On cooling, the sodium derivative crystallised in glistening plates; it was collected, washed with alcohol, and dried (25 g.). Acetylation by means of acetic anhydride and sodium

acetate for 1 hour at 100° afforded ω : 4-diacetoxyacetophenone, m. p. 98°.

ω-Hydroxy-4-methylcarbonatoacetophenone,

$$CO_2Me{\cdot}O{\cdot}C_6H_4{\cdot}CO{\cdot}CH_2{\cdot}OH.$$

—A solution of the sodium salt of ω : 4-dihydroxyacetophenone (5·5 g.) in warm water (50 c.c.) was rapidly cooled in order to cause the separation of small crystals. Methyl chloroformate (2·5 g.) was then gradually introduced with vigorous stirring. After ½ hour the mixture was cooled to 0° and the fine needles that had separated were collected (5·0 g.). The substance was crystallised from benzene–light petroleum (1 : 3), and then separated from water (charcoal) in long, colourless, prismatic needles, m. p. 84° (Found : C, 57·1; H, 4·7. $C_{10}H_{10}O_5$ requires C, 57·1; H, 4·8%). This carbonate is readily soluble in alcohol, moderately readily soluble in cold benzene and hot water, and sparingly soluble in light petroleum.

ω-Hydroxy-4-acetoxyacetophenone.—Acetic anhydride (10 c.c.) was added in three portions with vigorous shaking to a suspension of the sodium salt of ω : 4-dihydroxyacetophenone (10 g. finely powdered) in water (40 c.c.) and ether (75 c.c., to which further quantities were added as the product crystallised). After separation the aqueous layer was saturated with sodium chloride and twice extracted with ether; water (20 c.c.) was then added to the combined extracts and the original ethereal layer, and the ether distilled. The residual liquid deposited a white crystalline mass (7 g.), which was sufficiently pure for many purposes. The substance crystallised from water in rectangular, plank-shaped, white prisms, m. p. 95—96°, exhibiting a strong tendency to form clusters (Found : C, 61·8; H, 5·1. $C_{10}H_{10}O_4$ requires C, 61·9; H, 5·2%). The solubilities resemble those of the carbonate (above), the pure substance is sparingly soluble in dry ether. Further acetylation yielded ω : 4-diacetoxyacetophenone, m. p. 98°, and the substance is distinguished from the isomeric ω-acetoxy-4-hydroxyacetophenone by its insolubility in cold dilute alkali solution.

ω-Hydroxy-4-benzoyloxyacetophenone.—Benzoyl chloride (4 g.) was gradually added to a vigorously agitated solution of the sodium salt of ω : 4-dihydroxyacetophenone (5 g.) in water (100 c.c.). After 15 minutes, saturated, aqueous sodium bicarbonate (100 c.c.) was introduced and after 2 hours the solid was isolated (4 g.); it crystallised from ethyl alcohol in glistening, regular, rectangular plates, m. p. 140—141° (Found : C, 70·7; H, 4·6. $C_{15}H_{12}O_4$ requires C, 70·4; H, 4·6%). The substance is sparingly soluble in cold alcohol, moderately readily soluble in cold benzene, and readily soluble in hot alcohol and benzene.

ω : 4-*Dibenzoyloxyacetophenone*.—Benzoyl chloride (4·0 g.) was added in one portion to a vigorously stirred solution of the sodium salt of ω : 4-dihydroxyacetophenone (3·5 g.) and sodium hydroxide (2·5 g.) in water (80 c.c.), and the solid was isolated after 30 minutes and washed with aqueous sodium bicarbonate. This derivative is much more sparingly soluble in hot alcohol than the monobenzoate and crystallised in glistening, diamond-shaped plates, m. p. 180—182° after softening at 176° (yield, 2 g.) (Found : C, 73·3; H, 4·2. $C_{22}H_{16}O_5$ requires C, 73·3; H, 4·4%).

