311. *Experiments on the Synthesis of Anthocyanins. Part XIV. Cyanenin Chloride, Malvenin Chloride, and an Indication of the Synthesis of Cyanin Chloride.*

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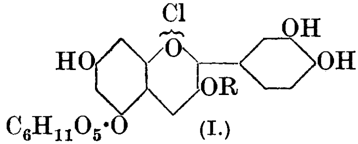
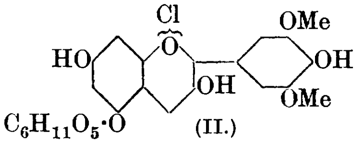
CONSIDERABLE interest attaches to the 5-glucosides of the chief anthocyanidins because they appear to be the products of partial hydrolysis of the more characteristic diglucosidic·anthocyanins. Thus pelargonenin chloride obtained by Willstätter and Bolton (*Annalen*, 1916, **412**, 133) was shown by its synthesis (Léon, Robertson, Robinson, and Seshadri, J., 1931, 2672) to be 5-β-glucosidylpelargonidin chloride. Although the method of synthesis leaves much to be desired, it has now been applied to the preparation of 5-β-glucosidylcyanidin chloride (I; R = H) and 5-β-glucosidylmalvidin chloride (II), which may be termed *cyanenin* and *malvenin chlorides* respectively.



(I.)



(II.)

Only small quantities of these salts have been accessible, but it has been possible to show that their properties in solution are identical with those of the monoglucosidic first hydrolytic products of cyanin and malvin chlorides. These comparisons were a repetition of those already described (J., 1931, 2735) and they gave the results formerly recorded, so no further description is necessary; an extract of flowers of *Malva sylvestris* was used for the preparation of natural malvenin.

Although other methods will be used in the substantiation of the claim, we have obtained cyanin chloride in small relative amount by the condensation of 2-glucosidylphloroglucinaldehyde (crude product from O-dibenzoylphloroglucinaldehyde by acetylglucosidation and hydrolysis) with the intermediate for chrysanthemin (Murakami, Robertson, and Robinson, J., 1931, 2665) by means of hydrogen chloride in ice-cold alcoholic solution. This affords support to our view that cyanin chloride has the formula (I; R = $C_6H_{11}O_5$). The solid product was not analysed, but was identified by by comparisons of distribution ratios and colour reactions of solutions.

EXPERIMENTAL.

Glucosidation of O-*Dibenzoylphloroglucinaldehyde.*—Some modifications of the process have been introduced. The reaction mixture was made as described by Léon, Robertson, Robinson, and Seshadri (*loc. cit.*) and with the same quantities, but during the first 20 mins. the temp. was not allowed to rise. The product was isolated as before and the crude glucoside (3 g.), dissolved in abs. EtOH (125 c.c.), was mixed with KOH (2 g.) in abs. EtOH (45 c.c.) and kept for 24 hrs. in an atmosphere of coal-gas. This solution is designated (A).

Cyanenin Chloride (I; R = H).—KOH (2·8 g.) in abs. EtOH (38 c.c.) was added to a hot abs. alc. solution of ω : 3 : 4-triacetoxyacetophenone (5 g.) and after the formation of the greenish-yellow Na salt HCl was introduced until the solution was acid. The solution (A) was mixed with anhyd. $MgSO_4$ and likewise acidified with HCl at 0°. The solutions were then mixed and saturated with HCl below 0°. After 24 hrs. the filtered solution was poured into much dry Et_2O, and the ppt. collected (0·25 g.); a further equal quantity was obtained by extracting the $MgSO_4$ with EtOH and precipitating the filtered solution with Et_2O. The crude cyanenin chloride (1·9 g.) was dissolved in hot 0·5% HCl aq. (30 c.c.), and cold sat. aq. picric acid (30 c.c.) added. The gelatinous deposit was collected at 0° (0·5 g.) and the filtrate was evaporated at room temp. in vac. over H_2SO_4; a cryst. solid (0·35 g.) was thus isolated. The crude picrate (0·5 g.) was well washed with Et_2O and dissolved in hot cold-saturated alc. picric acid; on cooling, a red cryst. powder separated. The analytical results showed that this specimen consisted of cyanenin picrate crystallised along with about 14% of picric acid. It was dissolved in MeOH and pptd. with Et_2O (Found in material dried in a high vac. at 100° : C, 47·6; H, 3·2; N, 6·8. $C_{27}H_{23}O_{18}N_3$ requires C, 47·9; H, 3·4; N, 6·2%), but it still contained traces of picric acid. The derivative (1·15 g.) was converted into chloride (0·9 g.) in the usual manner and the product was dissolved in the minimum of 1% methyl-alc. HCl. The conc. of HCl was increased to 4·2% by addition of 24·6% methyl-alc. HCl and after being kept for 3 days at 0° the dark greenish-brown, almost black, cryst. aggregates (indefinite prisms) were collected. Further fractions obtained from the mother-liquor could not be purified. The *cyanenin chloride* crystallised from 4% HCl aq. in prismatic needles exhibiting a weak green lustre (Found in air-dried material : C, 45·4; H, 4·9; Cl, 6·3. $C_{21}H_{21}O_{11}Cl,4H_2O$ requires C, 45·3; H, 5·2; Cl, 6·4%). Cyanenin chloride dissolves in Na_2CO_3 aq. to a bright bluish-green solution, and in faintly acid dil. solutions it is rapidly oxidised by ferric salts. These properties constitute a sharp distinction from chrysanthemin chloride.

