353. *Experiments on the Synthesis of Anthocyanins. Part XXV. Pelargonidin 3-Galactoside.*

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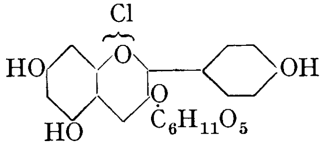
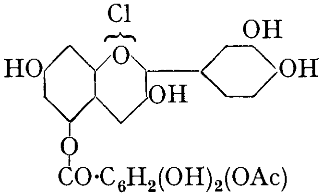
Pelargonidin 3-saccharides are widely distributed anthocyanins having similar colour reactions but distinguished by their ratios of distribution between *iso*amyl alcohol and dilute hydrochloric acid. As the result of qualitative tests it has been found that the orange-scarlet nasturtium contains a pelargonidin 3-bioside, the red *gloxinia* flowers are coloured by a pelargonidin rhamnoglycoside, and pelargonidin 3-glycosides occur in scarlet carnations, strawberries, certain of the more orange-red sweet-peas, and in other flowers (compare Robinson and Robinson, *Biochem. J.*, 1931, **25**, 1687; 1932, **26**, 1647). A description of the isolation in substance of pelargonidin 3-glycosides from certain of these natural sources will be submitted later; in the meantime we place on record the synthesis of pelargonidin 3-galactoside, which is one of the pigments that has been isolated.

The synthesis of pelargonidin 3-glycoside (callistephin) was the first of a naturally occurring anthocyanin (Robertson and Robinson, J., 1928, 1460) and the technique has been improved as the result of experience of other cases. A repetition of this investigation using the newer methods has therefore been undertaken.

Substituting galactose for glucose, we employed an analogous series of reactions for the synthesis of 3-galactosidyl pelargonidin chloride (I).

(I.)





(II.)

Some preliminary experiments on the preparation of hydroxylated *O*-acyl cyanidins (*e.g.*, II) are also mentioned. Such substances are of interest on account of the stable *O*-acylanthocyanidins frequently obtained by the hydrolysis of anthocyanins (Robinson and Robinson, *loc. cit.*). What is really desired is a series of 3-hydroxyacylcyanidins, but the 5-derivatives are more readily accessible and enable a study to be made of the preferential hydrolysis of acetoxy-groups in the molecule of the aromatic hydroxy-acid. Up to the present the results have not thrown light on the problem of the nature of acylanthocyanidins of natural origin; these have characteristic distribution properties which have not been reproduced by any of the synthetic compounds.

EXPERIMENTAL.

3-β-*Glucosidylpelargonidin Chloride* (*Callistephin Chloride*).—A solution of ω-*O*-tetra-acetyl-β-glucosidoxy-4-acetoxyacetophenone (5 g.) (Robertson and Robinson, *loc. cit.*) and 2-*O*-benzoylphloroglucinaldehyde (2·5 g.) in dry ethyl acetate (100 c.c.) was saturated with dry hydrogen chloride at room temperature and kept in the ice-chest for 3 days. Dry ether (500 c.c.) was added and the deep-red precipitate collected (5 g.) and dried. Aqueous sodium hydroxide (115 c.c. of 8%), cooled to 16°, was gradually added to the finely powdered flavylium chloride (5·1 g.) in an atmosphere of hydrogen, and the mixture maintained at room temperature for 3 hours. Hydrochloric acid (192 c.c. of 7%) was added to the black alkaline solution and the temperature raised to 60° to complete the formation of the oxonium salt. After cooling and filtration, an equal volume of saturated aqueous picric acid was added to the solution and the separation of the picrate was completed by keeping the mixture in the ice-chest for 12 hours. The crude picrate was collected, washed with dilute aqueous picric acid and ice-water, and dried (5·5 g.). The picrate was crystallised by the known method (Robertson and Robinson, *loc. cit.*), forming perfect, microscopic, rectangular plates (3 g.). It was converted into the chloride, which was crystallised as previously and analysed for confirmation of purity (Found: C, 49·6; H, 5·0. Calc. for $C_{21}H_{21}O_{10}Cl,2H_2O$: C, 49·9; H, 4·9%). As will be later described, this specimen has been found to be identical with the anthocyanin of scarlet carnations.

The distribution number for 8 concentrations over the range 1·44 mg. to 8·45 mg. in 25 c.c. of *iso*amyl alcohol and equilibrated 0·5% hydrochloric acid was found to vary from 38·9 to 32·0. The relation of log C_W to log C_{AA} is linear, but the slope is not so steep as in the cases of chrysanthemin and oenin and the degree of association in the aqueous layer must be smaller than in the other examples mentioned (Levy and Robinson, J., 1931, 2715).

