CCCLXXIII.—*Experiments on the Synthesis of Anthocyanins. Part VII. The Four Isomeric β-Glucosides of Pelargonidin Chloride.*

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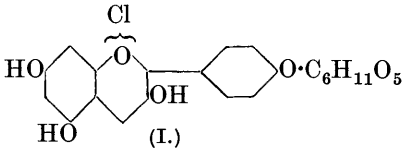
PELARGONIDIN 3-β-GLUCOSIDE (callistephin) has already been synthesised (Part V) and we now place on record the synthesis of the remaining isomeric β-glucosides.

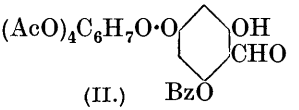
O-Benzoylphloroglucinaldehyde and 4-tetra-acetyl-β-glucosidoxy-ω-acetoxyacetophenone were condensed together under the influence of hydrogen chloride in ethyl acetate solution; the hydrolysis and reconstitution of the pyrylium ring offered no difficulty and the resulting pelargonidin chloride 4′-β-glucoside (I) crystallised with facility. It is quite unlike callistephin and pelargonenin in its colour reactions with alkalis.

Again, *O*-benzoylphloroglucinaldehyde was converted into a tetra-acetyl-β-glucoside, and this substance must have the constitution (II). This follows from the success of the flavylium salt synthesis, but it was also demonstrated in the following manner.

Methylation and subsequent hydrolysis of the glucoside furnished a monomethylphloroglucinaldehyde glucoside, and the same substance was prepared in stages from 4 : 6-dihydroxy-2-methoxybenzaldehyde.



(I.)



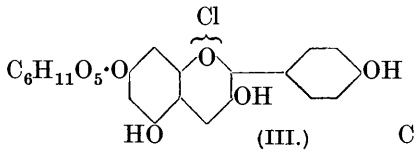
(II.)

The condensation of (II) with ω : 4-dihydroxyacetophenone provided, after the usual stages, pelargonidin chloride 7-β-glucoside (III). This substance bears some resemblance to pelargonenin, but its alcoholic solution does not exhibit fluorescence. By elimination of alternatives we were thus satisfied that pelargonenin chloride, the product of the partial hydrolysis of pelargonin (Willstätter and Bolton, *Annalen*, 1916, **412**, 133), must be pelargonidin chloride 5-glucoside (IV), and this view was confirmed by the preparation of the isomeric pelargonidin chloride monomethyl ethers (V and VI).

5-*O*-Methylpelargonidin chloride (V, R = OMe) (and the related flavylium salt in which Me replaces OMe) exhibits the colour reactions and fluorescence of pelargonenin chloride, but 7-*O*-methylpelargonidin chloride (**VI, R** = OMe) (and the related orcinol derivative) does

not exhibit fluorescence in alcoholic solution and is similar to the pelargonidin 7-β-glucoside chloride.

We were anxious to clinch this matter by a synthesis of pelargonenin chloride and although we ultimately succeeded in achieving our



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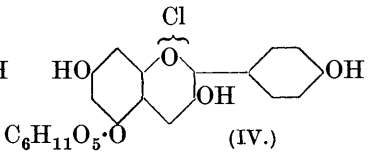

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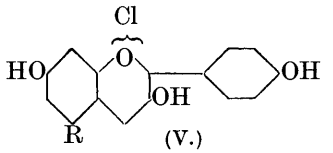

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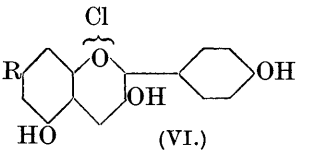


(VI.)

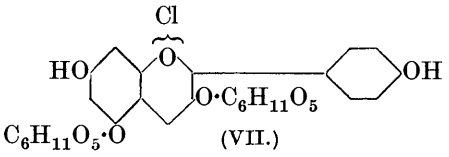
object the method which we were compelled to adopt is a highly unsatisfactory one. *O*-Benzoylphloroglucinaldehyde was benzoylated again and yielded 6-hydroxy-2 : 4-dibenzoyloxybenzaldehyde, as was proved by methylation and hydrolysis. This substance was converted into a tetra-acetylglucoside which could not be purified but, of course, must bear the glucosidoxy-group in the *o*-position to the formyl residue. On hydrolysis it furnished a solution of phloroglucinaldehyde glucoside, also not isolated, and the crude product was condensed in cold alcoholic solution with ω : 4-dihydroxyacetophenone. After purification of the resulting flavylium salt a pelargonidin 5-β-glucoside was isolated in orange-red needles, and this substance (IV) has the properties of pelargonenin chloride as recorded by Willstätter and Bolton (*loc. cit.*).

The position is, therefore, that pelargonin yields pelargonidin-5-glucoside on hydrolysis and it would be natural to conclude that the anthocyanin is a pelargonidin 5-bioside. However, there is strong evidence available that position 3 does not bear a hydroxyl group and we have convinced ourselves that this is so by an examination of the rate of destruction of various synthetic and naturally occurring members of the group by means of ferric chloride in very dilute solution. All the synthetic glucosides now described and pelargonidin are rapidly attacked, but pelargonin and callistephin are only very slowly broken down. This is an extension of the work of Karrer, Widmer, Helfenstein, Hürliman, Nievergelt, and Monsarrat-Thomas (*Helv. Chim. Acta*, 1927, **10**, 729) on the oxidation of anthocyanins by means of hydrogen peroxide and we accept the views of these authors in regard to the effect of the hydroxyl in position 3 in rendering the molecule vulnerable.

Our method can be used to test a large number of substances in

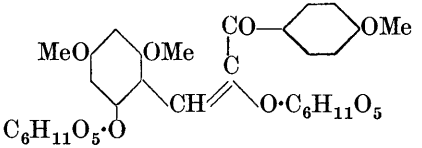
this connexion and we find that the contrast holds in all cases—hydroxyl in position 3, rapid loss of colour—hydroxyl in position 3 absent or modified, relative stability.

We are, therefore, convinced that pelargonin is pelargonidin 3 : 5-diglucoside (VII)



(VII.)

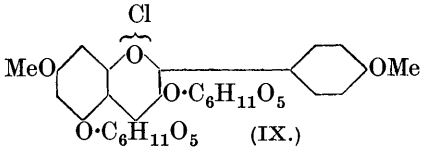
(VIII.)



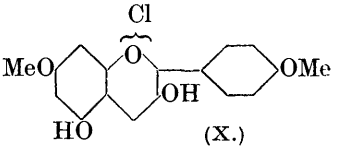
[Methylation of glucose rests is not represented.]

This theory can, we imagine, be easily reconciled with and is, indeed, supported by, the results of Karrer and others (*loc. cit.*) and Karrer and Widmer (*Helv. Chim. Acta*, 1928, **11**, 837) on the methylation of monardaein and other anthocyanins.

In the first series of experiments it is evident, as we have previously suggested, that the ring opened and the newly formed hydroxyl was methylated. This gave the substance (VIII) and naturally the action of hot hydrochloric acid split off the glucose residues and yielded the 5 : 7 : 4′-trimethyl ether of pelargonidin. This explanation applies also to the methylation of cyanin, peonin, and malvin chlorides, which we consider are also 3 : 5-diglucosides. In the experiments of Karrer and Widmer (*loc. cit.*) on the methylation of monardaein the pyran ring was probably not opened and the process may be represented in the following manner. Following the suggestion of Willstätter respecting delphinin, we assume that the *p*-hydroxycinnamoyl groups of monardaein are attached to the carbohydrate part of the molecule. Methylation affects then the phenolic groups in the acid and the free hydroxyl groups at positions 4′ and 7 in the pelargonidin nucleus. We obtain (IX) by hydrolysis of the product with alcoholic potassium hydroxide, followed by the action of hydrochloric acid.



(IX.)



(X.)

On further hydrolysis by hot 20% hydrochloric acid, this monardin

(pelargonin) dimethyl ether chloride yields pelargonidin dimethyl ether chloride (X).

The alkali colour reactions no longer offer a difficulty for reasons fully explained in subsequent papers of this series.

EXPERIMENTAL.

3 : 7 : 4′-Trihydroxy-5-methylflavylium Chloride. (V, R = Me).—A solution of 6-orcylaldehyde (2·5 g.) and ω-hydroxy-*p*-acetoxyacetophenone (2·0 g.) in dry ethyl acetate (70 c.c.) was saturated with dry hydrogen chloride and kept at room temperature for 36 hours. The product which had separated was then collected and washed with dry ethyl acetate (yield, 3·5 g.). This product was contaminated with a small amount of xanthylium salt formed by the self-condensation of 6-orcylaldehyde and this exhibited a strong greenish-yellow fluorescence in alcoholic solution. The separation of this impurity was rather tedious and was carried out as follows. The impure salt was dissolved in warm 70% alcohol containing 1% of hydrogen chloride, and hydrogen chloride was passed into the warm solution until the liquid began to deposit crystals; on cooling, the solution deposited the chlorides. After twelve recrystallisations in this way a product was obtained which did not exhibit the xanthylium salt type of fluorescence in alcoholic solution, and the salt was then twice recrystallised as before and obtained in orange-red, elongated, rectangular prisms—deep red in mass (Found in air-dried material: C, 60·0; H, 4·7. $C_{16}H_{13}O_4Cl,H_2O$ requires C, 59·6; H, 4·6%).

The salt is readily soluble in alcohol to a pelargonenin-type fluorescent solution, and is only slightly soluble in dilute hydrochloric acid (5—10%). With sodium carbonate it gives a blue-violet solution which is bluish in thin layers. Addition of sodium hydroxide gives the same colour, but this fades in about 2 minutes. In alcoholic solution a bluish-purple coloration is obtained on the addition of sodium carbonate.

3 : 5 : 4′-Trihydroxy-7-methylflavylium Chloride (VI, R = Me).—A solution of γ-orcylaldehyde (0·6 g.) (prepared from atranorin by the method of St. Pfau, *Helv. Chim. Acta*, 1926, **5**, 650) and ω-hydroxy-4-acetoxyacetophenone (1·4 g.) in dry ethyl acetate (40 c.c.) was saturated with dry hydrogen chloride, and the solution kept at room temperature for 36 hours. The product which had crystallised was collected and washed with dry ethyl acetate (yield, 1·0 g.). The salt was dissolved in warm alcohol, the solution filtered, and concentrated hydrochloric acid (⅓ vol.) added. Alcohol was evaporated until the solution showed signs of depositing solid. On cooling, the flavylium salt separated in dark red, rhombic, microscopic prisms

(Found in air-dried material : C, 56·1 ; H, 5·1. $C_{16}H_{13}O_4Cl,2H_2O$ requires C, 56·4 ; H, 5·0%).

