173. *Experiments on the Synthesis of Anthocyanins. Part XIX. 5-Glucosidylapigeninidin, believed to be identical with Gesnerin, an Anthocyanin of* Gesnera fulgens.

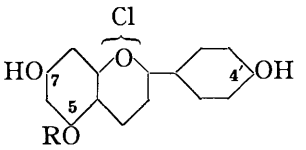
By (Mrs.) G. M. Robinson, Robert Robinson, and A. R. Todd.

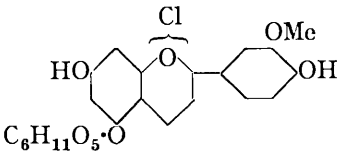
Although the great majority of natural anthocyanins are derivatives of pelargonidin, cyanidin, delphinidin or their methyl ethers yet exceptional cases do occur, the most interesting being the nitrogenous pigments of many *Chenopodiaceæ*.

Certain *Papaveraceæ* also contain abnormally constituted pigments of unknown constitution and, in the course of a broad qualitative survey of anthocyanins, only one further example was found in the orange flowers of a few species of *Gesnera* (Robinson and Robinson, *Biochem. J.*, 1931, **25**, 1687). In this case the anthocyanidin obtained on hydrolysis was isolated, analysed, and recognised as apigeninidin chloride (I, R = H) by comparison with a specimen synthesised (Pratt, Robertson, and Robinson, J., 1927, 1975) in the hope, now fulfilled, that it might ultimately be found in nature.

The synthetical specimen was obtained by demethylation of its 4'-methyl ether, acacetinidin chloride, itself obtained by an application of Bülow's method using *p*-anisyl β-hydroxyvinyl ketone. This method in itself offers no proof of constitution, because the product may be an *epi*flavylium salt with the anisyl group in position 4 of the pyran ring. Identity with the material prepared from phloroglucinaldehyde dimethyl ether and *p*-acetylanisole and subsequently demethylated was proved, however, and no further attention would have been paid to this aspect of the matter had not Asahina and Inubuse (*Ber.*, 1928, **61**, 1646) described as apigeninidin chloride a substance which they stated differed from the salt obtained by Pratt, Robertson, and Robinson. The Japanese chemists prepared their " apigeninidin chloride " by the reduction of apigenin with sodium amalgam and stated that, among other divergencies, the product dissolved in sulphuric acid without exhibiting fluorescence.

For purposes of further comparison, therefore, we have synthesised apigeninidin chloride by the *O*-benzoylphloroglucinaldehyde method (Robertson and Robinson, J., 1928, 1526), and the product showed all the properties, including a green fluorescence of a solution in sulphuric acid, described by Pratt, Robertson, and Robinson (*loc. cit.*).

(I.) 



(II.)

The anthocyanin of *Gesnera fulgens*, termed *gesnerin*, has not yet been isolated in substance, owing to lack of an adequate supply of the flowers, but highly purified solutions

have been obtained and the properties of these enabled us to draw the conclusion that the substance is an apigeninidin 5-glycoside. The colour reactions with alkaline solutions resembled those of apigeninidin and were quite different from those of apigeninidin 4′-methyl ether. For further guidance, apigeninidin 5- and 7-methyl ethers were prepared on a small scale from the 2- and 4-methyl ethers of phloroglucinaldehyde and *p*-hydroxyacetophenone, condensed together in ethyl acetate solution with the help of hydrogen chloride. The alkali-colour reactions of these isomeric salts were almost identical and also very similar to those of gesnerin. However, the 5-*O*-methylapigeninidin chloride exhibited a green fluorescence in alcoholic solution, whereas the 7-*O*-methylapigeninidin did not. Gesnerin also fluoresced in alcoholic solution, so it appeared likely that it is an apigeninidin 5-saccharide. The distribution number with *iso*amyl alcohol is so low that we were at first inclined to regard the substance as a bioside; this impression was removed by an examination of *apigeninidin 5-β-glucoside* (I, R = $C_6H_{11}O_5$), which was also found to have a low distribution number (*iso*amyl alcohol and 0·5% hydrochloric acid). This substance was synthesised by the general method from the acetylated glucoside of phloroglucinaldehyde (Robinson and Todd, J., 1932, 2302) as first component and *p*-hydroxyacetophenone as second component. The new glucoside was isolated both as a normal and as a basic chloride and it has very different general properties from those exhibited by the normal series of anthocyanins with a 3-glucosidoxy-group. Direct comparison of colour reactions and distribution properties rendered it almost certain that gesnerin is not merely closely similar to 5-glucosidylapigeninidin but is in fact identical with it.

