351. *Experiments on the Synthesis of Anthocyanins. Part XXIII. Glucosides of Petunidin Chloride.*

By (Miss) J. C. Bell and Robert Robinson.

The 3-monoglucosides and the 3 : 5-diglucosides of six of the seven anthocyanidins having been synthetically prepared, we were desirous of completing the series by the preparation of the corresponding petunidin derivatives. The intermediates are not readily accessible, but we have obtained the pure 3-monoglucoside and a specimen of the diglucoside contaminated with inorganic salts only. It is probable that this substance will prove to be identical with petunin which Willstätter and Burdick (*Annalen*, 1917, **412**, 217) isolated from a special variety of *Petunia* hybrids, and when a suitable opportunity offers, the synthesis and the isolation from the natural sources will be repeated; both are very laborious and in the meantime we can place on record certain characteristic properties of the diglucoside which are not affected by its content of inorganic matter.

Petunidin 3-monoglucoside has not been isolated in substance from natural sources, but there is reason to suppose that it is one of the anthocyanins of the bilberry, and the pigments of the purplish berries of *Berberis Darwinii* and of *B. stenophylla* appear to consist largely of this colouring matter (Robinson and Robinson, *Biochem. J.*, 1932, **26**, 1647).

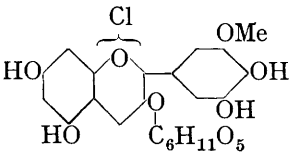
For the first component in the syntheses we have employed the known phloroglucinaldehyde derivatives, *i.e.*, 2-*O*-benzoylphloroglucinaldehyde for the monoglucoside and 2-*O*-tetra-acetyl-β-glucosidylphloroglucinaldehyde for the diglucoside.

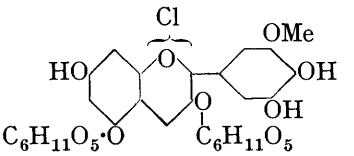
The second component was made by a method similar to that which proved successful in the delphinidin series (see Reynolds and Robinson, this vol., p. 1039). ω-Diazo-3-methoxy-4 : 5-diacetoxyacetophenone (Bradley, Robinson, and Schwarzenbach, J., 1930, 812) was converted by aqueous formic acid under narrowly defined conditions into *ω-hydroxy-3-methoxy-4 : 5-diacetoxyacetophenone*, $(AcO)_2(MeO)C_6H_2\cdot CO\cdot CH_2\cdot OH$ (I), a process first carried out by Miss T. M. Reynolds. The carbinol was tetra-acetylglucosidated in the usual manner to the intermediate of the formula

$$(AcO)_2(MeO)C_6H_2\cdot CO\cdot CH_2\cdot O\cdot C_6H_7O(OAc)_4 \text{ (II)}.$$

The coupling to flavylium salt was effected in ethyl acetate solution and the acetylated products were hydrolysed by means of methyl-alcoholic barium hydroxide and regenerated by treatment with sulphuric and hydrochloric acids. The monoglucoside (III) was purified

(III.)





(IV.)

through the picrate; as already stated, the diglucoside (IV) could not be separated from inorganic substances in view of the small amount available.

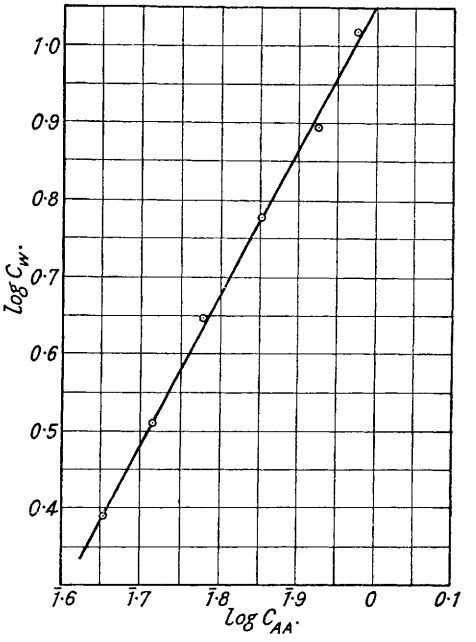
Experimental.