The salt is readily soluble in alcohol and the solution is quite non-fluorescent. It is practically insoluble in cold concentrated hydrochloric acid, and only slightly soluble in 5—10% hydrochloric acid. With sodium carbonate the salt gives a violet coloration, and sodium hydroxide gives the same colour which, however, fades almost instantly. An alcoholic solution on the addition of sodium carbonate assumes a bluish-violet tinge.

2-*Hydroxy*-4-*benzoyloxy*-6-*methoxybenzaldehyde*.—4 : 6-Dihydroxy-2-methoxybenzaldehyde (7 g.) (prepared by Gattermann's method from phloroglucinol monomethyl ether) and benzyl bromide (5·2 g.) were dissolved in acetone (60 c.c.), and anhydrous potassium carbonate (10 g.) added to the solution, which was refluxed during 1½ hours. After filtration the acetone was removed under diminished pressure ; the residue on trituration with cold water gradually solidified. Recrystallised from methyl alcohol and then from ethyl alcohol, the *ether* was obtained as elongated prisms, m. p. 101—102° (Found : C, 70·0 ; H, 5·4. $C_{15}H_{14}O_4$ requires C, 69·8 ; H, 5·4%).

This ether is practically insoluble in cold methyl or ethyl alcohol, but readily soluble in ether and in ethyl acetate. In alcoholic solution it gives a reddish-brown coloration on the addition of a drop of aqueous ferric chloride. The colour developed is identical with that given by dimethylphloroglucinaldehyde.

Proof of the constitution of this substance is afforded by the fact that it condenses with ω-hydroxy-4-acetoxyacetophenone to a flavylium salt.

3 : 4′-*Dihydroxy*-7-*benzyloxy*-5-*methoxyflavylium Chloride*.—A solution of the benzyl ether of 6-methylphloroglucinaldehyde (2·5 g.) and ω-hydroxy-4-acetoxyacetophenone in ethyl acetate (40 c.c.) was saturated with dry hydrogen chloride. After 3 days the product which had separated was collected and well washed with dry ethyl acetate (yield, 2·5 g.). The salt was dissolved in warm methyl alcohol, and concentrated hydrochloric acid ($\frac{1}{10}$ vol.) added to the filtered solution. Methyl alcohol was evaporated until the residue showed signs of crystallising ; on cooling, the solution deposited the salt as bright red, rhombic plates (brilliant green reflex) (Found in air-dried material : C, 66·6 ; H, 4·9. $C_{23}H_{19}O_5Cl$ requires C, 67·1 ; H, 4·6%).

The salt is very sparingly soluble in 1% hydrochloric acid and is readily soluble in warm alcohol to a solution which exhibits greenish-yellow fluorescence. An alcoholic solution becomes red-violet on the addition of aqueous sodium carbonate solution and the colour is stable.

Sodium hydroxide solution gives the same colour, but the latter fades in 1 minute.

3 : 7 : 4′-*Trihydroxy*-5-*methoxyflavylium Chloride* (V, R = OMe).—The benzyl derivative (0·8 g.) was suspended in a mixture of glacial acetic acid (300 c.c.) and concentrated hydrochloric acid (150 c.c.), which was then refluxed for 1 hour; the solid dissolved during the first 15 minutes. The solvent was then distilled until solid began to separate; on cooling, the residue deposited the 5-*methyl ether* of pelargonidin chloride as deep red, elongated prisms with a green reflex. This salt was dissolved in warm methyl alcohol, and concentrated hydrochloric acid (½ vol.) added. Methyl alcohol was then evaporated until the solution began to crystallise; on cooling, the chloride separated as red-brown, elongated, rectangular prisms with a faint green reflex (Found in air-dried material: C, 55·5; H, 5·0; MeO, 8·5. $C_{16}H_{13}O_5Cl,1\frac{1}{2}H_2O$ requires C, 55·4; H, 4·6; MeO, 8·9%). The salt is readily soluble in warm methyl and ethyl alcohol. It is nearly insoluble in cold dilute hydrochloric acid (1% to 5%) and is sparingly soluble in the warm solvents. An alcoholic solution exhibits a greenish-yellow fluorescence. With aqueous sodium carbonate the salt gives a blue-violet solution which is stable. Addition of sodium hydroxide produces no change in colour, but the latter rapidly fades to a dirty yellow.

3 : 5 : 4′-*Trihydroxy*-7-*methoxyflavylium Chloride* (VI, R = OMe).—6-Hydroxy-2-benzoyloxy-4-methoxybenzaldehyde (2·0 g.) and ω-hydroxy-4-acetoxyacetophenone (3 g.) were dissolved in dry ethyl acetate (50 c.c.), and the mixture saturated with dry hydrogen chloride. The solution quickly assumed a deep red colour and after 3 days part of the product had separated, and the remainder was precipitated by the addition of dry ether (300 c.c.). The benzoyl derivative was dissolved in warm alcohol, and warm concentrated hydrochloric acid (⅓ vol.) added. On cooling, the solid separated in red elongated prisms which have a greenish-yellow fluorescence in alcoholic solution.

The benzoyl derivative was not further purified but was debenzoylated at once. The solid (3 g.) was added to 8% sodium hydroxide solution (60 c.c.) in an atmosphere of hydrogen, and the mixture vigorously agitated until the solid dissolved (½ hour). The mixture was kept at room temperature during 3 hours, and was then acidified with concentrated hydrochloric acid (100 c.c.). The solution was heated on the water-bath (to 80°) for 20 minutes and kept at room temperature over-night; the 7-methyl ether of pelargonidin chloride then separated. The salt was collected and washed with 4% hydrochloric acid to remove sodium chloride and then with ether to remove benzoic acid. Since the solid could not be crystallised, it

was converted into picrate. The chloride was dissolved in hot saturated alcoholic picric acid, and water added until the solution became cloudy. On cooling, the solution deposited the picrate in red-brown needles, which were recrystallised from a warm aqueous-alcoholic solution of picric acid (50% alcohol saturated with picric acid in the cold), and the picrate obtained as red-brown needles.

The picrate was dissolved in warm methyl alcohol, and hot 15% hydrochloric acid ($\frac{1}{2}$ vol.) added. Methyl alcohol was evaporated until the solution showed signs of depositing the chloride. The cooled residue deposited the chloride as a crystalline solid, which was collected and washed with 4% hydrochloric acid to remove traces of picric acid.

The chloride was dissolved in warm methyl alcohol, and concentrated hydrochloric acid ($\frac{1}{2}$ vol.) added. The alcohol was evaporated to a certain extent and, on cooling, the salt separated as dense clusters of rectangular prisms, red-brown by transmitted light under the microscope, and in mass exhibiting a green reflex (Found : C, 59·4; H, 4·4; MeO, 9·6. $C_{16}H_{13}O_5Cl$ requires C, 59·8; H, 4·4; MeO, 9·7%). The salt is slightly soluble in 1—2% hydrochloric acid and is readily soluble in alcohol to a solution that shows no fluorescence. With sodium carbonate solution this salt gives a violet colour which is bluish-violet in thin layers. Sodium hydroxide gives a more bluish-violet, which rapidly fades. An alcoholic solution gives a bluish-violet with sodium carbonate and this violet is much redder than that given by the 5-methyl ether.

Potassium Salt of ω-4-*Dihydroxyacetophenone.*—ω-4-Diacetoxyacetophenone (Robertson and Robinson, J., 1928, 1464) (20 g.), mixed with potassium hydroxide solution (20 g. in 80 c.c. of water), was heated in a boiling water-bath, a clear solution resulting in a few minutes. The heating was continued for a further 15 minutes; the solution was then allowed to cool in an ice-chest and large, colourless, shining plates filled the liquid. The salt was collected, washed repeatedly with cold alcohol, and dried in an evacuated desiccator over soda-lime (yield, quantitative).

The potassium salt is stable in air, easily soluble in boiling water, much less readily soluble in cold water, and almost insoluble in 8% aqueous potassium hydroxide or boiling alcohol. It crystallises from a small volume of boiling water (1 g. in 2 c.c.) as colourless shining, rectangular plates, which were ground with alcohol, washed repeatedly, and finally dried over soda-lime in a desiccator. On heating, it loses water of crystallisation at 120°, turns brown at about 235°, and decomposes at 275° (Found : loss of weight at 120°, 15·6; K, 17·1. $C_8H_7O_3K,2H_2O$ requires $2H_2O$, 15·9; K, 17·3%).

The corresponding silver salt crystallised in clusters of colourless

needles when aqueous silver nitrate was added to a dilute aqueous solution of the potassium salt. Carefully washed, it remained unchanged in cold water for a considerable time, but on warming it darkened and a mirror was rapidly formed.

ω:4-*Dihydroxyacetophenone.*—When excess of concentrated hydrochloric acid was added to a warm concentrated aqueous solution of the potassium salt, and the solution cooled, the *ketone* separated as colourless flat needles. It is sparingly soluble in cold dilute hydrochloric acid, but is readily soluble in boiling water and separates on cooling. In boiling benzene, chloroform, or ether it is rather sparingly soluble, whereas it easily dissolves in cold alcohol or acetone; it is also easily soluble in aqueous sodium carbonate. The pure substance sinters at 170° and melts at 177—178° (Found: C, 63·3; H, 5·2. $C_8H_8O_3$ requires C, 63·2; H, 5·2%).

The substance reduces Fehling's solution in the cold within a minute and deposits silver from ammoniacal silver nitrate on warming. It furnished ω : 4-diacetoxyacetophenone when heated with sodium acetate and acetic anhydride for 2 hours on the steam-bath; the derivative crystallised from alcohol in colourless prisms, m. p. 98°, identical with the specimen obtained from ω-chloro-4-hydroxyacetophenone (*loc. cit.*). An *osazone* was readily obtained by mixing a hot aqueous solution of the ketone (0·5 g.) and a hot solution of phenylhydrazine (2 c.c.) in dilute acetic acid. In a few seconds turbidity appeared and a yellow crystalline precipitate filled the liquid. The reaction was completed by gentle heating for 15 minutes and the product was twice crystallised from alcohol, forming golden-yellow threads, m. p. 212—214° (Found: N, 16·6. $C_{20}H_{18}ON_4$ requires N, 17·0%).