ω-O-*Tetra-acetyl-β-glucosidoxy-4-benzoyloxyacetophenone*, $COPh{\cdot}O{\cdot}C_6H_4{\cdot}CO{\cdot}CH_2{\cdot}O{\cdot}C_6H_7O(OAc)_4$.—Freshly prepared, dry silver carbonate (10 g.) was added to a solution of ω-hydroxy-4-benzoyloxyacetophenone (4·5 g.) and *O*-tetra-acetyl-α-glucosidyl bromide (12 g.) in dry benzene (80 c.c.) at 35°. The mixture was vigorously stirred for 15 minutes and then refluxed for 30 minutes. The crude glucoside was precipitated from the filtered solution by means of light petroleum (400 c.c.) and the syrup was washed with hot water and triturated with cold water until it became semi-solid. A solution of this material in warm methyl alcohol deposited the glucoside as an opaque jelly which gradually crystallised; the substance then separated from ethyl alcohol in voluminous, colourless, hair-fine needles, m. p. 147° (yield, 5 g.) (Found : C, 59·4; H, 4·9. $C_{29}H_{30}O_{13}$ requires C, 59·4; H, 5·0%). This substance is readily soluble in hot methyl or ethyl alcohol and on cooling separates as a gel that is slowly resolved into crystals; it is sparingly soluble in ether, but easily soluble in cold chloroform. Attempts to condense this glucoside with *O*-benzoyl-phloroglucinaldehyde to give a derivative of callistephin were unsuccessful.

ω-O-*Tetra-acetyl-β-glucosidoxy-4-acetoxyacetophenone* (I).—The method previously employed for the preparation of ω-*O*-tetra-acetyl-glucosidoxyacetophenone (J., 1927, 244) was tried, but did not give satisfactory results in the present case.

Dry silver carbonate (15 g.) was added to a solution of ω-hydroxy-4-acetoxyacetophenone (7 g.) and *O*-tetra-acetyl-α-glucosidyl bromide (20 g.) in anhydrous benzene (100 c.c.) at 35°, and the mixture agitated for ½ hour. The reaction proceeded smoothly with a slight rise of temperature and was completed by refluxing for 15 minutes. The filtered solution was mixed with light petroleum (1000 c.c.) and the pale straw-coloured syrup obtained was well washed with hot water and then with cold water until it crystallised. The substance separated from 70% methyl alcohol (charcoal) in long, colourless, rectangular rods, m. p. 132° (yield, 10 g.) (Found : C, 54·8; H, 5·5. $C_{21}H_{28}O_{13}$ requires C, 55·0; H, 5·3%). This

glucoside is very sparingly soluble in anhydrous ether, somewhat readily soluble in the simple alcohols and benzene, and readily soluble in chloroform.

ω-*O*-Tetra-acetyl-β-glucosidoxyacetophenone (*loc. cit.*) was prepared in a similar manner from benzoylcarbinol (10 g.), *O*-tetra-acetylglucosidyl bromide (25 g.), and silver carbonate (20 g.). The product crystallised from 85% methyl alcohol (charcoal) in white, prismatic needles, m. p. 104—105° (yield, 12 g.), and was identical with the specimen previously obtained.

ω-O-*Tetra-acetyl-β-glucosidoxy-4-methoxyacetophenone*, $MeO{\cdot}C_6H_4{\cdot}CO{\cdot}CH_2{\cdot}O{\cdot}C_6H_7O(OAc)_4$.—This glucoside (4 g.) was obtained in the like manner from anisoylcarbinol (5 g.). It crystallised from 80% methyl alcohol in elongated prisms with pointed ends, m. p. 133° (Found: C, 55·6; H, 5·7. $C_{23}H_{28}O_{12}$ requires C, 55·6; H, 5·6%). The substance is moderately readily soluble in cold alcohol and benzene and sparingly soluble in ether.

Numerous analogies are available indicating that the silver carbonate method applied to *O*-tetra-acetyl-α-glucosidyl bromide (the ordinary acetobromoglucose) yields β-glucosides. A recent example is the synthesis of amygdalin (Campbell and Haworth, J., 1924, **125**, 1337). The use of a higher temperature than the normal, as in the above cases, effects a considerable improvement in the yields. The possibility that these glucosides obtained from benzoylcarbinols are really derivatives of the α-hydroxyphenylacetaldehydes is excluded by their condensation with *o*-hydroxybenzaldehydes to flavylium salts.

