**354.** *Experiments on the Synthesis of Anthocyanins. Part XXVI. Reso-callistephin and Reso-oenin Chloride.*

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By the term reso-anthocyanin we denote the analogues of the anthocyanins containing a resorcinol in the place of a phloroglucinol nucleus.

An example of the class is fisetinin chloride (I, R = H) or resochrysanthemin chloride (Fonseka and Robinson, J., 1931, 2732), the disclosure of the properties of which played an important part in the elucidation of the last details of the constitution of cyanin. Reso-oenin (II) was mentioned in that connexion also, but its preparation was not described. We are now able to do this and have also prepared reso-oxycoccicyanin (I, R = Me) as well as reso-callistephin (III) and the corresponding galactoside.

The methods employed need not be described in detail, as they do not diverge from the usual procedure adopted in this series. An examination of the distribution properties shows that, unlike chrysanthemin and oenin, which are associated in aqueous acid solution,

(I.) (II.) (III.)
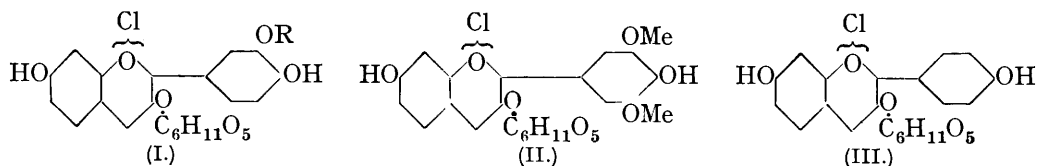
fisetinin and resocallistephin are in the same state of association in aqueous and in *iso*amyl alcoholic solution, the most probable assumption being that the salts are not associated in either medium.

As already observed, the colour reactions of these salts closely resemble those of their 5-glucosidoxy-derivatives, that is, of the normal series of diglucosidic anthocyanins.

A point of interest is that the distribution numbers of the reso-anthocyanins, except reso-oenin, are lower than those of the anthocyanins most closely related to them.

The introduction of a hydroxyl group in position 5 thus augments the distribution number of these salts. A similar phenomenon is the very low distribution number of gesnerin chloride (Robinson, Robinson, and Todd, this vol., p. 809) and it is evident that the distribution number is not connected in a simple manner with the number of hydroxyl groups in the molecule.

EXPERIMENTAL.

7 : 4'-*Dihydroxy*-3-β-*glucosidoxyflavylium Chloride* (III).—A solution of ω-*O*-tetra-acetyl-β-glucosidoxy-4-acetoxyacetophenone (5 g.) (Robertson and Robinson, J., 1928, 1465) and β-resorcylaldehyde (1·32 g.) in dry ethyl acetate (60 c.c.) was saturated with hydrogen chloride and kept in the ice-chest for 72 hours. The flavylium chloride was precipitated by the addition

FIG. 1.
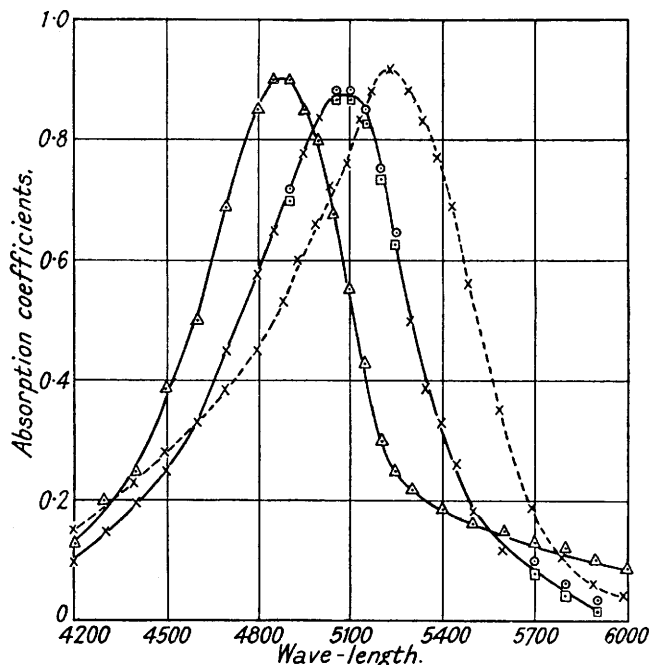
△ 3-*Glucosidyl*-4 : 7'-*dihydroxyflavylium chloride* (0·175*N*/10⁴).
× *Chrysanthemin chloride* (0·25*N*/10⁴).
× *Coincident points of* { ⊙ *Fisetinedin chloride* (0·2*N*/10⁴).
{ ⊡ 3-*Glucosidyl*-3'-*methylfisetinedin chloride* (0·175*N*/10⁴).

of dry ether (400 c.c.), collected, washed thrice with dry ether, and dried in a desiccator (yield, 5·2 g.) (Found : C, 52·5; H, 5·1. $C_{31}H_{31}O_{14}Cl,2\cdot5H_2O$ requires C, 52·6; H, 5·1%).