5-O-*Benzoylpelargonidin Chloride.*—A solution of the dihydroxyketone (0·5 g.) and 2-*O*-benzoylphloroglucinaldehyde (1 g.) in dry ethyl acetate (40 c.c.) was saturated with dry hydrogen chloride. Within a minute of passing the gas the solution became strongly fluorescent, appearing green by reflected and red by transmitted light, and crystals separated in a few minutes. After 24 hours an equal volume of ether was added and the brilliant indigo-like crystals (thick plates) of benzoylpelargonidin chloride were collected. The yield was theoretical and the substance was identical with the specimen obtained by Robertson, Robinson, and Sugiura (J., 1928, 1533) from diacetoxyacetophenone.

ω-*Hydroxy*-4-β-*tetra-acetylglucosidoxyacetophenone.*—A solution of the potassium salt of ω : 4-dihydroxyacetophenone (4 g.) in a mixture of water (40 c.c.) and acetone (40 c.c.) was treated gradually in the cold with α-tetra-acetylglucosidyl bromide (8·8 g.) in acetone (20 c.c.) with shaking. The clear colourless solution was kept for 24 hours

with occasional shaking; acetone was then removed under diminished pressure below 30°, and the viscous semi-solid residue washed repeatedly with cold water and kept in the ice-chest over-night. The resulting colourless solid was dissolved in a little cold methyl alcohol, and the solution rendered just turbid by addition of water; colourless flat needles were slowly obtained, m. p. 135—137° with sintering a few degrees lower (yield, 2·5 g.). The purified substance, m. p. 149—150°, was sparingly soluble in cold methyl and ethyl alcohols and especially so in 70% alcohol; it was easily soluble in the hot solvents and in chloroform and acetone. It was sparingly soluble in ether and reduced Fehling's solution in the cold and ammoniacal silver nitrate on heating (Found: C, 54·5; H, 5·3. $C_{22}H_{26}O_{12}$ requires C, 54·7; H, 5·4%).

5-O-*Benzoyl*-4′-O-*tetra-acetylglucosidylpelargonidin Chloride.*—ω-Hydroxy-4-β-tetra-acetylglucosidoxyacetophenone (2·5 g.) and 2-*O*-benzoylphloroglucinaldehyde (1·7 g.) were dissolved in a mixture of dry ether (80 c.c.) and chloroform (40 c.c.), and the solution saturated in the cold with dry hydrogen chloride. In a few minutes the liquid became fluorescent and after a few hours reddish-brown crystals began to be deposited from the reddish-violet solution. After 3 days, dry ether (120 c.c.) was added so as completely to precipitate the pyrylium salt, which was then filtered off, repeatedly washed with dry ether, and dried in an evacuated desiccator over sulphuric acid. The soft, thin, microscopic needles have a reddish-brown colour in bulk (yield, 3 g.). On heating, the salt darkens at 182°, sinters at 193°, and decomposes at 198° (Found: C, 58·6; H, 4·4. $C_{36}H_{33}O_{15}Cl$ requires C, 58·3; H, 4·5%).

The substance dissolves in glacial acetic acid to a yellowish-red solution with an intense green fluorescence which disappears on addition of water. Aqueous sodium carbonate dissolves it to a bluish-pink solution, which becomes pure blue on the addition of acetone. The solution in methyl alcohol is red and non-fluorescent and becomes colourless on dilution; on addition of concentrated hydrochloric acid the red colour reappears and the solution exhibits a green fluorescence.

4′-β-*Glucosidylpelargonidin Chloride.*—The tetra-acetylbenzoyl compound (2 g.) was mixed with 8% aqueous sodium hydroxide solution (40 c.c.) previously cooled below 10°; a deep blue colour, at first formed, faded through green and a clear brown solution resulted. After this had been kept at room temperature in an atmosphere of nitrogen for 3 hours, it was cooled in ice and treated with enough 14% hydrochloric acid to neutralise the alkali and make the acid concentration 2%. The deep crimson solution was slowly heated to 60° and kept for 12 hours; the acid concentration was then

increased to 5% by the addition of concentrated hydrochloric acid, and the solution extracted with an equal volume of ether. The deep red, crystalline precipitate was collected, extracted with boiling 2% hydrochloric acid (20 c.c.), and the salt separating from the filtrate was then crystallised twice from 50% methyl alcohol containing 2% of hydrochloric acid. It was obtained in shining, red, narrow plates, tapering at the ends (yield, 0·5 g.). When rapidly heated, it decomposed at 184° (Found in air-dried material: loss in weight at 105° in a high vacuum, 9·6; Cl, 6·9. $C_{21}H_{21}O_{10}Cl,2{\cdot}75H_2O$ requires H_2O, 9·6; Cl, 6·9%. Found in material dried at 105° in a high vacuum: C, 54·0; H, 4·7. $C_{21}H_{21}O_{10}Cl$ requires C, 53·8; H, 4·5%).

The substance is not readily soluble in cold water but dissolves on warming; it is only sparingly soluble in cold 5% aqueous hydrochloric acid or in cold acetone; but it dissolves easily in cold ethyl and methyl alcohols.

The aqueous and alcoholic solutions are non-fluorescent and deep red; on dilution, or keeping, the colour changes to bluish-violet and then it is discharged; addition of hydrochloric acid slowly restores the colour. Aqueous sodium acetate solution, added to a dilute acid solution of the pigment, brings about the same discharge of colour after a preliminary change to bluish-violet. Aqueous sodium carbonate and sodium hydroxide in aqueous and alcoholic solutions give reddish-violet colorations, which appear bluish-violet on dilution.

The distribution number was determined by the method of Willstätter and Zollinger (*Annalen*, 1916, **412**, 208) using pyridine-free amyl alcohol saturated in the cold with 0·5% aqueous hydrochloric acid. With the air-dried pigment (10·033 mg.), amyl alcohol (50 c.c.), and 0·5% hydrochloric acid (50 c.c.) the first extraction gave the distribution number 36·6 and the second, 35·8.

Hydrolysis. The glucoside was boiled for 5 minutes with 15% aqueous hydrochloric acid. The solution was almost decolourised and pelargonidin chloride crystallised in the characteristic shape of swallows' wings. It was completely extracted from 0·5% aqueous hydrochloric acid by amyl alcohol and it was found in every respect to be identical with an authentic specimen (J., 1928, 1535).

The picrate. The chloride was dissolved in water and mixed with a boiling aqueous solution of picric acid; the brown-red solution soon deposited deep red, rhombic prisms. The derivative was recrystallised from boiling aqueous picric acid, crushed under ether, washed repeatedly with this solvent, and finally dried in a vacuum over calcium chloride. It then decomposed at 146—148° with previous sintering (Found: N, 5·8. $C_{27}H_{23}O_{17}N_3,3H_2O$ requires N, 5·9%).

2-O-*Benzoylphloroglucinaldehyde.*—The method of preparation of this important intermediate was modified as described below.

Phloroglucinaldehyde which had been dried over concentrated sulphuric acid in an evacuated desiccator for 24 hours (18·6 g.) was mixed in a well-corked bottle with cold water (400 c.c.) and 10% potassium hydroxide solution (72 c.c.). The clear solution was then cooled in ice, and pure benzoyl chloride (14 c.c.) added in portions of 1 c.c., with vigorous shaking and occasional cooling in ice. After the addition of the acid chloride was completed (30—45 minutes) the mixture was mechanically shaken until the odour of benzoyl chloride disappeared (about 30 minutes). Cold aqueous sodium bicarbonate was then added, till the liquid reacted alkaline to litmus, and the vigorous shaking continued for a minute or so. The precipitate was collected, and the filtrate treated with more benzoyl chloride (5 c.c.) and excess of sodium bicarbonate solution. It was again shaken until the benzoyl chloride had completely reacted and the fresh precipitate was collected. By this means the yield of the benzoyl derivative was increased by nearly 15%. The total solid was dissolved in boiling alcohol (200 c.c.), a little charcoal added, and the filtered solution added to cold water (800 c.c.); a curdy precipitate was formed which was collected after 12 hours. The yield of this crude product after drying in an evacuated desiccator over sulphuric acid was 21 g.; m. p. 182—187°.

The dry crude product was ground into a paste with chloroform (50 c.c.), and the mixture boiled for a minute and allowed to cool. The solid was collected, washed with a little cold chloroform (10 c.c.), and crystallised from the minimum volume of boiling 95% ethyl alcohol. At this stage the substance was quite pure, m. p. 200—201° to a dark red liquid, but it had a light red colour. It was dissolved in acetone (100—150 c.c.), boiled with a little animal charcoal, and the filtered solution poured into water (1 litre). By this means a colourless crystalline solid was obtained, m. p. 201° (yield, a little more than 10 g.).

The alcoholic mother-liquor obtained from the crystallisations was poured into a large volume of water, and the precipitated solid collected. The product from several experiments was then boiled with chloroform, cooled, collected, and repeatedly crystallised from 95% alcohol, whereby a further quantity of pure benzoylphloroglucinaldehyde was obtained.

The chloroform extract contained most of the 2 : 4-dibenzoylphloroglucinaldehyde which had been produced in small yield (0·5—1·0 g.) along with some monobenzoylphloroglucinaldehyde and other impurities. It was first extracted with aqueous sodium bicarbonate and subsequently thrice with 8% aqueous sodium carbonate solution

in order to remove all the monobenzoylphloroglucinaldehyde. The carbonate solution was acidified with hydrochloric acid, and the precipitate crystallised from 95% alcohol.

The remaining chloroform solution was washed with water, and the solvent evaporated. The residue was washed with a little alcohol and crystallised twice from benzene (animal charcoal). The *dibenzoylphloroglucinaldehyde* separated in colourless plates, m. p. 139—140° (Found : C, 69·9; H, 4·0. $C_{21}H_{14}O_6$ requires C, 69·6; H, 3·8%).