3-O-*Tetra-acetyl-β-glucosidoxy-7-hydroxy-5-benzoyloxy-4'-acetoxyflavylium Chloride* (II).—A solution of ω-*O*-tetra-acetyl-β-glucosidoxy-4-acetoxyacetophenone (1·0 g.) and 2-benzoyloxy-4 : 6-dihydroxybenzaldehyde (1·5 g.) in dry chloroform (30 c.c.) and dry ether (100 c.c.) was saturated with dry hydrogen chloride at room temperature and protected from the access of moisture. In the course of 24 hours the liquid became deep red and exhibited an intense orange-green fluorescence. The condensation product crystallised on the sides of the vessel in red plates having a brilliant green reflex; it was collected after 3 days, washed with ether, and air-dried (0·4 g.) (Found: C, 55·8; H, 4·8. $C_{38}H_{35}O_{11}Cl,2H_2O$ requires C, 55·7; H, 4·8%). The original, decanted chloroform–ethereal solution gave a further 0·6 g. of more crude material when it was mixed with dry ether (100 c.c.), and in preparing larger quantities of this salt an equal volume of ether was added to the reaction product without first separating the red crystalline deposit. Recrystallisation was not a feasible operation. The salt is readily

soluble in alcohol to an orange-red solution exhibiting green fluorescence, and it is insoluble in cold 0·5% hydrochloric acid. The alcoholic solution gives a purplish-violet coloration with sodium hydroxide or concentrated aqueous sodium carbonate, but only a yellowish-red coloration with dilute aqueous sodium carbonate. We attribute this behaviour to the necessity for hydrolysis of the 4′-situated acetoxyl group as a preliminary to the exhibition of a strong alkali colour-reaction.

3-β-*Glucosidylpelargonidin Picrate.*—The crude benzoylpentaacetylglucosidylpelargonidin chloride (4·5 g.) was finely powdered and added to 8% sodium hydroxide solution (90 c.c.), cooled to 10°. Air was excluded from the apparatus by nitrogen, and 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 so that the concentration of hydrogen chloride was brought to 2%. The liquid immediately assumed a deep red colour and was heated at 60° until the formation of the oxonium salt appeared to be complete. Benzoic acid and other ether-soluble impurities were removed by five extractions and, on keeping, the separated aqueous solution deposited some tarry matter. The filtered liquid was evaporated over potassium hydroxide in a good vacuum at the ordinary temperature; more tar separated after 12 hours and was removed. The crude glucoside separated on further evaporation as a red gelatinous mass, which was collected and dried in the air. The product from three experiments was dissolved in 0·05% aqueous hydrochloric acid and precipitated by the addition of an excess of lead acetate solution. The mixture of the lead salt of the glucoside and lead chloride was collected, washed with 0·05% hydrochloric acid (100 c.c.), drained, and added to acetic acid (100 c.c.), which left the lead chloride undissolved. The solution was filtered, and the lead salt precipitated by the addition of ether (500 c.c.) as a dark red sticky mass, which after decantation of the mother-liquor was washed with fresh ether. The residual lead salt was then decomposed by means of propyl alcohol (150 c.c.) and 25% methyl-alcoholic hydrogen chloride (25 c.c.), and the lead chloride removed by filtration. On the addition of dry ether (1000 c.c.) the anthocyanin chloride was precipitated in an amorphous condition as a bright red solid (yield, 1·2 g.). At this stage the hydrochloride could not be crystallised, but it exhibited the correct reactions.

The salt was dissolved in 0·01% hydrochloric acid (10 c.c.), and cold saturated aqueous picric acid (70 c.c.) added. After 24 hours the bright red precipitate was collected, washed with saturated aqueous picric acid, and dried (1·2 g.). The product had a dull

golden reflex. Another specimen was prepared by the addition of aqueous picric acid to an alcoholic solution of the crude chloride.