The distribution number was found to be 22·8 [5·102 mg. in 25 c.c. each of equilibrated *iso*amyl alcohol and 0·5% HCl aq. The quantities of oenin chloride mentioned in the tables (J., 1931, 2720) were dissolved in 50 c.c. of the mixed solvents and not in 50 c.c. of each solvent].

Under the conditions prescribed by Robertson and Robinson (*Biochem. J.*, 1929, **23**, 35) the following colour reactions were observed in a series of solutions of graded p_H: (1) rose, fading rather slowly; (3) cherry-red, fading rapidly, almost colourless in 1 min.; (5) bright reddish-violet, fading more slowly than (3); (7) bluer reddish-violet; (9) a little bluer; (11) a little bluer; (13) blue-violet; (15) blue without violet tinge. After 2 hrs.: (1), (3), and (5) faint pink; (7), (9), and (11) reddish-violet; (13) violet; (15) colourless. After 5 hrs.: (1), (3), (5), and (15) colourless; (7) almost colourless; (9), (11) reddish-violet; (13) violet. After 24 hrs., only (11) was weak reddish-violet and the colourless solutions gave no recovery of pyrylium salt on acidification.

Cyanin Chloride.—The following exp. is only put forward as a preliminary indication, but the results of it convinced us that cyanin had actually been obtained.

ω-*O*-Tetra-acetyl-β-glucosidoxy-3 : 4-diacetoxyacetophenone (3 g., m. p. 144°) was dissolved in abs. EtOH (50 c.c.) and hydrolysed with KOH (1·7 g.) in EtOH (28 c.c.); the solution (A) was prepared on one-third of the usual scale. Both solutions were acidified with dry HCl at 0°, then mixed, anhyd. $MgSO_4$ added, and the whole saturated with HCl at 0°. After 12 hrs. the filtered solution was added to Et_2O (700 c.c.), and the reddish-violet gelatinous ppt. collected (0·1 g.); a further quantity (0·25 g.), less rich in anthocyanin, was recovered from the $MgSO_4$ by solution in hot abs. EtOH and pptn. with Et_2O. This crude material (0·35 g.) was dissolved in 0·5% HCl aq. (5 c.c.) and the conc. of HCl increased to 6% by the addition of conc. acid (2 c.c.); after 2 days a small cryst. deposit was collected, consisting essentially of cyanin chloride. In order to effect comparisons with the natural anthocyanin the whole product (brownish-violet crystals with green metallic glance) was dissolved in hot 0·5% HCl aq., and a test of a small portion with Et_2O and picric acid showed that anthocyanidin was absent. The distribution with *iso*amyl alcohol, however, appeared to indicate the presence of some monoglucoside, and in order to remove this the solution was extracted twice with *n*-butyl alcohol and then several times with *iso*amyl alcohol; the distribution number with the latter solvent now appeared to be very low and indeed lower than that of a specimen of cyanin chloride.

The latter was kindly supplied by Geheimrat Professor R. Willstätter, to whom we are deeply indebted, and the pigment had been isolated from dahlia flowers. Although very finely cryst., this specimen also contained some monoglucoside (or rhamnoglucoside) and accordingly a solution of the natural colouring matter in 0·5% HCl aq. was also washed with *n*-butyl alcohol and with *iso*amyl alcohol. The synthetic and the natural solution were then made of the same conc. (perfect match in the colorimeter) by addition of 0·5% HCl aq., saturated with *iso*amyl alcohol, to the former.