Salts of 2-O-*Benzoylphloroglucinaldehyde.*—Pure colourless 2-*O*-benzoylphloroglucinaldehyde (12 g.) was dissolved in dry acetone (100 c.c.), and the solution cooled in ice and treated with a cold solution of potassium (1·7 g.) in absolute alcohol (25 c.c.). The faint brown liquid was shaken and after 10 minutes treated gradually with dry ether (500 c.c.), with vigorous shaking. The *potassium* salt slowly separated as a light yellow powder. It was collected, repeatedly washed with dry ether, and kept in an evacuated desiccator over solid caustic potash for 24 hours (yield, 13 g.) (Found : K, 11·8. $C_{14}H_9O_5K$ requires K, 13·2%).

The salt underwent vigorous decomposition when heated above 70° and it did not undergo condensation with α-tetra-acetylglucosidyl bromide in cold dry acetone, chloroform, or benzene solution.

The monosilver salt. The potassium salt (4 g.) was dissolved in cold acetone (50 c.c.), a little cold water (2 c.c.) being added to clarify the solution. It was then gradually treated at 0° with an aqueous solution of silver nitrate (4 g. in 20 c.c.) with vigorous shaking. The brownish-red precipitate was collected, washed repeatedly with cold water and subsequently with cold acetone, and dried on porous porcelain in an evacuated desiccator. The dark brown powder weighed 2·5 g. This preparation was carried out in the dark room.

2 - *Benzoyl* - 4 - β - *tetra-acetylglucosidylphloroglucinaldehyde.—First method.* The above-described silver salt (2·5 g.) was shaken with a solution of α-tetra-acetylglucosidyl bromide (6 g.) in dry benzene (80 c.c.) during 24 hours. The filtered solution was twice extracted with 10% aqueous sodium carbonate (25 c.c. each time) in order to remove any benzoylphloroglucinaldehyde present, washed with water, and the solvent removed under reduced pressure. The residue was dissolved in a little acetone, mixed with a little aqueous sodium acetate, and after 12 hours poured into water (500 c.c.). The colourless precipitate after two crystallisations from 95% ethyl alcohol (charcoal) was obtained in colourless, thin, flat needles, m. p. 144—145° (yield, 0·2 g.) (Found : C, 57·2; H, 4·8. $C_{28}H_{28}O_{14}$ requires C, 57·1; H, 4·8%). The substance is readily soluble in cold

chloroform, benzene, and acetone, rather less readily soluble in ether, and very sparingly soluble in light petroleum. It is best crystallised from boiling methyl or ethyl alcohol. Ferric chloride imparts a brown-red colour to the alcoholic solution.

Second method. Pure 2-*O*-benzoylphloroglucinaldehyde (6·8 g.) was dissolved in a mixture of acetone (30 c.c.) and aqueous potassium hydroxide (2·4 g. in 48 c.c.), and a solution of α-tetra-acetylglucosidyl bromide (16·8 g.) in cold acetone (30 c.c.) was added with vigorous shaking. The whole was brought, by the addition of acetone (30 c.c.), into a homogeneous solution, which was kept for 30 hours with occasional shaking. It was then poured into water (1 litre) containing a few drops of acetic acid and kept for 12 hours. The pasty solid was collected, dried in a desiccator, ground with chloroform (60 c.c.), boiled for a minute, and cooled; most of the unreacted benzoylphloroglucinaldehyde was then deposited in an almost pure condition (3·0 g.). The chloroform filtrate was twice shaken with 10% aqueous sodium carbonate (40 c.c. and 30 c.c. respectively), by which means it was completely freed from unreacted monobenzoylphloroglucinaldehyde. The emulsion which was invariably produced during this process had to be resolved by means of a centrifuge. The chloroform layer was then washed with water containing a little acetic acid, the solvent evaporated, and the brown viscous residue crystallised from 95% ethyl alcohol (10 c.c.). When the dark brown solution was inoculated with a crystal of the pure glucoside the whole was filled with a mass of needle-like crystals; these were collected and the coloured impurities were completely removed by washing with cold 95% alcohol (5 c.c.). The solid was then quite colourless and had m. p. 143° (yield, 1·6—1·8 g.). A second crystallisation from dilute alcohol raised the melting point to 144—145°. This specimen was identical with that obtained by the first method.

Methylation of 2-Benzoyl-4-β-tetra-acetylglucosidylphloroglucinaldehyde. Formation of 2-Benzoyl-4-β-tetra-acetylglucosidyl-6-O-methylphloroglucinaldehyde.—The tetra-acetylglucoside (0·7 g.) was dissolved in pure acetone (20 c.c.), methyl iodide (3 c.c.) and silver carbonate (1·5 g.) were added, and the whole was shaken vigorously for 48 hours. The residue after filtration was repeatedly washed with acetone. After removal of the solvent, the colourless semi-solid residue was taken up in ether, and the solution shaken twice with 5% aqueous potassium hydroxide and twice with water; the alkaline extract gave no precipitate on acidification, thus showing that methylation was complete.

The glass resulting from the removal of the ether was easily soluble in the ordinary organic solvents and was therefore purified by dissolution in a little boiling ethyl alcohol and addition of water

so that the solution was just clear at the boiling point. On cooling, a colourless semi-solid was deposited and this slowly became hard and crystalline. It was then collected, powdered, and washed with cold water, and this process repeated; the purified substance was dried over concentrated sulphuric acid in an evacuated desiccator for 48 hours. It sintered at about 85°, became vitreous and then easily fluid at about 120°. It did not give any colour with ferric chloride in alcoholic solution and was only slowly attacked by aqueous 10% potassium hydroxide. The yield was satisfactory (Found: C, 58·1; H, 5·2. $C_{29}H_{30}O_{14}$ requires C, 57·8; H, 5·0%).

The hydrolysis of the foregoing substance was attempted with aqueous-alcoholic hydrochloric acid with the idea of first removing the glucose residue, leaving the benzoyl group to be removed by subsequent treatment with aqueous potash so that 2-*O*-methylphloroglucinaldehyde could be isolated from the alkaline solution. This was contemplated, as it was found that 2-*O*-methylphloroglucinaldehyde was rather sensitive to hydrochloric acid and was transformed into a dark product which was strongly fluorescent in alkaline solution. The process of hydrolysis with aqueous alcoholic hydrochloric acid, however, took a different course. The benzoyl and the acetyl groups were removed and 4-β-*glucosidyl*-2-O-*methylphloroglucinaldehyde* was obtained in fair yield. The methylation product (0·4 g.) was dissolved in ethyl alcohol (8 c.c.), the solution heated to 50—60° in a water-bath, and 15% aqueous hydrochloric acid (4 c.c.) added. The turbidity that appeared at first soon disappeared and the clear solution was maintained at 50—60° for 4 hours and then kept for 12 hours. The alcohol was distilled in a vacuum until a light yellow crystalline solid appeared; more water (2—4 c.c.) was then added, and the mixture extracted with ether in order to remove benzoic acid. The solid was collected and crystallised twice from 80% ethyl alcohol (animal charcoal), forming colourless, compact, elongated plates and prisms which sintered at 235°, melted at 237—239°, and decomposed at a higher temperature.

4-β-Glucosidyl-2-*O*-methylphloroglucinaldehyde is almost insoluble in ether, chloroform, and benzene; it is slightly soluble in cold water or alcohol, but it is easily soluble in the hot solvents. It dissolves in aqueous potassium hydroxide, and an alcoholic solution gives a brownish-red coloration with ferric chloride (Found: C, 51·1; H, 5·7. $C_{14}H_{18}O_9$ requires C, 50·9; H, 5·5%).

The removal of the benzoyl group under the above conditions of hydrolysis is not an isolated instance. This method is also applicable in the parallel case of 6-*O*-benzoyl-2:4-*O*-dimethylphloroglucinaldehyde (Robertson, Robinson, and Struthers, J., 1928, 1455), from which 2:4-*O*-dimethylphloroglucinaldehyde could be

obtained in good yield : the former (0·25 g.) was dissolved in ethyl alcohol (12 c.c.), and the solution treated with 15% aqueous hydrochloric acid (6 c.c.) and kept at 50—60° for 4 hours. Subsequently the dimethyl ether, m. p. 70°, could be isolated.

The hydrolysis of the methylation product proceeded easily in cold alcoholic potash and a pure product was obtained after one crystallisation. The benzoyltetra-acetyl compound (0·2 g.) was treated with 10% methyl-alcoholic potassium hydroxide (4 c.c.) in the cold, and the clear solution kept for 3 hours. The alcohol was then distilled in a vacuum at room temperature, the residue dissolved in the minimum quantity of water (2—3 c.c.), and the solution saturated with carbon dioxide. On vigorous stirring, a colourless crystalline precipitate was formed and this was collected, washed with a little water, and crystallised from 80% ethyl alcohol. The crystalline substance, m. p. 237—239° (decomp.), was identical with the product obtained by acid hydrolysis.

The final hydrolysis of 4-β-glucosidyl-2-*O*-methylphloroglucinaldehyde to 2-*O*-methylphloroglucinaldehyde could not be accomplished because the glucoside was stable in dilute acid solutions, and concentrated acids, especially on warming, produced a dark colouring matter which exhibited a characteristic fluorescence in alkaline solutions. Hence the identity of the methylglucosidylphloroglucinaldehyde was established by its synthesis from 2-*O*-methylphloroglucinaldehyde.

2-*O*-Methylphloroglucinaldehyde (1 g.) was dissolved in acetone (10 c.c.), and potassium hydroxide (0·5 g.) in water (10 c.c.) added; the solution was then slowly treated, with shaking, with α-tetra-acetylglucosidyl bromide (3·7 g.) dissolved in acetone (10 c.c.) and kept at room temperature for 24 hours with occasional shaking. This mixture was poured into cold water (200 c.c.) containing a little acetic acid and after 12 hours the colourless solid was collected and crystallised from 95% ethyl alcohol (15 c.c.). Almost the whole of the *tetra-acetylglucoside* crystallised on cooling in sheaves of colourless long needles (0·6 g.), m. p. 147—148°. A second crystallisation from 95% alcohol raised the melting point to 149—150° (Found : C, 53·0; H, 5·2. $C_{22}H_{26}O_{13}$ requires C, 53·0; H, 5·3%). Almost pure 2-*O*-methylphloroglucinaldehyde (0·4 g.) was recovered from the alcoholic mother-liquor.