ω-O-*Tetra-acetylgalactosidoxy*-4-*acetoxyacetophenone*.—Freshly prepared, dry silver carbonate (15 g.) was added to a solution of ω-hydroxy-4-acetoxyacetophenone (7 g.) and *O*-tetra-acetylgalactosidyl bromide (20 g.) in dry benzene (100 c.c.) at 35—40°, and the mixture agitated at that temperature for 45 minutes. The reaction was completed by refluxing on the steam-bath for 20 minutes and the solution was filtered hot and mixed with light petroleum (1000 c.c.). The cream-coloured syrup obtained was washed with hot water and then with ice-cold water repeatedly, but the substance could not be crystallised. After draining, the syrup was emulsified with ether, in which it was sparingly soluble, and the emulsion was gradually freed from ether by slow evaporation under reduced pressure. At the end of 12 hours the emulsion had changed to a solid mass (8—9 g.) and this was purified by repeated dissolution in methyl alcohol and precipitation with water (yield, 7·5 g. of a colourless semi-crystalline solid) (Found : C, 54·5; H, 5·4. $C_{24}H_{28}O_{13}$ requires C, 54·9; H, 5·3%). This substance is readily soluble in most organic solvents but it is sparingly soluble in ether and in light petroleum.

7-*Hydroxy*-3-O-*tetra-acetylgalactosidoxy*-5-*benzoyloxy*-4′-*acetoxyflavylium Chloride*.—A solution of ω-*O*-tetra-acetylgalactosidoxy-4-acetoxyacetophenone (6 g.) and 2-*O*-benzoylphloroglucinaldehyde (3 g.) in dry ethyl acetate (100 c.c.) was saturated with hydrogen chloride at room temperature, protected from the access of moisture, and kept in the ice-chest for 72 hours. Addition of dry ether (750 c.c.) to the deep crimson solution precipitated the flavylium chloride, which was washed twice with dry ether and dried over potassium hydroxide in a vacuum (yield, 5·8 g.) (Found : C, 55·9; H, 4·4. $C_{38}H_{35}O_{16}Cl,2H_2O$ requires C, 55·7; H, 4·8%).

3-*Galactosidylpelargonidin Picrate*.—Aqueous sodium hydroxide (90 c.c. of 8%), cooled to 10°, was added to the finely powdered, crude flavylium chloride (4·2 g.), air having been displaced by hydrogen. After 3 hours aqueous hydrochloric acid (150 c.c. of 7%) was introduced; the solution then became dark red and the formation of the oxonium salt was completed by heating at 60° for a few minutes. An equal volume of saturated aqueous picric acid was added to the cooled solution, and the mixture kept in the ice-chest until the separation of the picrate was complete; the dark brown solid was collected, washed, and dried. This crude picrate contained a considerable amount of anthocyanidin, which was eliminated as follows. The crude picrate was dissolved in warm 0·5% hydrochloric acid and the cooled solution, after addition of saturated aqueous picric acid, was repeatedly shaken with ether, until the ethereal layer was only lemon-yellow. The aqueous layer was separated and freed from ether by air-blowing; an equal volume of saturated picric acid was then added, and the mixture kept in the ice-chest for 24 hours. The separated solid was well washed and dried (2·4 g.). The still impure picrate was crystallised from saturated alcoholic picric acid and again twice from more dilute alcoholic picric acid (1 vol. of saturated alcoholic picric acid and 4 vols. of alcohol), finally forming well-formed reddish plates with a golden reflex (1·2 g.) (Found : C, 48·0; H, 3·6; N, 6·1. $C_{27}H_{25}O_{18}N_3$ requires C, 47·7; H, 3·7; N, 6·2%). The *picrate* is sparingly soluble in cold water, but is freely soluble in alcohol and hot water.

3-*Galactosidylpelargonidin Chloride* (I).—The pure picrate (0·8 g.) was dissolved in methyl-alcoholic hydrogen chloride (35 c.c. of 5%), and the chloride precipitated by the addition of dry ether (300 c.c.), collected by centrifuging, washed with dry ether, and dried in air. The salt was crystallised according to Willstätter and Burdick's method (*Annalen*, 1916, **412**, 149) for callistephin chloride, *viz.*, by the slow evaporation of a methyl-alcoholic solution to which one-quarter its volume of 12% aqueous hydrochloric acid has been added; a crop of dark red, microscopic needles was obtained. Recrystallisation in a similar manner afforded clusters of very fine needles, dark red in mass, but orange by transmitted light (yield, 0·3 g.) (Found : C, 46·2; H, 5·4. $C_{21}H_{21}O_{10}Cl,4H_2O$ requires C, 46·6; H, 5·4. Found in material dried at 110° in a high vacuum : C, 53·9; H, 4·6. $C_{21}H_{21}O_{18}Cl$ requires C, 53·8; H, 4·5%).

Concentrated aqueous acid solutions of the anthocyanin are deep red and very stable to aerial oxidation; dilute aqueous acid solutions have an orange-red colour. The anthocyanin dissolves easily in alcohol to reddish-pink solutions, but it is very sparingly soluble in acetone, a pale pink colour being produced in the solution. The colour reactions in buffered solutions of graded p_H, also with ammonia, sodium acetate, carbonate, hydroxide, etc., are identical with those of callistephin chloride.