Equal vols. of the solutions were again extracted with *iso*amyl alcohol and the colorimetric identity was confirmed. The distribution ratio (*n*-butyl alcohol) of the pigments was proved to be identical by shaking each solution with *n*-butyl alcohol and comparing the filtered butyl-alcoholic layers. The intensity of the colour was identical in the two cases. A second and a third shaking gave the same results. Further equal volumes of the two solutions were gradually mixed with AcONa aq., with Na_2CO_3 aq., and with NaOH aq. In this way the effect of every variation in p_H of the solutions was noted and again no divergences were observed as the colour changed from red to reddish-violet, bluish-violet, pure blue, greenish-blue, green, and yellow.

Again the rate of oxidation by means of $FeCl_3$ aq. was noted after dilution and the results were identical. Other tests of a similar nature, including rate of pseudo-base formation, were carried out and the behaviour of the solutions was not divergent in any respect.

Malvenin Chloride (II).—The following expts. were carried out in collaboration with Mr. Daniel Sanroma of the Laboratorio de Quimica Organica de la Junta de Ampliacion de Estudios, Madrid. The solution (A) was mixed with one of ω-hydroxy-4-acetoxy-3 : 5-dimethoxyacetophenone (1·05 g.) in abs. EtOH (20 c.c.) and anhyd. Et_2O (40 c.c.). After the addition of anhyd. $MgSO_4$ the solution was saturated with HCl at 0°. Next day the filtered liquid was poured into much Et_2O, and the chocolate-red ppt. collected and dried (0·2 g.). The crude *malvenin chloride* (1·5 g.) was converted into picrate by solution in 0·5% HCl aq. (50 c.c.) and addition of cold-saturated aq. picric acid (50 c.c.). The brown-red ppt. (0·45 g.) was collected after 24 hrs. and a further quantity could be obtained from the mother-liquor by addition of solid picric acid, by gentle heating, filtration, and long keeping in the ice-chest; this material contained much picric acid. The whole product (1·8 g.) was triturated with dry Et_2O, collected (0·9 g.), and crystallised from aq.-alc. picric acid, forming dark chocolate, prismatic needles, and again by the addition of Et_2O to a solution in MeOH (Found in material dried in a high vac. at 100° : C, 48·1; H, 3·6; N, 5·9. $C_{29}H_{27}O_{19}N_3$ requires C, 48·3; H, 3·7; N, 5·8%).

A solution of the picrate (0·5 g.) in 5% methyl-alc. HCl (35 c.c.) was added to dry Et_2O (200 c.c.), the brownish-red powder (0·35 g.) was collected and dissolved in 1% methyl-alc. HCl (acid conc. later increased to 4·2%), and the solution kept in Et_2O vapour. After 24 hrs. the semi-cryst., dark violet deposit was collected (0·14 g.) (satisfactory value for C; 1% low in Cl content). Malvenin chloride is very difficult to crystallise; it separated, on slow evaporation of a solution in EtOH–8% HCl aq., in dark brownish-violet, opaque, cryst. aggregates (Found in air-dried material: C, 48·7; H, 4·5; Cl, 6·2. $C_{23}H_{25}O_{12}Cl,2H_2O$ requires C, 48·9; H, 4·4; Cl, 6·3%). The solution of the pigment in Na_2CO_3 aq. is bright greenish-blue and, in comparison with œnin chloride, oxidation in dil. solution in presence of $FeCl_3$ is very rapid. The distribution number (4·75 mg. in 25 c.c. each of equilibrated *iso*amyl alcohol and 0·5% HCl aq.) was 8·3.

The colour reactions in a range of buffered solutions (*loc. cit.*) were the following: (1) bluish salmon-red; (3) fading cherry-red; (5) reddish-violet, rapidly fading and becoming more orange; (7) violet; (9) bluish-violet; (11) blue; (13) greenish-blue; (15). Decolorisation was most rapid in (3) and after 2 hrs. (1), (3), and (5) were colourless, (7) and (9) were pale reddish-violet, (11) was pale greenish-blue, and (13) and (15) were bluish-green. After 24 hrs. all were decolorised and again the free hydroxyl in position 3 was indicated by the failure to recover the pyrylium salt on the addition of an excess of HCl aq.

We wish to thank the Academy of Sciences of Madrid for a Ramsay Memorial Fellowship awarded to one of us.

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[*Received, June 9th*, 1932.]