The tetra-acetyl compound (0·1 g.) was mixed with 10% aqueous potassium hydroxide (2 c.c.) and the clear solution was kept for 1 hour and then saturated with carbon dioxide; a colourless crystalline precipitate quickly filled the liquid. This was collected (m. p. 227°) and crystallised from 80% ethyl alcohol. It then melted at 237—239° and was identical with the specimen obtained by the hydrolysis

of 2-*O*-benzoyl-4:β-tetra-acetylglucosidyl-6-*O*-methylphloroglucinaldehyde.

3:4′-*Dihydroxy*-5-*benzoyloxy*-7-β-*tetra-acetylglucosidoxyflavylium Chloride.*—2-*O*-Benzoyl-4:β-tetra-acetylglucosidylphloroglucinaldehyde (1·7 g.) and ω:4-dihydroxyacetophenone (0·7 g.) were dissolved in a mixture of dry chloroform (40 c.c.) and ether (45 c.c.), and the solution rapidly saturated with dry hydrogen chloride. Very soon a deep red colour and a strong green fluorescence developed and after 4 hours the liquid was filled with a mass of dark brown crystals. It was kept for 60 hours and filtered, and the solid washed repeatedly with dry ether. The product, a brown-red solid (prisms), was powdered and kept in an evacuated desiccator for 24 hours (2 g.); a small specimen was exposed to the air for several days (Found: C, 57·7; H, 4·7. $C_{36}H_{33}O_{15}Cl,0{\cdot}5H_2O$ requires C, 57·6; H, 4·5%).

The pure substance sinters at 177°, then shrinks and decomposes at 184—186°; it is insoluble in water, but readily soluble in methyl and ethyl alcohol and in aqueous alkalis. The solution in glacial acetic acid has an orange colour and a green fluorescence. Aqueous sodium carbonate dissolves the salt to a deep violet solution, the colour of which very rapidly fades; on addition of acetone to the violet solution the colour becomes purple.

7-β-*Glucosidylpelargonidin Picrate.*—The benzoyloxyflavylium chloride (1 g.) was dissolved in pure methyl alcohol (22·5 c.c.) in a flask which was then filled with nitrogen. Methyl-alcoholic potassium hydroxide (5 c.c. of 25%) was then added, whereupon a small precipitate appeared; this was brought into solution by addition of a few drops of water and the clear solution was kept in the atmosphere of nitrogen for ½ hour. The whole of the alcohol was then removed under diminished pressure at the ordinary temperature, the residue taken up with water (12·5 c.c.), and concentrated hydrochloric acid (2·5 c.c.) added. The acid solution was shaken thrice with ether (20 c.c. each time) to remove benzoic acid and then more concentrated hydrochloric acid (6 c.c.) was added and the mixture kept for 2 days. The dark red crystalline product was recrystallised from 2% aqueous hydrochloric acid and then treated with a boiling aqueous solution of picric acid. The deep red solution first formed rapidly deposited a mass of shining red crystals (thin rectangular plates) and after cooling and keeping for 12 hours the crystals were collected and washed repeatedly with ether (dry solid, 0·4 g.). It was easily soluble in saturated ethyl-alcoholic picric acid and crystallised from a small volume of this solvent in deep red, tiny, rectangular plates (0·3 g.). On heating it sintered at 120°, grew dark at about 140°, and melted to a dark liquid and decomposed at

about 180° (Found in a specimen dried in air : loss of weight at 105° in a vacuum, 4·3; N, 6·3. $C_{27}H_{23}O_{17}N_3,1{\cdot}5H_2O$ requires H_2O, 3·9; N, 6·1%. Found in a specimen dried at 105° in a vacuum : C, 48·5; H, 3·4. $C_{27}H_{23}O_{17}N_3$ requires C, 49·0; H, 3·5%). The use of ethyl-alcoholic potash in place of methyl-alcoholic potash for the hydrolysis of the benzoyl compound was found to be very disadvantageous.

7-β-*Glucosidylpelargonidin Chloride.*—The pure picrate (0·3 g.) was dissolved in 5% methyl-alcoholic hydrochloric acid (6 c.c.), and ether (80 c.c.) was added to the clear solution with vigorous shaking. The chloride was precipitated as a red powder; it was collected, washed repeatedly with ether, and dissolved in hot 2% aqueous hydrochloric acid. Even so it separated as a gelatinous solid, but crystallisation of this from ethyl alcohol containing 2% of hydrochloric acid resulted in the production of deep red, thin needles (methyl alcohol was unsuitable for this crystallisation) (Found in an air-dried specimen : C, 48·5; H, 5·2; Cl, 7·2; loss at 110° in a vacuum, 9·2. $C_{21}H_{21}O_{10}Cl,3H_2O$ requires C, 48·2; H, 5·2; $3H_2O$, 10·3; Cl, 6·8%. Found in anhydrous substance : C, 53·5; H, 4·5. $C_{21}H_{21}O_{10}Cl$ requires C, 53·8; H, 4·5%).

The substance dissolves easily in water to an orange-red solution; sodium carbonate gives a deep violet-red colour; on dilution the violet develops a bluish tinge. An alcoholic solution containing a little hydrochloric acid is deep red without fluorescence; on addition of sodium hydroxide it turns into a bright reddish-violet with bluish tinge in thin layers; dilution with acetone turns it violet-blue, blue and finally colourless. Sodium hydroxide added to an aqueous solution gives an unstable violet-red but the colour (orange-red) returns rapidly on addition of concentrated hydrochloric acid.

The distribution number was determined in the same way as for the 4′-glucoside except that only 6·50 mg. were dissolved in 0·5% hydrochloric acid (50 c.c.) and shaken with amyl alcohol (50 c.c.). After the first extraction the amyl alcohol layer contained 46·7% of the pigment and the second extraction removed only 32·5% of the remainder. This apparent discrepancy is probably due to the readiness with which the substance passes into the pseudo-base. The table opposite summarises the main characteristics of the different glucosides of pelargonidin.

Colour intensity comparisons of the glucosides of pelargonidin were made in dilute methyl-alcoholic hydrochloric acid solution (1 c.c. of aqueous hydrochloric acid, *d* 1·16, made up to 1000 c.c. with methyl alcohol). The anthocyanins derived from pelargonidin may be arranged with regard to their colour intensities in decreasing order as 4′-glucoside (20), callistephin (8), 7-glucoside (5), pelargonin

(disaccharide) (2). No allowance was made for the additional mass of the disaccharide unit in pelargonin and hence the figure should be corrected to about (3). Solutions of equal concentration of the 5- and 7-methyl ethers of pelargonidin have about equal tinctorial intensity; the 5-methyl ether has the feebler colour in the ratio 3·7/4.

Callistephin (3-*glucoside*).	4′-*Glucoside*.
Dark brown mass of hair-fine needles with bronze reflex.	Glistening, scarlet-red, narrow plates tapering at the ends.
Readily soluble in water.	Not easily soluble in water.
Readily soluble in 0·5% up to 7% aqueous hydrochloric acid; moderately soluble in 10% hydrochloric acid. From concentrated acid solutions it separates as a gelatinous mass.	Rather sparingly soluble in 0·5% aqueous hydrochloric acid and almost insoluble in 5% acid solutions.
Easily soluble in methyl and ethyl alcohols.	Easily soluble in methly and ethyl alcohols.
Alcoholic solutions are red tinged with violet and much yellower than pelargonidin solutions. They exhibit no fluorescence.	Alcoholic solutions are non-fluorescent and deep red.
Aqueous solutions are yellowish-red and on dilution reddish-yellow.	Aqueous solutions are deep red and on dilution turn bluish-violet.
With aqueous sodium carbonate a reddish-violet to violet-red solution is obtained; with sodium hydroxide in alcoholic solution it is a shade bluer.	Sodium carbonate or sodium hydroxide added to aqueous or alcoholic solutions produces a reddish-violet colour which turns bluish-violet on dilution.
7-*Glucoside*.	*Pelargonenin* (5-*glucoside*).
Scarlet-red fine needles.	Scarlet-red needles.
Easily soluble in water.	Sparingly soluble in water.
Easily soluble in 0·5% aqueous hydrochloric acid, and much less soluble in 5% acid. Exhibits a great tendency to separate as a gelatinous mass from aqueous acid solutions.	Very difficultly soluble in 0·5% hydrochloric acid or acid of higher concentration.
Easily soluble in alcohols.	Easily soluble in methyl alcohol, but less soluble in ethyl alcohol.
Alcoholic solutions are non-fluorescent and deep red.	Colour of alcoholic solution between those of callistephin and pelargonidin; strongly fluorescent.
Aqueous solutions are orange-red; not intense.	The acid aqueous solutions are red, having a more bluish tinge.
Aqueous sodium carbonate gives a deep pink colour; alcoholic sodium hydroxide produces violet-red.	Aqueous sodium carbonate solution gives a violet-blue and in alcoholic solution it is pure blue. Addition of acetone also gives a pure blue colour.

Attempts to prepare 2-Tetra-acetylglucosidylphloroglucinaldehyde from Phloroglucinaldehyde.—The usual methods for obtaining phenolic glucosides were employed without success. We attempted to condense phloroglucinaldehyde and tetra-acetyl-α-glucosidyl bromide in the presence of potassium hydroxide (1 mol.) in aqueous-acetone solution. After 24 hours the solution was poured into water, but the brown semi-solid could not be crystallised or used in

condensations to flavylium salts. The second attempt was on the same lines as Fischer's synthesis of phloroglucinol-*d*-glucoside (*Ber.*, 1912, **45**, 2470) and various organic solvents were used for the glucosidyl bromide but without success. Ultimately, we achieved a part of our object through the use of dibenzoylphloroglucinaldehyde after failing to obtain the triphenylmethyl ether of *O*-benzoylphloroglucinaldehyde although six methods were tried. These included the use of triphenylmethyl chloride and iodide and potassium hydroxide, pyridine and silver oxide as agents. Further trials are in progress.

Dibenzoylphloroglucinaldehyde.—This derivative was obtained as a by-product in the preparation of 2-benzoylphloroglucinaldehyde as already described. The yield was very variable and the larger the quantity employed the greater the percentage yield of the dibenzoyl compound; for example, 18·6 g. of phloroglucinaldehyde yielded 0·5 to 2·0 g. of the by-product, whereas in an experiment with 40 g. of phloroglucinaldehyde, a little more than 7 g. of pure dibenzoylphloroglucinaldehyde was isolated from the chloroform extract.