The salt dissolved with difficulty in boiling alcoholic picric acid (saturated in the cold) and separated gradually in the course of 3—4 days from the cooled solution as a mass of irregular, bright red plates having a brilliant golden reflex. On recrystallisation from more dilute alcoholic picric acid (4 vols. of alcohol mixed with 1 vol. of cold saturated alcoholic picric acid) perfect, microscopic, rectangular plates were obtained; the separation occupied 3—4 days in this case also. The derivative was once more crystallised and washed with alcoholic picric acid and then with ether (Found: N, 6·0. $C_{27}H_{23}O_{17}N_3$ requires N, 6·35%). The salt is moderately readily soluble in ethyl alcohol and readily soluble in methyl alcohol and water. The alcoholic solutions are red in colour, tinged with violet, and become more orange on dilution with alcohol.

3-β-*Glucosidylpelargonidin Chloride* (*Callistephin Chloride*) (III).—The pure crystalline picrate (0·8 g.) was dissolved in 5% methyl-alcoholic hydrogen chloride (35 c.c.), and the chloride precipitated as an amorphous red solid by the addition of ether (300 c.c.). This material (0·5 g.) could be crystallised, by the method described by Willstätter and Burdick (*loc. cit.*) for callistephin chloride, by the slow evaporation of a methyl-alcoholic solution to which one-quarter of its volume of 12% hydrochloric acid had been added. The anthocyanin crystallised in hair-fine, orange-red needles and it was recrystallised in the same manner and obtained in the same form. The crystals are voluminous, filling the liquid and matting together on the filter. In mass they have a dark brownish-red colour and bronze reflex, but are orange by transmitted light under the microscope. A specimen was washed with 12% hydrochloric acid and dried in the air (Found: C, 49·9, 49·8; H, 5·2, 5·0; Cl, 7·1; loss at 105° in a high vacuum, 7·1, 7·2. Calc. for $C_{21}H_{21}O_{10}Cl,2H_2O$: C, 49·9; H, 5·0; Cl, 7·1; $2H_2O$, 7·1%. Found in anhydrous material: C, 53·8, 53·7; H, 4·8, 4·8. Calc. for $C_{21}H_{21}O_{10}Cl$: C, 53·8; H, 4·5%). Willstätter and Burdick record concordant analyses of anhydrous callistephin chloride (C, 53·84; H, 4·61%), but in regard to the air-dried material they merely give the loss at 105° (8·05%), which is between that calculated for $2H_2O$ (7·1%) and $2·5H_2O$ (8·8%).

Every statement made by Willstätter and Burdick (*loc. cit.*, p. 161) regarding the properties of callistephin chloride applies to the synthetic glucoside and therefore there can be no doubt that the substances are identical. The annexed table illustrates this and also the contrast with the isomeric pelargonenin chloride, the

properties of which have been recorded by Willstätter and Bolton (*loc. cit.*).

Callistephin chloride.	3-β-Glucosidyl-pelargonidin chloride.	Pelargonenin chloride.
Dark brown-red mass of hair-fine needles with bronze reflex.		Scarlet-red needles.
Readily soluble in water to a yellowish-red solution that becomes more violet on dilution and then colourless.		Sparingly soluble in water.
Readily soluble in 0·5%, 7%, and moderately readily soluble in 10%, aqueous hydrochloric acid; much less readily soluble in 12% hydrochloric acid.		Very difficultly soluble in 0·5% hydrochloric acid or acid of greater concentration.
When concentrated solutions in aqueous hydrochloric acid are kept, the anthocyanin separates as a gelatinous mass.		
Readily soluble in 7% sulphuric acid.		Sparingly soluble in 7% sulphuric acid.
Easily soluble in methyl alcohol containing hydrogen chloride.		Easily soluble in methyl alcohol.
Easily soluble in ethyl alcohol.		Moderately readily soluble in ethyl alcohol.
The alcoholic solutions are red, tinged with violet, and much yellower than pelargonidin solutions.		Colour of alcoholic solutions intermediate between those of callistephin and pelargonidin.
The alcoholic solutions do not exhibit any fluorescence and give no ferric chloride colour reaction.		The alcoholic solution exhibits strong fluorescence.
Moderately concentrated aqueous acid solutions are yellowish-red and dilute solutions are reddish-yellow.		The acid aqueous solutions have a more bluish tinge.
With aqueous sodium carbonate a reddish-violet to violet-red solution is obtained. The same colour is produced by sodium hydroxide and even in alcoholic solution the colour is reddish-violet, only a shade bluer than in aqueous solution.		Aqueous sodium carbonate gives a violet-blue coloration and in alcoholic solution the coloration is pure blue.