This *penta-acetate* of resocallistephin chloride was dissolved in aqueous sodium hydroxide (80 c.c. of 8%) below 10°, air having been excluded by nitrogen, and the solution kept at room temperature for 3 hours. Hydrochloric acid (134 c.c. of 7%) was then added, and the mixture heated to 60°, cooled, filtered, and the solution shaken with ethereal picric acid in order to remove traces of anthocyanidin. An equal volume of saturated aqueous picric acid was added to the separated aqueous solution; a voluminous dark red precipitate was formed, and after keeping in the ice-chest for 12 hours, the picrate was collected, washed, and dried (5·0 g.). The crude product (1·5 g.), twice crystallised from the minimum of boiling dilute alcoholic picric acid (1 vol. of saturated alcoholic picric acid and 8 vols. of alcohol), formed well-shaped orange-red needles with a golden reflex and bluish-violet in transmitted light (0·75 g.) (Found : N, 6·4. $C_{27}H_{25}O_{17}N_3$ requires N, 6·3%). The pure *picrate* (0·7 g.) dissolved in methyl-alcoholic hydrogen chloride (25 c.c. of 5%) to a clear red solution, from which the red chloride was precipitated by the addition of dry ether (300 c.c.) and collected by centrifuging (yield, 0·4 g.).

(1) The crude chloride was dissolved in 2% ethyl-alcoholic hydrogen chloride, the acid concentration increased to about 6%, and the solution allowed to evaporate in the air for a few minutes; crystallisation of clusters of orange-red needles quickly followed.

(2) The crude chloride was dissolved·in absolute methyl alcohol, and a few drops of 38% methyl-alcoholic hydrogen chloride were added to the solution; a thick mass of needles separated on slow evaporation in a desiccator. Recrystallised in the same way, sharply cut needles were obtained and these were washed with methyl-alcoholic hydrogen chloride and ether and dried in the air (Found : C, 50·4; H, 4·7. $C_{21}H_{21}O_9Cl,2\cdot5H_2O$ requires C, 50·7; H, 5·2. Found in material dried at 110° in a high vacuum over phosphoric oxide : C, 55·4; H, 4·5. $C_{21}H_{21}O_9Cl$ requires C, 55·7; H, 4·6%).

Concentrated aqueous solutions are deep reddish-orange, but on dilution become yellow-orange; alcoholic solutions are brown-orange and pink in thin layers. Addition of sodium acetate to an aqueous solution gives a bright cherry-red coloration. Sodium carbonate gives a rich violet-red coloration and this becomes violet-blue on the addition of an excess of acetone. Aqueous sodium hydroxide dissolves the salt to a plum-red coloured solution and the coloration is rather stable, but fades in a few minutes to yellow. The anthocyanin is stable in the ferric oxidation test.

The distribution number was found to be independent of the concentration and had the value 17·0 for 6·1 mg. in 25 c.c. each of equilibrated *iso*amyl alcohol–0·5% hydrochloric acid. Approximately the same value was found at half and at double the concentration.

7 : 4′-*Dihydroxy-3-galactosidoxyflavylium Chloride* (III).—This salt was prepared like the last-mentioned, galactose being substituted for glucose (compare Nair and Robinson, preceding paper, for the necessary second component). The intermediate crude *penta-acetate* was obtained in amount equal to the galactoside employed (Found : C, 52·4; H, 5·0. $C_{31}H_{31}O_{14}Cl,2\cdot5H_2O$ requires C, 52·6; H, 5·1%). This derivative (2·7 g.) was hydrolysed as before and a *picrate* was prepared (crude, 1·8 g.); it crystallised from alcoholic picric acid in small red plates with a golden reflex (pure, 0·9 g.) (Found : N, 6·5. $C_{27}H_{25}O_{17}N_3$ requires N, 6·3%). The *chloride* was obtained in a crude condition by the usual method, and crystallised from 2% alcoholic hydrogen chloride by the gradual addition of 10% alcoholic hydrogen chloride and evaporation in fine red, microscopic needles (Found in air-dried material : C, 51·2; H, 5·1; Cl, 7·1. $C_{21}H_{21}O_9Cl,2H_2O$ requires C, 51·5; H, 5·1; Cl, 7·3. Found in material dried at 110° in high vacuum over phosphoric oxide : C, 55·5; H, 4·7. $C_{21}H_{21}O_9Cl$ requires C, 55·7; H, 4·6%). The colour reactions of this salt were almost identical with those of the glucoside; its aqueous acid solutions were a little more intensely coloured and the stability towards sodium hydroxide in aqueous solution was markedly greater.