Preparation of Dibenzoylphloroglucinaldehyde.—(A) Phloroglucinaldehyde (2·3 g.) was dissolved in acetone (20 c.c.), 10% aqueous potassium hydroxide (9 c.c.) and pure benzoyl chloride (1·7 c.c.) were added, and the whole was shaken on the machine for ½ hour, at the end of which further acetone (20 c.c.), potassium hydroxide solution (9 c.c.), and benzoyl chloride (1·7 c.c.) were introduced and the shaking was continued as before. At this stage the solution was deep red and contained some precipitated oily matter. Enough acetone was added to produce a clear solution, along with more potassium hydroxide (4·5 c.c.) and benzoyl chloride (0·9 c.c.), and the benzoylation was completed by shaking for ½ hour. The acetone was largely removed under reduced pressure, and a large volume of water added and decanted from the semi-solid mass precipitated. This residue was dried and its chloroform solution extracted twice with a cold saturated solution of sodium bicarbonate, and then twice with 10% sodium carbonate, which removed about 0·1 g. of monobenzoylphloroglucinaldehyde. The chloroform solution was finally washed with water and evaporated under reduced pressure. The brown-red pasty residue solidified in contact with ether, and this solid dissolved in benzene to form a yellowish-red solution, which was filtered from an insoluble residue and gradually treated with light petroleum until the precipitation of brown impurity was complete, leaving a clear, almost colourless solution. After filtering, the solution was concentrated to crystallisation and the product was recrystallised from benzene, forming colourless plates, m. p. 139—

140°. The yield of the pure substance varied considerably; the best yield obtained was 1·2 g. When larger quantities of phloroglucinaldehyde were benzoylated, the yield of the dibenzoyl compound was poor and tribenzoylphloroglucinaldehyde was obtained as the main product.

(B) *From monobenzoylphloroglucinaldehyde.* This method produces almost regular yields, though when more than 2 g. of monobenzoylphloroglucinaldehyde were used the yield and the purity of the product were considerably reduced.

2-*O*-Benzoylphloroglucinaldehyde (2 g.) was dissolved in dilute aqueous potassium hydroxide (0·5 g. in 20 c.c.), and acetone (16 c.c.) added. The clear solution was treated with benzoyl chloride (0·9 c.c.) and shaken for 15 minutes. Enough sodium bicarbonate solution was now added to make the liquid alkaline and also more benzoyl chloride (0·2 c.c.) and the mixture was shaken for ½ hour. By working as before, we obtained the pure product (1·0 g.), m. p. 139—140° (Found: C, 69·9; H, 4·0. $C_{21}H_{14}O_6$ requires C, 69·6; H, 3·8%). The *dibenzoyl* compound was rather sparingly soluble in alcohol and ether and very sparingly soluble in light petroleum; but it dissolved readily in chloroform, benzene, or acetone. An alcoholic solution gives a brownish-red coloration with ferric chloride.

(C) The use of pyridine enabled the preparation to be carried out on a larger scale. Benzoyl chloride (10·6 c.c.) was added in portions of about 0·5 c.c. to a vigorously agitated solution of *O*-benzoylphloroglucinaldehyde (20 g.) in dry pyridine (160 c.c.) cooled in melting ice. After 4 hours the solution was poured into water (800 c.c.) with stirring; the viscous red material solidified after 20 hours to a rose-coloured mass (24·8 g., m. p. *ca.* 110—120°). The dried crude product was extracted with chloroform (120 c.c.), leaving a residue of unchanged monobenzoate (5·4 g., m. p. 195°), and the red solution was washed twice with 10% aqueous sodium carbonate (200 c.c.) (acidification of these extracts afforded 2·4 g. of the monobenzoate, m. p. 182°) and then with very dilute acetic acid, dried, and the solvent removed. The residue was crystallised from 96% alcohol (> 500 c.c.) (yield, 9·2 g. of m. p. 136°, or 54%).

The constitution of dibenzoylphloroglucinaldehyde was established by methylation with methyl iodide and silver carbonate and hydrolysis of the product with alkali. By this process 6-*O*-methylphloroglucinaldehyde was obtained and thus the substance is 2 : 4-*O*-dibenzoylphloroglucinaldehyde.

2 : 4-*Dibenzoyl*-6-O-*methylphloroglucinaldehyde.*—Dibenzoylphloroglucinaldehyde (1·8 g.) was dissolved in pure acetone (40 c.c.), mixed with methyl iodide (3 g.) and silver carbonate (3 g.), and the whole mechanically agitated for 30 hours. The solution was filtered, the

solid washed with warm acetone, and the solvent removed under reduced pressure. When the colourless semi-solid residue was treated with a little boiling ether a clear solution was at first formed, but colourless crystals were soon deposited (0·9 g.). The substance was insoluble in water and light petroleum and was readily soluble in cold acetone, chloroform, and benzene. After three crystallisations from ethyl alcohol it occurred as spear-shaped plates, m. p. 133—134° (Found: C, 70·3; H, 4·4. Calc. for $C_{22}H_{16}O_6$: C, 70·3; H, 4·3%). A mixture with dibenzoylphloroglucinaldehyde melted below 110°. This substance has been made by Karrer and Helfenstein (*Helv. Chim. Acta*, 1927, **11**, 793) from 2-methylphloroglucinaldehyde by benzoylation and they give the melting point as 131°.

The methylation product (0·6 g.) was heated with 10% aqueous potassium hydroxide (4 c.c.) at 40—50° for ½ hour and subsequently kept for 3 hours. When the clear, light yellow solution was rapidly saturated with carbon dioxide, straw-coloured crystals separated; recrystallised from dilute ethyl alcohol, these formed extremely long, straw-coloured needles which sintered slightly at 202° and melted to a dark liquid and decomposed at 208—210°. The substance is identical with 2-methylphloroglucinaldehyde obtained from monomethylphloroglucinol by Gattermann's method.

Tribenzoylphloroglucinaldehyde.—If, in the preparation of dibenzoylphloroglucinaldehyde from phloroglucinaldehyde in one operation, exactly twice the quantities were used and the experiment was carried out as before, very little dibenzoyl derivative could be obtained. On the other hand, a colourless crystalline product insoluble in cold dilute sodium hydroxide solution and giving no ferric chloride colour reaction could be isolated by the following procedure: The chloroform solution which had been shaken with aqueous sodium carbonate was dried over fused calcium chloride and evaporated, and the viscous residue repeatedly treated with small volumes of warm ether and decanted from the dark red insoluble residue. The ethereal solution on slow evaporation yielded an almost colourless crystalline solid. After repeated crystallisation from benzene–light petroleum it was obtained in colourless hexagonal plates, m. p. 121—122°. It could be more readily purified when it was dissolved in chloroform, the solution shaken twice with 2% aqueous potassium hydroxide, washed with water containing a little acetic acid, and dried over fused calcium chloride, and the solvent removed. The residue crystallised readily from benzene–light petroleum; m. p. 121—122° (Found: C, 72·0; H, 4·0. $C_{28}H_{18}O_7$ requires C, 72·1; H, 3·9%). The substance is very easily soluble in chloroform, acetone, or benzene, much less soluble in ether, and very sparingly soluble in light petroleum. It

crystallises from methyl and ethyl alcohols in colourless needles which, when rapidly heated, melt at 80° with vigorous decomposition; on slow heating, there is sintering at 80° and the solid then melts at about 118°. A specimen which has been first crystallised from methyl alcohol retains its composition and melting point in spite of subsequent crystallisations from other anhydrous solvents (Found in an air-dried specimen: C, 71·1, 70·8; H, 4·3, 4·1; loss in weight at 110° in a vacuum, 3·5. $C_{28}H_{18}O_7,0{\cdot}5CH_4O$ requires C, 71·0; H, 4·2; CH_4O, 3·3%). The alcohol of crystallisation is completely removed at 110° in a high vacuum: the anhydrous substance, after crystallising from benzene–light petroleum, melts at 121—122° without any previous sintering at 80°.

Attempts to prepare 2 : 4-*Dibenzoyl*-6-*tetra-acetyl*-β-*glucosidyl-phloroglucinaldehyde.*—(A) Dibenzoylphloroglucinaldehyde (0·4 g.) was mixed with acetone (4 c.c.), water (4 c.c.), and 10% potassium hydroxide solution (1·3 c.c.), and tetra-acetylglucosidyl bromide (1 g.) in acetone (6 c.c.) gradually introduced with cooling. After 24 hours (occasional shaking) the red solution was added to water (100 c.c.) containing a few drops of acetic acid and kept for 24 hours. The pasty solid that separated was dissolved in chloroform and extracted once with 5% potassium hydroxide solution. It was subsequently washed with water and with very dilute acetic acid. The residue after removal of the solvent crystallised from 95% ethyl alcohol in colourless plates and prisms, m. p. 144—145° (mixed m. p. with dibenzoylphloroglucinaldehyde, 115—122°; with 2-benzoyl-4-tetra-acetyl-β-glucosidylphloroglucinaldehyde, 120—125°). A second crystallisation produced no change in the shape of the crystals or the melting point. The substance was insoluble in cold dilute potassium hydroxide solution and its alcoholic solution gave no colour with ferric chloride. The air-dried substance suffered no loss in weight when it was heated at 105° in a vacuum (Found: C, 56·1, 55·8; H, 5·6, 5·5%). The tetra-acetylglucoside of dibenzoylphloroglucinaldehyde ($C_{35}H_{32}O_{15}$) requires C, 60·7; H, 4·7%, and we were unable to determine the true nature of this well-defined substance.

(B) Powdered silver carbonate (3 g.) was added to dibenzoyl-phloroglucinaldehyde (2 g.) and tetra-acetyl-α-glucosidyl bromide (8·8 g.) dissolved in dry benzene (80 c.c.), and the mixture shaken for 48 hours; the liquid was then filtered, and the residue repeatedly washed with cold benzene. The combined filtrates were thrice extracted with 5% potassium hydroxide solution, then washed twice with water containing a little acetic acid, and the solvent was evaporated. The colourless glassy mass was dissolved in ethyl alcohol (10—20 c.c.), and the solution poured into water (200 c.c.).