ω-Hydroxy-3-methoxy-4 : 5-diacetoxyacetophenone (I).—ω-Diazo-3-methoxy-4 : 5-diacetoxyacetophenone (6 g.) (Bradley, Robinson, and Schwarzenbach, *loc. cit.*), mixed with 50% formic acid (33 c.c.), was heated on the steam-bath for 10 minutes; evolution of nitrogen was rapid at first. An equal volume of water was added, and the hot solution filtered and neutralised with potassium carbonate. The semi-solid mass which separated was taken up in chloroform, the extract dried, and the solvent removed in a vacuum; the syrupy residue crystallised from alcohol in colourless needles, m. p. 83—84° (yield, 4 g. or 70%) (Found: C, 55·2; H, 5·2. $C_{13}H_{14}O_7$ requires C, 55·3; H, 5·0%). When this substance was crystallised 4 times from benzene (m. p. raised to 86—87°) it retained $0·5H_2O$ (Found: C, 53·8; H, 5·3; MeO, 10·4; loss in a vacuum at 160°, 2·0. $C_{13}H_{14}O_7,0·5H_2O$ requires C, 53·6; H, 5·2; MeO, 10·7; $0·5H_2O$, 3·1%). This carbinol had little action on Fehling's solution in the cold, probably owing to its sparing solubility in water, but reduction occurred on heating.

ω-O-Tetra-acetyl-β-glucosidoxy-3-methoxy-4 : 5-diacetoxyacetophenone (II).—ω-Hydroxy-3-methoxy-4 : 5-diacetoxyacetophenone (3·2 g.) and tetra-acetylglucosidyl bromide (7 g.) (compare Freudenberg, *Ber.*, 1927, **60**, 241, for the preparation) were mixed with dry benzene (20 c.c.) and heated to 40°. Dry silver carbonate (9 g.) was added to the solution; a reaction then supervened, accompanied by a rise of temperature and evolution of carbon dioxide. The mixture was shaken for 30 minutes, filtered through kieselguhr, and light petroleum added to the filtrate. The semi-solid white syrup which was precipitated was washed with hot and then with cold water and purified by solution in warm methyl alcohol (4 c.c.) and precipitation with water with cooling in ice. This process was repeated until the glucoside crystallised in colourless prisms, m. p. 67—69° after softening at 60° (yield, 2·2 g. or 32%) (Found in material dried in a vacuum over phosphoric oxide: C, 52·5; H, 5·1. $C_{27}H_{32}O_{16}$ requires C, 52·9; H, 5·2%).

Fig. 1.

Distribution of petunidin 3-monoglucoside chloride.



3-O-Glucosidylpetunidin Chloride (III).—A solution of tetra-acetylglucosidylmethoxydi-acetoxyacetophenone (2·2 g.) and 2-*O*-benzoylphloroglucinaldehyde (2·2 g.) in dry ethyl acetate (44 c.c.) was saturated with hydrogen chloride at 0°, protected from access of moisture, and kept in the ice-chest for 72 hours. The deep red liquid was added to dry ether (250 c.c.) and the red solid which was precipitated was collected and washed with ether. In a vacuum desiccator over phosphoric oxide and sodium hydroxide it became deeper red in colour and appeared to be crystalline (yield, 1 g. or 32%).

This benzoylhexa-acetylpetunidin monoglucoside chloride (1 g.) was dissolved in 1% methyl-alcoholic hydrogen chloride (10 c.c.); air was displaced by hydrogen and anhydrous conditions were maintained. A solution of anhydrous barium hydroxide in absolute methyl alcohol (60 c.c.) was gradually added and a slow stream of hydrogen passed for 6 hours. Sufficient methyl-alcoholic sulphuric acid to neutralise the barium hydroxide was then added, and the mixture shaken for 10 minutes in order to facilitate the deposition of the precipitated barium sulphate before centrifuging. The supernatant liquid from the latter process was filtered and an equal volume of cold saturated aqueous picric acid was added to the filtrate. The anthocyanin picrate separated after keeping for 12 hours in the ice-chest; it was collected and washed with ether, which removed a little picric acid to a yellow solution, showing that the product was free from anthocyanidin. It was crystallised by solution in hot alcohol and addition of hot aqueous