The distribution number (*iso*amyl alcohol and 0·5% hydrochloric acid) was found to be 13·8 and independent of the concentration in the very dilute solutions employed.

Fisetinin Chloride.—The distribution number under the usual conditions is 14. The absorption spectrum (0·2N/10⁴ in 1·0% methyl-alcoholic hydrogen chloride in a cell of 20 mm.) (Fig.) is practically identical with that of its methyl ether mentioned below. The other spectra illustrated in the Fig. relate to similar conditions with small variations of concentration as indicated under the diagram.

Reso-oxycoccicyanin Chloride (I, R = Me).—A solution of ω-*O*-tetra-acetyl-β-glucosidoxy-3-methoxy-4-acetoxyacetophenone (2·0 g.) and β-resorcylaldehyde (1·6 g.) in dry ethyl acetate

(35 c.c.) was saturated with hydrogen chloride at 0° and kept in the ice-chest for 2 days. It was then filtered into dry ether and the bright-red flavylium salt so precipitated was collected, washed, and dried (yield, 2·0 g.) (Found : C, 51·5; H, 5·5. $C_{22}H_{23}O_{10}Cl,2·5H_2O$ requires C, 50·2; H, 5·3%). The finely powdered salt (2·0 g.) was hydrolysed by means of 8% sodium hydroxide under nitrogen in the usual manner, and the picrate was precipitated from the acidified solution as a heavy orange-red precipitate (yield, 1·5 g.). Very fine orange-red needles with golden reflex were obtained by dissolving the crude picrate in hot methyl alcohol (100 c.c.), filtering and diluting the solution with an equal volume of cold saturated aqueous picric acid (yield, 0·5 g.) (Found : N, 6·1. $C_{28}H_{25}O_{17}N_3,2H_2O$ requires N, 5·9. Found in material dried at 105° in a high vacuum : C, 49·8; H, 3·7. $C_{28}H_{25}O_{17}N_3$ requires C, 49·7; H, 3·7%).

The crystalline picrate (0·9 g.) was dissolved in absolute methyl alcohol (125 c.c.), and the filtered solution mixed with methyl-alcoholic hydrogen chloride (26 c.c. of 20%); the addition of ether (500 c.c.) precipitated the chloride as a dark brown crystalline powder. The product was collected (0·6 g.) and crystallised by solution in warm methyl alcohol (100 c.c.), addition of 20% methyl-alcoholic hydrogen chloride (20 c.c.), and keeping in the ice-chest. The *chloride* separated as microscopic, dark red-brown needles with a bronze reflex (yield, 0·45 g.) (Found : C, 53·6; H, 4·9; Cl, 7·8. $C_{22}H_{23}O_{10}Cl,0·5H_2O$ requires C, 53·7; H, 4·9; Cl, 7·2. Found in material dried at 110° in a high vacuum : C, 54·4; H, 5·0; Cl, 7·8. $C_{22}H_{23}O_{10}Cl$ requires C, 54·7; H, 4·8; Cl, 7·5%). This substance has a normal distribution between *iso*amyl alcohol and 0·5% hydrochloric acid, the number being 17·4. This salt is sparingly soluble in 1% hydrochloric acid, but readily dissolves on heating to an orange-red solution with brown tinge and appearing salmon-red in thin layers; on the addition of alcohol the solution becomes bluish-red. In aqueous solution, sodium acetate gives a permanganate-red coloration, rapidly decolorised in dilute solution with formation of the pseudo-base; sodium carbonate gives a rich violet coloration, blue in thin layers and becoming royal blue on the addition of acetone. The coloration on the addition of sodium hydroxide is violet, then almost blue, but change to a yellowish-brown supervenes very rapidly. The anthocyanin is stable in the ferric-oxidation test.