Owing to the fact that gesnerin is accompanied in the flower petals by a similar colouring matter having a bluer tone, a long and tedious process of purification was necessary before the natural and the synthetic pigment could be equated. The second anthocyanin gave no ferric reaction and the related anthocyanidin was not easily oxidisable in weakly acid solution in presence of traces of ferric salts; hence we thought it might be *3′-methoxygesnerin* (II). This was prepared and attempts were made to simulate the nearly pure gesnerin by mixtures of the two synthetic glucosides. When, however, the colours were matched, the distribution numbers became divergent, and it now appears more probable that 3′ : 5′-dimethoxygesnerin is the second anthocyanin. This idea fits the occurrence of malvin as a secondary anthocyanin of the scarlet pelargonium (Robinson and Robinson, *loc. cit.*), and also the suggestion that gesnerin may represent a reduced pelargonin (compare the formation of trimethyleneglycol derivatives from fats in fermentation processes), but we have not yet had the opportunity to test the hypothesis. Further work in this field and on the isolation of gesnerin is contemplated.

Experimental.

Apigeninidin Chloride (I, R = H).—A solution of 2-*O*-benzoylphloroglucinaldehyde (2 g.) and 4-hydroxyacetophenone (4 g.) in dry ethyl acetate (70 c.c.) was saturated with hydrogen chloride at 0° and kept in the ice-chest for 48 hours. The solid was then collected (1·9 g.), and dilution with ether afforded a further quantity of the same material (1 g.). This crude product was dissolved in boiling alcohol (*ca.* 300 c.c.), and an equal volume of 12% hydrochloric acid added. On cooling, the *benzoylapigeninidin chloride* separated in small crimson prisms with a metallic lustre (Found: C, 65·0; H, 4·1; Cl, 8·8. $C_{22}H_{15}O_5Cl,0·5H_2O$ requires C, 65·4; H, 4·0; Cl, 8·8%). The substance decomposes at 203°. It is sparingly soluble in 0·5% hydrochloric acid to a yellow solution, which becomes rose-coloured on the addition of sodium acetate and magenta with sodium carbonate. The orange-yellow solution in alcohol shows a weak green fluorescence.

This derivative (1·6 g.) was debenzoylated by treatment with 10% aqueous sodium hydroxide during 5 hours at room temperature in an atmosphere of hydrogen. When the brown-red solution was acidified with a mixture of alcohol (20 c.c.) and concentrated hydrochloric acid (6 c.c.) and heated on the steam-bath for a short time, the apigeninidin chloride separated as a mass of small salmon-red crystals. Recrystallised according to the method of Pratt, Robertson, and Robinson (*loc. cit.*), minute rhombic prisms, which were orange-yellow by transmitted light and salmon-red in mass, were obtained. The properties of this specimen were identical with those described by Pratt, Robertson, and Robinson (*loc. cit.*) for apigeninidin chloride prepared by the demethylation of acacetinidin chloride. In accordance with the results of the earlier work,

the crystalline substance was found to be a monohydrate (Found : C, 58·5; H, 4·4; Cl, 11·3. Calc. for $C_{15}H_{11}O_4Cl,H_2O$: C, 58·4; H, 4·6; Cl, 11·5%). The characteristic green fluorescence of the solution in concentrated sulphuric acid can be observed on mixing, but it rapidly becomes more intense, probably as the result of sulphonation.