The solid was dissolved in the minimum of 95% ethyl alcohol (15 c.c.) and the semi-solid obtained on cooling was removed from the mother-liquor and triturated with cold water, causing crystallisation. After the process had been repeated twice, the material sintered at about 85°, became a glassy mass at 110° and fluid at 145°. It dissolved only slowly in dilute potassium hydroxide solution, and an alcoholic solution did not give any appreciable colour with ferric chloride (Found: C, 62·0; H, 4·5. $C_{27}H_{24}O_{11}$ requires C, 61·8; H, 4·7%). $C_{27}H_{24}O_{11}$ is the formula of the de-acetylated glucoside. However, repeated attempts to hydrolyse it with aqueous and alcoholic potash, aqueous and alcoholic baryta, and aqueous sodium hydroxide did not yield any definite products except benzoic acid. Acetylation by Fischer's method (*Ber.*, 1917, **50**, 1055) with acetic anhydride and pyridine failed to give rise to any easily crystallisable acetyl derivative.

(C) The method of Takahashi (*J. Pharm. Soc. Japan*, 1925, **525**, 4), which has also been employed by Zemplén (*Ber.*, 1928, **61**, 2486), is probably the most effective known glucoside synthesis and we have applied it to the present case. In other examples, one of us (A. R.) has been able to show that the products are β-glucosides that can be hydrolysed by emulsin.

Dibenzoylphloroglucinaldehyde (2 g.), tetra-acetylglucosidyl bromide (4·5 g.), and freshly prepared silver oxide (2·2 g.) (compare Helferich and Klein, *Annalen*, 1926, **450**, 225) were dried over phosphoric oxide in a vacuum for 48 hours, powdered together, and pure quinoline (7 c.c.) added. The mixture was stirred for 15 minutes; the temperature then rose to 50° and the mass thickened. After the whole had been kept in the desiccator for 2 hours, acetic acid (35 c.c.) was introduced and the filtered solution poured in a very fine stream into ice-water (300 c.c.). 12 Hours later the process was repeated. The product, a yellow powder, was collected after 5—6 hours, dried, and dissolved in chloroform (50 c.c.); the solution was washed twice with 5% aqueous potassium hydroxide and then with very dilute acetic acid and dried over sodium sulphate. Evaporation of this solution and crystallisation of the residue from alcohol (long keeping) gave a substance, m. p. 224—226°, in spherical, dense aggregates of colourless needles; the nature of this substance was not elucidated, since we were unable to make use of the analytical results. Other methods of working up the crude product were the following.

(A) The chloroform was removed on the steam-bath, and the yellowish-green residue dissolved in hot alcohol and added to ice-water; the process was repeated (using animal charcoal) (yield, 3·8 g.). This material (4·6 g.) along with acetic anhydride (15 c.c.)

and pyridine (10 c.c.) was heated on the steam-bath for 1 hour, and the product decomposed by means of ice-water (300 c.c.); solidification occurred after 4 hours. The product was thrice dissolved in ethyl alcohol and precipitated by means of ice-water; the white powder was amorphous and melted over a range below 100° (Found : C, 55·3; H, 5·0%).

(B) The chloroform solution was diluted with light petroleum (2 vols.), precipitating a reddish oil; on standing, the substance, m. p. 223—224° (after crystallisation from alcohol), separated in white balls on the sides of the vessel. The solvent was then removed; the residual yellow oil partly solidified on keeping. The substance crystallised from absolute alcohol in long needles, but the separation of these was instantly followed by that of a second substance. By working with benzene–light petroleum mixtures the main product could be ultimately separated in colourless prisms, m. p. 143—144°; this substance crystallised from alcohol in long needles, m. p. 144—145° (Found : C, 55·7; H, 5·4. $C_{35}H_{32}O_{15}$,3·5H_2O requires C, 55·6; H, 5·1%). The substance is evidently identical with the compound previously obtained (see p. 2693) and we give the composition as a tentative suggestion; direct proof of the existence of solvent of crystallisation was not forthcoming. Dibenzoyltetra-acetylglucosidylphloroglucinaldehyde has the composition $C_{35}H_{32}O_{15}$. The substance is easily soluble in chloroform and its alcoholic solution does not become coloured on the addition of ferric chloride.

5-β-*Glucosidylpelargonidin Chloride* (*Pelargonenin Chloride*) (IV).—The crude glucoside as obtained by the Takahashi process was hydrolysed by means of methyl-alcoholic baryta and in other experiments by means of ethyl-alcoholic potassium hydroxide, but the resulting phloroglucinaldehyde could not be purified. In the condensation to pelargonenin chloride it was therefore not attempted to isolate the glucoside and the experiment was carried out under the following conditions, which succeed only because the 5-glucosidylanthocyanidins are relatively resistant to acid hydrolysis (hence their production by partial hydrolysis of the di-monosides). The present instance is the first of an anthocyanin synthesis effected in a hydroxylic solvent.

The crude " glucoside " (2 g.) in alcohol (80 c.c.) under nitrogen was hydrolysed in the cold by the addition of potassium hydroxide (1·8 g.) in alcohol (30 c.c.), and the mixture kept for 3 days with frequent shaking. A solution of the sodium salt of ω : 4-dihydroxyacetophenone (0·5 g.) in alcohol (12 c.c.) which had been acidified by means of dry hydrogen chloride was then added, and the whole slowly saturated with hydrogen chloride at 0°. The alcoholic solution became dark red and acquired a strong green fluorescence;

it was filtered into ether (450 c.c.) and the chocolate-red precipitate was collected and dried (average yield in 29 operations, 0·32 g.). This product (9·65 g.), which was far from homogeneous, was dissolved in 0·5% hydrochloric acid (500 c.c.), and the filtered solution extracted with amyl alcohol (350 c.c.); this involved loss but ensured the removal of pelargonidin. The aqueous solution was mixed with saturated aqueous picric acid (500 c.c.); after 16 hours, the picrate slowly separated as a red precipitate. This was collected, dried, and converted into chloride by solution in methyl-alcoholic hydrogen chloride and precipitation with ether. The crude chloride was triturated with cold 5% methyl-alcoholic hydrogen chloride; it then became crystalline and, on drying, the deep red substance acquired a green lustre. At this stage the salt could be crystallised by Willstätter and Bolton's method (*loc. cit.*), namely, from 2% aqueous hydrochloric acid, and this was carried out twice (Found in air-dried material: loss in a vacuum over sulphuric acid, 3·8; loss at 110° in a high vacuum, 7·6. $C_{21}H_{21}O_{10}Cl,2H_2O$ requires H_2O, 3·5; $2H_2O$, 7·0%. Found in anhydrous material: C, 53·9; H, 4·6. $C_{21}H_{21}O_{10}Cl$ requires C, 53·8; H, 4·5%). The bright red needles had all the properties of pelargonenin recorded by Willstätter and Bolton (who also recorded the loss of half the solvent of crystallisation in a desiccator) and we have recently prepared a solution of pelargonenin from pelargonin and made a direct comparison of the colour reactions of the natural and the synthetic substance. The solutions in 0·5% hydrochloric acid used in this case were colorimetrically matched, extracted with amyl alcohol containing 10 volumes % of benzene, and the organic layers were found to be a perfect match. The distribution number of pelargonenin in very dilute solution was found as high as 48—50, but when this experiment was performed we were unaware of the variation of distribution number with the concentration and a further report on the distribution number of pelargonenin will be submitted. The alkali colour reactions of pelargonenin closely resemble those of pelargonin, but the former gives a bluer violet than the latter in aqueous sodium carbonate. We were at first inclined to attribute the divergence to impurity of the pelargonin, especially since a gladiolus, "Flaming Sword," appeared to contain a pelargonin which gave a reaction identical with that of pelargonenin. However, the gladiolus anthocyanin was later found to contain a trace of cyanin, which accounted for the blue shade of its solution in soda, and a very pure specimen of pelargonin, kindly sent to us by Professor Karrer, gave the redder tinge. Finally, the pelargonenin which we have prepared from pelargonin is identical with our synthetic material in respect of the alkali colour reaction.

We consider this slight divergence of pelargonenin from pelargonin is further evidence in favour of the 3 : 5-monoside constitution for the latter anthocyanin.

Comparison of the Stability of Certain Flavylium Salts in the Presence of Ferric Chloride.—Synthetic pelargonenin chloride (2·002 mg.) was dissolved in 1% hydrochloric acid (50 c.c.), and the solution colorimetrically matched by solutions (50 c.c.) of pelargonin chloride, salvinin chloride, monardin chloride, and pelargonidin (and, in an experiment made at a different time, approximately similar solutions of callistephin, pelargonidin 4′-glucoside and 7-glucoside were tested, all in 1% hydrochloric acid). A solution (50 c.c.) of ferric chloride (5 g. of anhydrous salt in 2000 c.c. of water with a drop of hydrochloric acid; 0·125%) which was yellow-brown in colour was added. All the solutions, with four exceptions, faded rapidly and decolorisation was complete in 35 minutes.

The callistephin, pelargonin, monardin, and salvinin (Karrer has stated that monardin and salvinin are identical with pelargonin) solutions were unchanged after 3 days.

Note on the Methylation of γ-Orcylaldehyde.—We are much indebted to Dr. St. Pfau for a specimen of atranorin with the aid of which we have prepared γ-orcylaldehyde by his method (*Helv. Chim. Acta*, 1926, **9**, 650).

A mixture of the aldehyde (0·5 g.), methyl iodide (5 c.c.), potassium carbonate (2 g.), and acetone (15 c.c.) was refluxed for 2 hours; the product crystallised from light petroleum in colourless needles, m. p. 91—92° (alone or mixed with the specimen synthesised from *p*-orsellinonitrile); Robertson and Robinson (J., 1927, 2201) give the m. p. 90—91°.

Characterisation of Anthocyanins derived from Pelargonidin by Means of their Colour Reactions in Alkaline Solutions.—The procedure of Robertson and Robinson (*Biochem. J.*, 1929, 35) was adopted. The numbers refer to buffered solutions of graded p_H as prescribed in that memoir.