Reso-oenin Chloride (II).—The condensation of β-resorcylaldehyde and the second component used for oenin was carried out in the usual manner and the hydrolysis of the crude flavylium salt first produced also presented no new feature until the alkaline solution was acidified with hydrochloric acid. At this stage the glucoside chloride largely separated from the solution, as it is much more sparingly soluble in aqueous hydrochloric acid than the analogues herein described. The salt could be crystallised from 2% aqueous hydrochloric acid, but the material that separated was not quite free from reso-oenidin. All the available specimens were therefore combined and dissolved in hot 0·1% hydrochloric acid, and the filtered solution extracted with *iso*amyl alcohol until the distribution to this solvent had reached a minimum (5 extractions). The aqueous layer, first washed with benzene, was mixed with an equal volume of saturated aqueous picric acid and the scarlet flocculent precipitate was collected by centrifuging. It was washed in the cups with aqueous picric acid and then dissolved in boiling methyl alcohol containing a little picric acid. The filtered solution was mixed with half its volume of ethyl alcohol, also containing picric acid; the picrate then crystallised in very short, dark, extremely slender, microscopic needles. This specimen was free from reso-oenidin (ether–picric acid test); it was dissolved in 2·5% methyl-alcoholic hydrogen chloride, and the chloride precipitated as a dark chocolate-brown powder by means of ether. The salt crystallised from a mixture of methyl and ethyl alcohols and 5% aqueous hydrochloric acid in dark violet, prismatic needles (brownish-red by transmitted light) exhibiting an intense green reflex (Found in air-dried material : C, 50·0; H, 5·4; Cl, 6·4. $C_{23}H_{25}O_{11}Cl,2H_2O$ requires C, 50·3; H, 5·3; Cl, 6·5%. Found in material dried at 105° in a high vacuum over phosphoric oxide : C, 54·1; H, 4·7; Cl, 6·6; MeO, 12·4. $C_{23}H_{25}O_{11}Cl$ requires C, 53·9; H, 4·9; Cl, 6·9; 2MeO, 12·1%).

Reso-oenin chloride is readily soluble in methyl-alcoholic hydrogen chloride, less readily soluble in ethyl-alcoholic hydrogen chloride or in aqueous hydrochloric acid. Its solutions are rich bluish-red, especially in the simple alcohols. It dissolves in aqueous sodium carbonate to a pure blue solution without any violet tinge; in sodium acetate the coloration is a bright reddish-violet and these reactions are similar to those exhibited by malvin.

The distribution number was determined by the standard method at different concentrations and showed variation suggesting association in the aqueous solution. However, the effect is by no means so marked as in the case of oenin and the degree of association is relatively small. The results were : 3·9 mg. of anhydrous reso-oenin in 25 c.c. each of equilibrated *iso*amyl alcohol and 0·5% hydrochloric acid, 14·3; 5·2 mg., 14·0; 6·5 mg., 13·8; 7·8 mg., 13·6; 9·1 mg., 13·4;

10·4 mg., **13·3**; 11·7 mg., **13·2**; 13·0 mg., **13·0**; 14·3 mg., **12·9**; 15·6 mg., **12·7**. Over a similar range of concentration oenin shows a much larger effect : 4·1 mg. (11·5) to 14·8 mg. (5·9) (compare Levy and Robinson, J., 1931, 2720).

Reso-oenidin chloride.—This salt was obtained by hydrolysing reso-oenin with boiling 15% hydrochloric acid and also by direct condensation of β-resorcylaldehyde with ω : 4-dihydroxy-3 : 5-dimethoxyacetophenone in ethyl acetate solution with the participation of hydrogen chloride. The products obtained by these methods were identical. The anthocyanidin closely resembles malvidin in appearance and properties. It is very sparingly soluble in dilute hydrochloric acid, but crystallises in prismatic needles from boiling 1% hydrochloric acid containing a little ethyl alcohol. The dark chocolate-brown material had a dull bronze reflex (Found in material dried in a desiccator : C, 55·3; H, 4·6. $C_{17}H_{15}O_6Cl,H_2O$ requires C, 55·4; H, 4·6. Found in material dried in stages up to 110° in a high vacuum over phosphoric oxide : C, 58·5; H, 4·4; Cl, 10·0; MeO, 17·8. $C_{17}H_{15}O_6Cl$ requires C, 58·3; H, 4·3; Cl, 10·1; 2MeO, 17·7%).