5-β-Glucosidylapigeninidin Chloride (I, R = $C_6H_{11}O_5$).—A solution of 2-*O*-tetra-acetyl-β-glucosidylphloroglucinaldehyde (1 g.) and 4-hydroxyacetophenone (1 g.) in dry ethyl acetate (25 c.c.) was saturated with hydrogen chloride at 0° and kept for 60 hours at this temperature. The yellowish-brown product had partly separated and after the addition of ether it was collected (1·05 g.) (Found : C, 52·7; H, 5·1; Cl, 5·8. $C_{29}H_{29}O_{13}Cl,2H_2O$ requires C, 53·0; H, 5·0; Cl, 5·4%). This *7 : 4′-dihydroxy-5-O-tetra-acetyl-β-glucosidoxyflavylium chloride* (1·0 g.) was added to aqueous sodium hydroxide (10 c.c. of 10%) and kept under hydrogen with stirring for 90 minutes. The dark brownish-red solution was then acidified with hydrochloric acid (4·3 c.c. of 30%) and the 2% acid solution so obtained was heated at 50° for a few minutes; the product separated as a red powder. The crude material was dissolved in hot 0·1% hydrochloric acid, and an equal volume of 5% methyl-alcoholic hydrogen chloride added; small brownish needles were deposited on cooling (Found in air-dried material : C, 48·2; H, 5·5; Cl, 6·5; loss at 110° in a high vacuum, 14·4, 14·7. $C_{21}H_{21}O_9Cl,4H_2O$ requires C, 48·1; H, 5·5; Cl, 6·8%). The dried material appears to have lost about $3H_2O$ and 31% of its chlorine (Found : C, 54·9; H, 4·9; Cl, 5·4. Theory for losses indicated : C, 54·9; H, 5·0; Cl, 5·3%). When the acid concentration of a solution in 0·1% hydrochloric acid was raised to 2%, the glucoside separated in minute brown crystals of a *basic chloride* (Found in air-dried material : C, 49·1; H, 5·3; Cl, 3·7; loss at 110° in a high vacuum over phosphoric oxide, 12·6, 12·1. $2C_{21}H_{20}O_9,HCl,8H_2O$ requires C, 49·8; H, 5·6; Cl, 3·5; $7H_2O$, 12·4%. Found in dried material : C, 55·7; H, 4·8; Cl, 4·4. $2C_{21}H_{23}O_9,HCl,H_2O$ requires C, 56·8; H, 4·8; Cl, 4·0%). The low values for carbon are associated with somewhat high values for chlorine, indicating some content of normal chloride.

The salt is almost insoluble in 2% hydrochloric acid or in acetic acid; it dissolves sparingly in 0·5% hydrochloric acid to a yellowish-brown solution and the distribution to *iso*amyl alcohol is superficially similar to that of a normal diglucoside. Under the conditions laid down in Parts XV and XVII of this series (J., 1932, 2296, 2492) the distribution number (*n*-butyl alcohol) was 50·0 (3·18 mg. in 50 c.c. of the mixed solvents). The orange-yellow alcoholic and *n*-butyl-alcoholic solutions exhibit a green fluorescence. The aqueous or alcoholic solution develops a fine bluish-rose coloration on the addition of sodium acetate, sodium carbonate, ammonia or sodium hydroxide. The glucoside is rapidly hydrolysed by hot 10% hydrochloric acid.

4-Hydroxy-3-methoxyacetophenone.—This substance was prepared from ω-chloroaceto-vanillone (Pratt and Robinson, J., 1923, **123**, 753; Levy and Robinson, J., 1931, 2715) because a specimen of the latter was available. A mixture of the chloro-ketone (3 g.), sodium iodide (0·3 g.), iron filings (3 g.), alcohol (30 c.c.), and dilute sulphuric acid (30 c.c. of 10%) was agitated at 40—50° for 1 hour, and the product isolated from the filtered and diluted solution by means of ether. After recrystallisation from water the acetovanillone was obtained as small colourless prisms, m. p. 114—115°.

7 : 4′-Dihydroxy-3′-methoxy-5-β-glucosidoxyflavylium Chloride (*3′-Methoxygesnerin Chloride*) (II).—A solution of 2-*O*-tetra-acetyl-β-glucosidylphloroglucinaldehyde (1·5 g.) and acetovanillone (1·5 g.) in dry ethyl acetate (40 c.c.) was saturated at 0° with hydrogen chloride and kept for 48 hours in the ice-chest; dry ether (400 c.c.) was added, and the precipitated flavylium *salt* was collected, washed, and dried (1·45 g.) (Found : C, 52·7; H, 5·0; Cl, 3·6. $C_{30}H_{31}O_{14}Cl,2H_2O$ requires C, 52·5; H, 5·1; Cl, 5·2%). The low value for chlorine indicates replacement of some HCl by H_2O.

This crude product (1·3 g.) was hydrolysed under the usual conditions by means of aqueous sodium hydroxide (13 c.c. of 10%) and on addition of 20% hydrochloric acid (6·4 c.c.) immediate precipitation of the amorphous glucoside occurred. Great difficulty was experienced in attempts to crystallise this substance, but it was ultimately obtained as a brown microcrystalline powder by solution in boiling 0·1% alcoholic hydrogen chloride, in which it was very sparingly soluble, and increase of the acid concentration of the filtered solution to 2% (Found in air-dried material : C, 50·0; H, 5·0; Cl, 6·0. $C_{22}H_{25}O_{10}Cl,1·5H_2O$ requires C, 50·0; H, 5·3; Cl, 6·7%). This *glucoside* resembles gesnerin chloride in many of its properties; its acid solutions are redder and the alkaline solutions bluer in tone than those of the apigeninidin glucoside; the solution in aqueous sodium carbonate has a rich bluish-crimson colour. The alcoholic solutions do not exhibit any fluorescence. A solution in 0·5% hydrochloric acid (1·14 mg. in 50 c.c.) was pale reddish-brownish-orange and the distribution to *iso*amyl alcohol was almost zero; on the addition

of salt the glucoside merely separated as an orange film at the interface. The distribution to butyl alcohol was also lower than in the case of gesnerin.