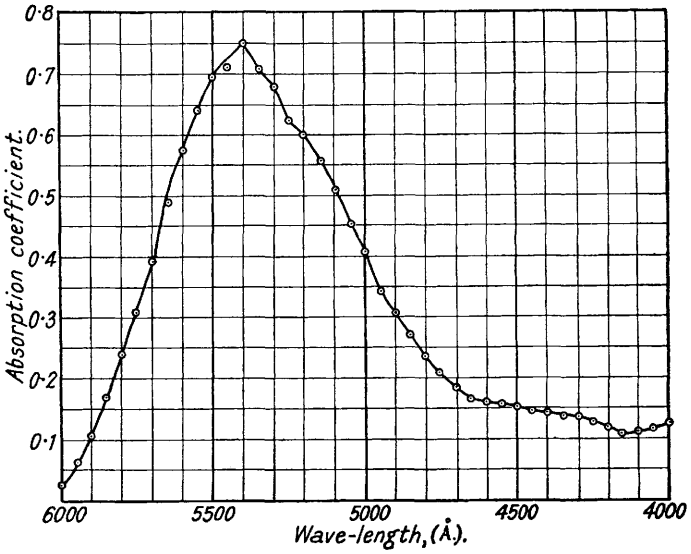
picric acid; on cooling, the picrate gradually separated as bright red, slender needles (yield, 0·4 g. or 74%).

The picrate (0·1 g.) was dissolved in a little 4% methyl-alcoholic hydrogen chloride, and an amorphous chloride separated by the addition of ether; this was collected, washed with dry ether, and found to be free from anthocyanidin (yield, 60 mg. or 82%). The crude chloride was dissolved in cold 1% methyl-alcoholic hydrogen chloride, and the acid concentration increased to about 4%. After keeping for 2 days in the ice-chest, very dark prisms with a dark green metallic lustre had separated [Found in air-dried material: C, 42·9; H, 5·3; Cl, 5·2. Found after drying in a desiccator (loss, 1·5): C, 44·5; H, 5·3; Cl, 6·3. $C_{22}H_{23}O_{12}Cl,5·5H_2O$ requires C, 43·0; H, 5·5; Cl, 5·8%. $C_{22}H_{23}O_{12}Cl,4·5H_2O$ requires C, 44·3; H, 5·4; Cl, 6·0; loss from $5·5H_2O$ to $4·5H_2O$, 1·8%. Loss on further drying at 110° in a high vacuum over phosphoric oxide, 12·8. Found in anhydrous material: C, 51·1; H, 4·5; Cl, 6·9. $C_{22}H_{23}O_{12}Cl$ requires C, 51·3; H, 4·5; Cl, 7·0%].

The crystals are dark red by transmitted light under the microscope and give a violet smear on paper. The solution in aqueous hydrochloric acid is brownish-red, becoming bluish-red on dilution with alcohol. The violet solution in aqueous sodium acetate is rapidly decolorised

FIG. 2.

Absorption spectrum of petunidin monoglucoside chloride.



with formation of the pseudo-base and the rich blue solution, with violet tinge, in aqueous sodium carbonate becomes pure blue on the addition of sodium hydroxide.

The Distribution Number.—The distribution of petunidin monoglucoside chloride between 0·5% aqueous hydrochloric acid and *iso*amyl alcohol, mutually saturated, was determined by the standard method at several concentrations. When the log. of the concentration in the *iso*amyl alcohol was plotted against the log. of the concentration in the aqueous layer, the points lay on a straight line (Fig. 1) and the ratio of the square of the concentration in the *iso*amyl alcohol to that in the hydrochloric acid was approximately constant.

Concentration in mg.	Concentration in g.-mols. $\times 10^{-6}$.	D.	Log C_w.	Log C_{AA}.	K.
7·484	11·40	8·34	1·0191	1·9778	8·64
5·674	8·64	9·83	0·8915	1·9289	9·25
4·365	6·65	10·79	0·7731	1·8558	8·88
3·310	5·04	11·98	0·6474	1·7809	8·21
2·488	3·80	13·72	0·5159	1·7169	8·28
1·891	2·88	15·67	0·3856	1·6538	8·36

The absorption of light in the visible region was measured (Fig. 2) in a 0·1% methyl-alcoholic hydrogen chloride solution (1·05 mg. of the hydrated salt in 100 c.c.).