Callistephin (3-*glucoside of pelargonidin*). 1% Hydrochloric acid gives an orange solution, pink in thin layers. Saturated sodium carbonate, reddish-violet, gradually fading. (1) Orange, rapidly fading to pink; (2) similar loss of colour, but more rapid and giving a fainter pink; (3) intense pink at first, rapidly loses colour; (4) bluish-pink, also loses colour but less quickly than (3); (5) bluish-red, still slower loss of colour. At this time (2) faint orange-pink, (3) very faint bluish-pink, and (4) feebler than (3). (6) Duller bluish-red; (7) browner red; (8) brown-red with violet tinge; (9) rather dull reddish-brown-violet; (6)—(9) colour more stable than (2)—(5). (10) Violet-red; (11) and (12) reddish-violet, rather

bright colour; (12)—(17) give same reddish-violet. After 20 minutes: (3), (4), (5) nearly colourless; (6) pink; (7), (8), (9) brown-red, faded; (10) intermediate; (11)—(17) still reddish-violet; sodium carbonate, faded to a weak pink. After 1 hour: 1% hydrochloric acid unchanged; sodium carbonate pale yellow; (1) orange; (2) weak pink descending series to (5), which is very faint pink; (6) stronger pink and ascending series brown-red to (9); (9) brownish-red; (10) violet-brown-red; (11, 12, 13, 14) reddish-violet; (15) much more faded reddish-violet; (16) very faded and much duller. After 24 hours: 1% hydrochloric acid retains colour undiminished; sodium carbonate very pale yellow; (1) unchanged orange; (2), (3), (4) descending series, (4) being faint pink; (5) colourless; (6) very faint pink ascending series to (9), which is not much changed; (10)—(12) reddish-violet; (13) faint violet, much faded; (14) and (15) colourless; (16) very pale yellow. This series as a whole after a day was more intensely coloured than the 4′-glucoside. The colourless solution (5) regained its colour immediately after addition of concentrated hydrochloric acid in the cold. *4′-Glucoside of pelargonidin.* 1% Hydrochloric acid, orange-red, pink in thin layers and bluer than callistephin; sodium carbonate, greenish-blue with violet-red dichroic effect; (1) orange-pink; (2) bluer pink; (3) bluish-red; colour fades, (1) losing its orange colour last and (3) most rapidly; (4) bluish-red; (5) violet-red; (6) reddish-violet; (2), (3), and (4) now very pale; (7) and (8) dull reddish-violet; (9) dull violet; (10) bluish-violet (dichroic red); (11) dull violet-blue with red dichroic effect; (12) and (13) blue with violet tinge (red dichroic); (14)—(17) same as (13). After 20 minutes: (1) pink, weaker than callistephin; (2)—(4) colourless; (5) bluish-pink faded; (6)—(9) ascending series of colour intensity; (9) distinctly violet tinged; (10) violet; (11)—(17) blue tinged violet (dichroic); sodium carbonate colour about the same as (10)—(17) and fades much less easily than callistephin. After 1 hour: (1)—(4) colourless; (5) very faint pink; the rest as before, the colour slightly faded. After 24 hours: (5) colourless; (6) almost colourless, having only a tinge of the original colour; (7)—(10) faint pink, equal in intensity, only slightly faded from the previous day; (11, 12) very faint violet-blue, much more faded; (13, 14) almost colourless, only a tinge of blue; (15, 16, 17) colourless; sodium carbonate colourless; 1% hydrochloric acid, very faint orange-red, much faded. (1)—(5) do not recover colour immediately on addition of concentrated hydrochloric acid; the colour appears only slowly and after 24 hours it is completely recovered. This series as a whole is much less intense in colour after a day than the callistephin series.

7-Glucoside of pelargonidin. 0·5% Hydrochloric acid, red-orange;

(1)—(4) weaker orange; (5) violet-red; (6) bluish-pink; (1)—(6) rapidly decolourised, quicker than pelargonin; (7) duller red-violet, at this point there is a gap. (8) and (9) violet; (10) violet, duller than pelargonin; (11, 12, 13) similar to (10); (14) similar colour, diminishing, but not so rapidly as pelargonin; (15) similar to (14); sodium carbonate deep pink, decolourising rapidly. After 20 minutes: 0·5% hydrochloric acid unchanged; (1)—(5) colourless; (6)—(8) increasing to dull violet; (9)—(14) like (8), and so the 7-glucoside is more stable at the alkaline end than pelargonin; sodium carbonate, very faint pink. After 2 hours: (1)—(7) quite colourless, whereas pelargonin has always a very weak pink colour; (8) weak pink; (9)—(11) increasing violet; (12) and (13) similar to (11); (14) weaker reddish-violet; (15), and sodium carbonate, almost colourless (weak pink). After 24 hours: 0·5% hydrochloric acid, almost decolourised (very faint pink); (1)—(7) colourless; (8)—(11) increasing to weak brownish-pink; (12)—(14) equal to (11); (15) a trace bluer; sodium carbonate, colourless. Unlike the pelargonin series, the yellow colour at the alkaline end does not appear and pseudo-base formation is quicker throughout in the 7-glucoside series; this probably explains the anomalous distribution numbers that were obtained. *Pelargonin.* 0·5% Hydrochloric acid gives red-orange; (1) and (2) reddish-violet turning into weak pink; (3) bluish-pink, rapidly decolourised; (4) more intense bluish-pink, decolourised less readily; (5) violet-red, decolourises more slowly; at this point there is a gap. (6) and (7) permanganate colour, slower decolorisation; (8) reddish-violet; (9) and (10) a little bluer, but still reddish-violet; (11)—(13) similar to (10); (14, 15) similar, but rapidly decolourised; sodium carbonate, reddish-violet with a bluish tinge. After 20 minutes: (1)—(5) colourless; (6)—(8) increasing reddish-violet; (9)—(13) red-violet; (14)—(15) almost decolourised, only a greenish shade; sodium carbonate, yellow. After 2 hours: (6, 7) also decolourised; (8, 9, 10) increasing red-violet; (11) equal to (10); (12) weaker; (13) decolourised, faint greenish colour; (14) greenish-yellow; (15) and sodium carbonate, brownish-yellow. After 24 hours: 0·5% hydrochloric acid unchanged; (1)—(4) diminishing series, very weak pink; (5) colourless; (6)—(9) increasing to weak violet; (10) equal to (9); (11)—(15) and sodium carbonate, yellow. 7-*Methyl ether of pelargonidin.* A methyl-alcoholic hydrochloric acid solution was employed and it had the same molar concentration as the anthocyanin solutions. 0·5% Hydrochloric acid, orange, pink in thin layers, same as the 5-methyl ether; (1) and (2) as 0·5% hydrochloric acid; (3) and (4) a little bluer; (5) bluer pink; (6)—(9) dull reddish-violet; (10) same as above, but violet in thin layers; (11)—(15) and sodium carbonate,

similar to (10). After 2 hours : 0·5% hydrochloric acid almost same as before; (1)—(7) colourless; (8)—(11) increasing red-violet, much duller and redder than 5-methyl ether; (12) and (13) equal to (11); (14) and (15) very weak red-violet; sodium carbonate, almost colourless (very faint pink). After 24 hours : 0·5% hydrochloric acid, weak colour; (1)—(8) colourless; (9)—(11) increasing colour to dull red-violet (weak); (12) and (13) weak red-violet; (14) and (15) almost colourless, tinge of pink; sodium carbonate, colourless. 5-*Methyl ether of pelargonidin.* A methyl-alcoholic hydrochloric acid solution was employed. 0·5% Hydrochloric acid, orange, pink in thin layers; (1) and (2) a little bluer; (3) still bluer; (4), (5) violet-red; (6)—(8) more intense violet-red; (9) reddish-violet; (10) violet; (11)—(15) and sodium carbonate, a little bluer violet. After 2 hours : (1)—(5) colourless; (6)—(10) increasing red-violet with (10) a shade bluer; (11)—(15) increasing bluer-violet, but intensity about the same; sodium carbonate, faint pink. After 24 hours : 0·5% hydrochloric acid unchanged; (1)—(7) colourless; (8)—(11) colourless (tinge of violet); (12) and (13) bluish-violet; (14) weak violet; (15) weak violet-red; sodium carbonate, colourless.

For purposes of comparison with other anthocyanidins (Robertson and Robinson, *loc. cit.*) the colour reactions of the methyl ethers of pelargonidin were also studied under the standard conditions.

5-*Methyl ether of pelargonidin.* 0·5% Hydrochloric acid, orange-red, pink in thin layers; (1) bluer pink; (2) very much bluer; (3), (4), (5) violet-red : these colours are brighter than those of the 7-methyl ether in agreement with the behaviour of the corresponding glucosides; (6) permanganate; (7) same; (8) a shade bluer; (9) red-violet; (10) violet; (11) bluish-violet; (12) violet-blue; (11) and (12) blue in thin layers; (13) blue, violet tinge due to dichroism; (14), (15) and sodium carbonate, blue with violet-tinge. After 20 minutes : (1) much weaker; (2) colourless; (3) weaker than (1); (4) much weaker than (5) and contains an opalescent precipitate of colour base. After 4 hours : (1)—(3) colourless; (4) and (5) very nearly colourless; between (6) and (5), a gap; (6)—(8) increasing red-violet; (9)—(12) also increasing and bluer; (13)—(15) equal to (12); sodium carbonate, colourless. After 24 hours : hydrochloric acid unchanged; (1)—(6) colourless; (7) has only a tinge of red-violet; (8)—(10) increasing red-violet; (11)—(14) bluish-violet, getting bluer; (15) faint bluish-violet; sodium carbonate, colourless with a tinge of yellow. On addition of ammonia to an alcoholic solution it becomes violet-blue with red fluorescence, blue in thin layers; the colour rapidly fades to yellowish-green and finally is yellow with a green fluorescence. 7-*Methyl ether.* Hydrochloric acid, orange-red, pink in thin layers as the 5-methyl ether; (1)—(3)

same as above; (4) browner and bluer shade; (5) dull red-violet; (6) and (7) dull permanganate; (8) a shade bluer, but still duller than the 5-methyl ether; (9) red-violet duller than 5-methyl ether; (10)—(15) and sodium carbonate, same as (9). After 20 minutes: (1) very weak colour; (2)—(4) colourless; (5)—(8) increasing series; the rest intense as before. After 4 hours: (1)—(6) colourless; (7) very faint violet; (8) weak violet; (9)—(14) fairly good red (but dull violet); (15) very weak red-violet; sodium carbonate, almost colourless (a little brown). After 24 hours: hydrochloric acid, unchanged; (1)—(7) colourless; (8) almost colourless; (9)—(11) increasing red-violet (dull); (12) and (13) same as (11); (14) weak red-violet; (15) and sodium carbonate, almost colourless, just a trace of brown. On addition of ammonia to an alcoholic solution it develops a greener blue with deeper red dichroism than the 5-methyl ether; this is also blue in thin layers at first but in a few seconds degenerates and the colour changes to orange.

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