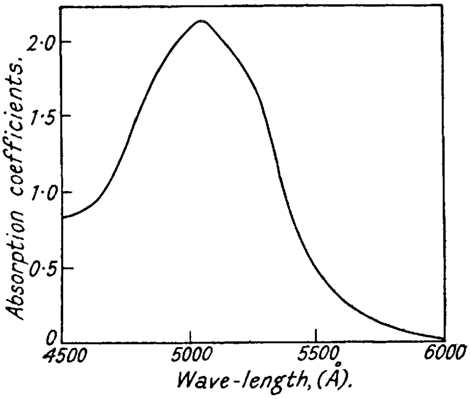
Unfortunately the distribution number was determined in a series of concentrations with a specimen of the anthocyanin not entirely free from pelargonidin, but as the error caused thereby is in the opposite direction, it can be stated that the effect of concentration is similar to that observed in the case of callistephin. A specimen was later freed from every trace of anthocyanidin and the distribution number was found to be 24·3, 24·3, 24·4 for 8·00, 8·01 and 7·93 mg. respectively in 25 c.c. each of equilibrated *iso*amyl alcohol and 0·5% hydrochloric acid.

The absorption spectrum was determined with a solution of the anthocyanin (0·997 mg.) in methyl-alcoholic hydrogen chloride (100 c.c. of 0·5%) (Fig.).

Experiments on the Preparation of Various 5-O-*Hydroxyacylcyanidins*.—These experiments will be described very briefly, because we do not claim to have worked out the best conditions. Phloroglucinaldehyde (10 g.) was *O*-triacetylgalloylated by means of aqueous sodium hydroxide and *O*-triacetylgalloyl chloride (10 g., dissolved in chloroform) at 0°. The product (3·2 g.) crystallised in small colourless needles, m. p. 148—150° (Found : C, 55·3; H, 3·8. $C_{20}H_{16}O_{11}$ requires C, 55·6; H, 3·7%).

This 2-O-(O-*triacetylgalloyl*)*phloroglucinaldehyde* (2 g.) was condensed with ω : 3 : 4-trihydroxyacetophenone (1·2 g.) in ethyl acetate–alcohol solution (2 : 1) by means of hydrogen chloride at 0°. The dark violet product (2·2 g.) was crystallised similarly to 5-*O*-benzoylcyanidin chloride (Robertson and Robinson, J., 1928, 1528); it was obtained in dark green needles (0·45 g.) (Found in air-dried material: C, 49·9; H, 4·3. $C_{28}H_{21}O_{13}Cl,4H_2O$ requires C, 49·9; H, 4·3. Found in material dried at 105° in a high vacuum : C, 56·2; H, 3·6. $C_{28}H_{21}O_{13}Cl$ requires C, 56·0; H, 3·5%). The substance has the properties expected of an *O*-5-aroylcyanidin chloride.

When boiled with 5% alcoholic hydrogen chloride, the substance appears to lose only two of its acetyl groups. The product crystallised from aqueous hydrochloric acid (from 1 to 3%) in greenish-black needles (Found in air-dried material: C, 51·5; H, 4·0. $C_{24}H_{17}O_{11}Cl,3H_2O$ requires C, 51·4; H, 4·1. Found in material dried at 105° in a high vacuum: C, 56·7; H, 3·8. $C_{24}H_{17}O_{11}Cl$ requires C, 56·8; H, 3·4%). This salt is a partly *acetylated* 5-O-*galloylcyanidin chloride* and its colour reactions are entirely like those of 5-*O*-benzoylcyanidin.



3-*Galactosidylpelargonidin chloride.*

The 4-acetoxybenzoylation of phloroglucinaldehyde, under the usual conditions for mono-aroylation and the acid chloride being applied in ethereal solution, appears to have furnished as main product a 2 : 4-*di*-O-4-*acetoxybenzoylphloroglucinaldehyde*. The substance in question crystallises from alcohol in prismatic needles, m. p. 180—182° (Found : C, 61·7; H, 3·5. $C_{25}H_{18}O_{10},0·5H_2O$ requires C, 61·6; H, 3·8%). This view is provisional, but is thought to be correct because the flavylium salt made by condensation with ω : 3 : 4-trihydroxyacetophenone in the usual manner and crystallised from aqueous alcoholic hydrogen chloride appears to be 5 : 7-*di*-O-4-*hydroxybenzoylcyanidin chloride* (Found in air-dried material: C, 56·2; H, 4·3. $C_{29}H_{19}O_{10}Cl,3H_2O$ requires C, 56·4; H, 4·1. Found in material dried at 110° in a high vacuum : C, 62·4; H, 3·7; Cl, 6·1. $C_{29}H_{19}O_{10}Cl$ requires C, 61·9; H, 3·4; Cl, 6·3%). Not only the analysis but also the colour reactions suggest the introduction of two acyl groups into the cyanidin molecule. For example, the solution in aqueous sodium carbonate is not blue, as in the case of benzoylcyanidin, but intense violet-red. On hydrolysis with hot aqueous sodium hydroxide under nitrogen and acidification of the solution, cyanidin was produced and recognised by means of the tests prescribed by Robinson and Robinson (*loc. cit.*).

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