The colour reactions of a solution of the pigment (6·00 mg.) in ethyl alcohol (25 c.c.) were examined in buffered solutions of graded p_H (Robertson and Robinson, *Biochem. J.*, 1929, **23**, 35). (1) Rose; (3) slightly bluer rose; (5) reddish-violet; (7) duller reddish-violet; (9) plum colour; (11) plum colour; (13) blue-violet; (15) violet-blue; (16) blue; (17) greenish-blue. After 10 minutes: (1) Rose; (3) pale violet-pink; (5) reddish-violet; (7) plum colour; (9), (11) redder than (7); (13), (15) slatey violet; (16) duller violet; (17) pale violet-grey. After 1½ hours: (1) Rose; (3) pale violet-pink; (5) violet; (7) reddish-violet, dull; (9) paler and redder; (11) the same as (9); (13)—(16) pale greyish-red; (17) pale orange-red. After 24 hours: (1) Rose; (3) pinkish violet; (5) violet; (7) dull violet; (9) plum colour; (11) duller than (9); (13)—(16) pinkish yellow; (17) pale, slightly orange-yellow.

7-Hydroxy-3 : 5-di-(O-tetra-acetyl-β-glucosidoxy)-3′-methoxy-4′ : 5′-diacetoxyflavylium Chloride.—A solution of tetra-acetylglucosidoxymethoxydiacetoxyacetophenone (1·3 g.) and 2-*O*-tetra-acetyl-β-glucosidylphloroglucinaldehyde (1 g.) (Robinson and Todd, J., 1932, 2299) in dry ethyl acetate (18 c.c.), cooled in ice-water, was saturated with dry hydrogen chloride under anhydrous conditions and kept in the ice-chest for 3 days. The dark red solution was poured into dry ether (200 c.c.) and the bright red precipitated solid was collected, washed with dry ether, and dried in a vacuum desiccator over phosphoric oxide and sodium hydroxide (yield, 1·4 g. or 60%) (Found: C, 50·8; H, 4·9. $C_{48}H_{53}O_{27}Cl,HCl$ requires C, 50·8; H, 4·8%). This salt dissolves in aqueous sodium carbonate to a pure blue solution, gradually fading to a greenish-yellow; the red colour was restored on acidification.

Petunidin 3 : 5-Diglucoside Chloride (IV).—The foregoing chloride (1·3 g.) was dissolved in 0·5% methyl-alcoholic hydrogen chloride (10 c.c.) and a stream of hydrogen was passed through the flask, which was cooled in ice-water. A solution of anhydrous barium hydroxide in dry methyl alcohol (80 c.c.) was gradually introduced and hydrogen was slowly passed for 6 hours. Sufficient methyl-alcoholic sulphuric acid was added to precipitate all the barium as sulphate, the solution was shaken vigorously for 10 minutes, and next day centrifuged, and the supernatant liquid filtered into ether, which precipitated the anthocyanin. The precipitate was collected, washed with dry ether, and dried in a vacuum desiccator, but was found to contain a barium compound in spite of the precipitation as sulphate.

Many attempts were made to free the anthocyanin from barium, and the method finally adopted was dissolution in absolute ethyl alcohol to which a trace of hydrogen chloride had been added, filtration, and concentration at the room temperature in a high vacuum; the petunidin 3 : 5-diglucoside then separated. After keeping in the ice-chest for some hours, the anthocyanin was collected and washed with a little cold 2% ethyl-alcoholic hydrogen chloride; it was microcrystalline but hygroscopic.

The chloride was dissolved in 0·5% aqueous hydrochloric acid, and the acid concentration increased in the cold to 2%. After the solution had been kept for some time and concentrated in a vacuum desiccator, the anthocyanin did not separate; accordingly an equal volume of *n*-propyl alcohol was added, precipitating a small quantity of a hygroscopic chloride, and when this had been twice triturated with alcohol and the alcohol concentrated, the substance was obtained in a crystalline condition as dark, black violet prisms. Addition of ethyl alcohol to the mother-liquor precipitated more of the anthocyanin and this was obtained crystalline by the same treatment.