A specimen of pure callistephin chloride was unfortunately not available for a direct comparison, but Professor R. Willstätter very kindly sent us a specimen of partly purified material (" kugeln ") and in addition some asterin chloride. The callistephin specimen gave a ferric chloride reaction (due to asterin) and its alkali-colour reaction was bluer than that of our synthetic glucoside. We therefore took a dilute methyl-alcoholic solution of the latter (containing a trace of hydrochloric acid) and added a solution of asterin chloride of the same concentration in the same faintly acid methyl alcohol, drop by drop; a solution was finally obtained which had exactly the same colour and behaviour with ferric chloride and sodium carbonate as a solution of the crude callistephin (also in the faintly acid methyl alcohol).

Distribution Number.—Willstätter and Burdick state that the partition of callistephin chloride between amyl alcohol and 0·5% hydrochloric acid characterises it as a normal monoglucosidic anthocyanin, but did not determine the distribution number. The synthetic glucoside exhibits an unusually high number. This was determined according to the method of Willstätter and Zollinger (*Annalen*, 1916, **412**, 208). The air-dried pigment was employed and a standard was prepared from 2·130 mg. dissolved in methyl-alcoholic hydrochloric acid (1 c.c.) and made up to 50 c.c. with amyl alcohol previously saturated with 0·5% aqueous hydrochloric acid. The pigment (10·023 mg.) was dissolved in 0·5% hydrochloric acid and shaken with pyridine-free amyl alcohol previously brought into equilibrium with 0·5% hydrochloric acid. It was found useful to clear the solutions centrifugally before making the colorimetric observations; these showed that 31·9% of the total colouring matter had passed into the amyl-alcoholic layer. On a second extraction 32·1% of the material remaining in the aqueous solution passed into the amyl alcohol. The distribution number is therefore 32.

It is difficult to criticise this value because of the paucity of data relating to monoglucosidic anthocyanins. Willstätter and Bolton found the value 19·5 for chrysanthemin (*Annalen*, 1916, **412**, 146). The distribution is visibly affected by the strength of the aqueous acid, and especially in the presence of picric acid a much larger proportion of the glucoside passes to the amyl-alcoholic layer.

Absorption.—The solvent employed was 98% alcohol (475 c.c.) mixed with 20% hydrochloric acid (25 c.c.). The following observations were made through a thickness of 10 cm.: *N*/5000, end of band at 5825; *N*/10,000, at 5757; *N*/20,000, at 5690, and, on addition of ammonia, shift to 6138; *N*/40,000, 5598 (fairly sharp) . . . 4660 (indefinite); *N*/80,000, 5500 (not sharp) . . . 4865—4790. At *N*/40,000 the light admitted in the blue region is of feeble intensity, but at *N*/80,000 the band is obvious.

Hydrolysis.—An equal volume of concentrated hydrochloric acid was added to a boiling solution of the glucoside in 0·5% hydrochloric acid, and after the mixture had been maintained at the point of incipient ebullition for about a minute crystals began to separate; thereafter the solution was soon almost decolorised. The salt was collected, washed with 1% hydrochloric acid, and dried at 80—90° in the air (Found: C, 56·1, 56·1; H, 4·0, 4·1. Calc. for $C_{15}H_{11}O_5Cl,H_2O$: C, 55·6; H, 4·0%). A somewhat high carbon content has previously been observed in pelargonidin chloride hydrate (compare Willstätter and Zechmeister, *Sitzungsber. Preuss. Akad. Wiss. Berlin*, 1914, 886; Pratt and Robinson, J., 1924, **125**,

188). In every respect this substance exhibited the recorded properties of pelargonidin chloride and it was shown by careful comparison of reactions, solubility, and crystal form to be identical with a specimen, prepared from benzoylpelargonidin chloride, which we hope to describe in detail in a later paper.

We wish to thank the Chemical Society and the Royal Society for grants which have defrayed a part of the cost of this investigation.

THE UNIVERSITY, MANCHESTER. [*Received, March 27th*, 1928.]