This salt dissolves in aqueous sodium acetate and sodium carbonate to rich red-violet and pure blue solutions respectively, closely resembling the corresponding reso-oenin solutions. Oenin and malvidin, however, do not exhibit the same alkali-colour reactions and this is another instance of a phenomenon which is especially characteristically exhibited in the cyanidin series. The rule may be stated as follows. In flavylium salts closely related to the anthocyanidins the substitution of OR for OH in position 3 has a large effect on the colour reactions, only if there is a free hydroxyl group in position 5. Thus in respect of alkali-colour reactions chrysanthemin diverges from cyanidin, but cyanin and cyanenin are identical with one another, because cyanin is the 3-glucoside of a cyanidin derivative not bearing a hydroxyl in position 5.

7-β-Glucosidylmalvidin Chloride.—2-O-Benzoyl-4-O-tetra-acetyl-β-glucosidylphloroglucin-aldehyde was prepared by the method of Léon, Robertson, Robinson, and Seshadri (J., 1931, 2672); an improved method of crystallisation is from 90% methyl alcohol. The condensation of the glucoside with ω : 4-dihydroxy-3 : 5-dimethoxyacetophenone in dry ethyl acetate with the participation of hydrogen chloride gave the expected product in 91% yield. The yield was inferior when the acetylated second components were employed. With ω-hydroxy-4-acetoxy-3 : 5-dimethoxyacetophenone the yield was 75% (Found : C, 52·3; H, 5·0; Cl, 4·2. $C_{40}H_{39}O_{18}Cl,4H_2O$ requires C, 52·5; H, 5·1; Cl, 3·9%). With ω : 4-diacetoxy-3 : 5-dimethoxy-acetophenone the yield fell still further to 50% and the condensation was very much slower than in the other examples. Either of the three crude products was hydrolysed by the usual method and the same results were obtained in all cases. A picrate could not be precipitated from the solution resulting from the acidification of the reaction product and accordingly this solution was evaporated to dryness in a vacuum at room temperature, the residue extracted with 2% methyl-alcoholic hydrogen chloride, and the crude chloride precipitated by the addition of ether. This specimen was not free from malvidin and it was dissolved in 0·1% hydrochloric acid and the anthocyanidin removed by washing with several successive small volumes of *iso*amyl alcohol. The solution was again concentrated in a vacuum and it was then found possible to precipitate a picrate as brown-red flocks. This derivative was centrifuged, washed, dried in a vacuum desiccator, and converted into chloride in the usual way. All attempts to crystallise the resulting dark brown-violet powder in a recognisable form were unsuccessful; therefore the precipitation was repeated from a filtered solution, and the product dried in a vacuum, first at room temperature and then at 110° (Found : C, 52·2; H, 4·8; Cl, 6·4; MeO, 11·9. $C_{23}H_{25}O_{12}Cl$ requires C, 51·9; H, 4·7; Cl, 6·6; 2MeO, 11·7%). This glucoside, possibly because it was not crystallised, appears to be unusually readily soluble in aqueous and alcoholic hydrogen chloride; it dissolves in aqueous sodium acetate to a bluish-violet solution and in aqueous sodium carbonate to a pure blue solution. It is unstable in the ferric chloride oxidation test (Léon and Robinson, J., 1931, 2732) and under the standard conditions it was decolorised in 7 minutes, whereas the isomeric oenin was not destroyed at the end of 80 hours. The distribution number was determined in the usual manner and the value 18·0 was found for 4·99 mg. in 25 c.c. each of equilibrated *iso*amyl alcohol and 0·5% hydrochloric acid.

A crude specimen of malvidin 3 : 7-diglucoside was prepared from the requisite components, and although this was diglucosidic when placed in the tube, it suffered hydrolysis in its amorphous condition and after 2 years the specimen consisted almost entirely of the 7-glucoside now described. The salt was dissolved in 0·5% hydrochloric acid and washed with *iso*amyl alcohol in order to free it from malvidin (only a trace was present). The solution was then shown to be monoglucosidic (distribution number, 18·3) and it must therefore be the 7-glucoside or 3-glucoside of malvidin. The alkali-colour reactions and the ferric-oxidation test proved conclusively

that the substance consisted essentially of the 7-glucoside. It is already known that a **3** : **5**-di-glucoside of an anthocyanidin furnishes the 5-monoglucoside on part hydrolysis; this experience would appear to indicate a similar preferential hydrolysis of the **3**-positioned glucose residue in the **3** : **7**-diglucosides.

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