To our great surprise, analysis of this substance indicated the presence of a large proportion of inorganic impurity, which must be barium in some form, possibly in a co-ordinated complex, possibly in a colloidal state. The actual value found for the content of carbon was 28·1%. If all the organic material present is petunidin diglucoside chloride (calc.: C, 49·7%), this would correspond to an anthocyanin content of 56·5%. On this basis the required percentage of methoxyl is 2·56, the value found was 2·50. Since, therefore, we find the expected carbon/methoxyl ratio, we consider that the organic constituent is essentially petunidin chloride diglucoside.

Colour Reactions in Solutions of Graded p_H.—A solution of the pigment in 0·5% aqueous hydrochloric acid (about 5 mg. in 25 c.c.) was used, the method and solutions being the same as those previously described. This specimen of the anthocyanin contained a trace of a xanthylium salt as an impurity, as shown by the faint greenish fluorescence of a solution in aqueous sodium carbonate.

Colours on mixing: (1) Rose; (3) slightly redder, fading; (5) reddish-purple, fading; (7) violet; (9), (11) bluer violet; (13), (15) violet-blue; (16) the same, going greener; (17) greenish-blue. After 1½ hours: (1) Yellowish-rose; (3) paler; (5), (7) very pale yellowish-pink; (9) pale blue-violet; (11) blue-violet; (13) greenish-blue; (15) paler; (16), (17) yellow.

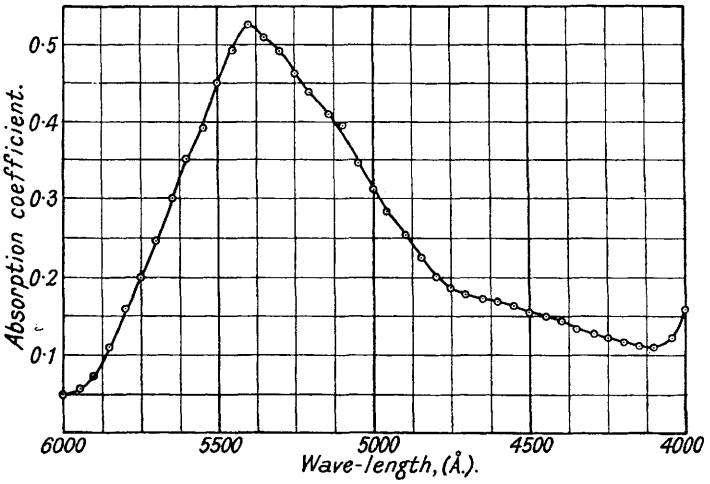
After 18 hours: (1) Yellowish-rose; (3) paler; (5), (7) very pale yellowish-pink; (9) pale greyish-blue; (11) bluer and darker; (13)—(16) pale pinkish-yellow; (17) yellow.

The solution in aqueous sodium acetate is blue with violet tinge and in aqueous sodium carbonate, pure greenish-blue; addition of sodium hydroxide causes rapid decomposition to a yellow solution.

Absorption Spectrum.—The specimen used was part of that which was analysed, so the concentration of anthocyanin can be calculated. The amount necessary to give a solution of

FIG. 3.

Absorption spectrum of petunidin 3 : 5-diglucoside chloride.



1·1 mg. of the pure anthocyanin in 100 c.c. of 0·1% methyl-alcoholic hydrogen chloride was used (Fig. 3).

The distribution number was determined, using mutually saturated *n*-butyl alcohol and 0·5% aqueous hydrochloric acid. Under conditions equivalent to 3·5 mg. of the pure anthocyanin in 50 c.c. of the mixed solvents the distribution number found was 13·6. This result serves to confirm our view of the nature of the specimen, for it is comparable with the values 14·6 and 11·7 for cyanin and delphin respectively (Robinson and Todd, J., 1932, 2488; Reynolds, Robinson, and Scott-Moncrieff, this vol., p. 1235).

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