Preparation of a Solution of Gesnerin Chloride from Flower Petals of Gesnera fulgens.—The darker bluer-red parts of the petals were excised and the pigment was extracted by hot 0·2% hydrochloric acid; the clear orange aqueous solutions were centrifuged and exhaustively extracted by means of *n*-butyl alcohol after the addition of salt (for the later extractions only); the oxonium salt was then returned to 0·1% hydrochloric acid after the addition of an equal volume of light petroleum. The filtered solution was treated with a few c.c. of dilute ferric chloride solution and kept for 3 months. The colouring matter was then thrice similarly transferred to *n*-butyl alcohol and 0·5% hydrochloric acid and finally to 0·5% hydrochloric acid. This solution (A) was a little browner and bluer than one of pure gesnerin chloride and at this stage an attempt was made to imitate it by synthetic mixtures. It should be stated clearly that even at this stage the identity of the chief pigment with glucosidylapigeninidin was obvious. Addition of 8% of methoxygesnerin chloride to gesnerin chloride (synthetic) gave a solution closely resembling the natural in colour and in behaviour towards alkalis, but in a comparison of behaviour towards *n*-butyl alcohol the distribution of the synthetic mixture was far too low (too much remained in the watery layer). Hence the impurity in the natural pigment solution is probably a substance of greater tinctorial intensity than 3′-methoxygesnerin. It must be a related compound and it gives no indication of an iron reaction.

The further purification of (A) was difficult and wasteful. The acid concentration was increased to 5%, and the solution heated on the steam-bath until about a third of the anthocyanin was hydrolysed; the resulting anthocyanidin was removed by means of *iso*amyl alcohol and the solution was cooled, mixed with a few drops of ferric chloride, and kept for 6 weeks. The salt was transferred between *n*-butyl alcohol and 0·1% hydrochloric acid four times and then had the pure orange-yellow colour of solutions of the synthetic substance; the last aqueous solution was free from iron but was too dilute for a determination of the distribution number. It was concentrated in a vacuum, and the chlorine in the solution estimated; the requisite amount of silver carbonate was then added in order to reduce the concentration to 0·5%, but this did not work according to plan and the acid concentration of the filtrate was too high. The solution was therefore shaken with butyl alcohol and adjusted so that 20·5 c.c. of each layer were obtained in which the acid concentration of the aqueous layer was 0·46% and the amount of pigment was 1·41 mg. as determined by colorimetric comparison with the synthetic standard.

The distribution number actually found was 49·3, so the total amount of pigment was about 2·85 mg. and the conditions approximated closely to those of the experiment with the synthetic glucoside. No difficulty was experienced in the colorimetric matching.

The natural pigment was returned from the butyl alcohol to the aqueous layer (addition of light petroleum) and the solution was washed with *iso*amyl alcohol. Colour-reaction comparisons were made between this solution and the correspondingly treated synthetic pigment solution. The green fluorescence noted on the addition of much alcohol was identical in both cases, and parallel experiments with buffered solutions of graded p_H as well as with sodium acetate, sodium carbonate and sodium hydroxide gave identical results. The behaviour on treatment with aqueous sodium hydroxide was also the same, an orange-yellow solution being quickly obtained. Finally, equal volumes of the acid solutions were placed in narrow test-tubes and carefully covered with a dilute solution of sodium carbonate; the appearance of the two tubes remained identical as the liquids diffused and the coloured zones ranging from yellow to bluish-rose gradually extended.

The leaves and stems of *Gesnera zebrina* and *Gesnera fulgens* contain a different anthocyanin, the anthocyanidin from which does not tally with any known substance but is apparently unhydroxylated in position 3.

The authors are grateful to the Royal Commissioners for the Exhibition of 1851 for a Senior Studentship awarded to one of them. They also wish to thank Messrs. Carters Ltd. for a supply of flowers of *Gesnera fulgens*.

THE DYSON PERRINS LABORATORY, OXFORD UNIVERSITY. [*Received, April 4th*, 1934.]