CCCLXXVIII.—*Experiments on the Synthesis of Anthocyanins. Part XII. Fisetinidin and Luteolinidin Chlorides.*

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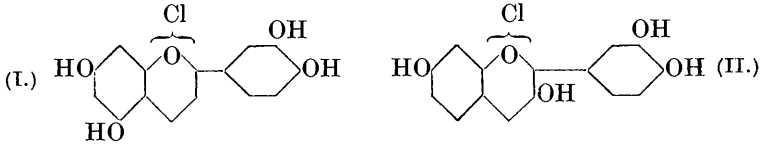
THE object of the work described in this communication was to make a more careful comparison of the colour reactions of fisetinidin and luteolinidin (Pratt and Robinson, J., 1925, **127**, 1128) with those of cyanin chloride and chrysanthemin chloride. Eventually another interest of the work emerged and we describe it in this series in order to complete the evidence for the 3 : 5-diglucoside constitution of pelargonin, cyanin, and malvin.



Luteolinidin (I) is stable to oxidation by means of ferric chloride, but cyanidin and fisetinidin (II) are easily attacked. Fisetinin chloride (see preceding communication), in which position 3 bears the glucose residue, is stable and so is chrysanthemin chloride. Cyanidin 5-glucoside has been prepared in solution by two methods, the first following the pelargonenin synthesis (Part VII) and the second involving partial hydrolysis of cyanin chloride. A distribution test showed that the two methods gave the same substance. This 5-glucoside is unstable to ferric chloride. Cyanin chloride is as

stable as chrysanthemin chloride and fisetinin chloride. Furthermore, malvin is found to be stable and the 5-glucoside of malvidin, which will be described in detail later, is as unstable as malvidin chloride itself. Œnin chloride is stable. Fisetinidin and luteolinidin chlorides were previously obtained by the demethylation of their methyl ethers (*loc. cit.*) so that it is improbable that the salts were quite homogeneous. We have now prepared these substances by applications of the newer method utilising hydroxy-aldehydic and hydroxy-ketonic components in which the hydroxyl groups are either unprotected or acylated.

EXPERIMENTAL.

Some Derivatives of β-Resorcylaldehyde.—The Schotten–Baumann benzoylation of β-resorcylaldehyde under conditions similar to those employed for the preparation of *O*-benzoylphloroglucinaldehyde (see Part VII) yields as main product a substance that crystallises from alcohol, benzene or water in colourless needles, m. p. 103° (Found: C, 69·6; H, 4·2. $C_{14}H_{10}O_4$ requires C, 69·4; H, 4·1%). On methylation by means of methyl iodide and potassium carbonate in boiling acetone solution, it yields a *methyl* ether, which crystallises from methyl alcohol in colourless needles, m. p. 85—86° (Found: C, 70·2; H, 4·8. $C_{15}H_{12}O_4$ requires C, 70·3; H, 4·7%). Hydrolysis of this benzoyloxymethoxybenzaldehyde by means of aqueous-alcoholic sodium hydroxide furnishes the known 4-hydroxy-2-methoxybenzaldehyde, m. p. 154—155° (compare Gattermann and Kölner, *Ber.*, 1899, **32**, 278). Consequently the benzoylation of β-resorcylaldehyde affects the hydroxyl in the *p*-position to the formyl group, and this is in contrast to the behaviour of phloroglucinaldehyde, which is benzoylated in the *o*-situated hydroxyl group.

3 : 4-*Diacetoxyacetophenone.*—The most convenient method of preparation is one that depends on the reduction of the ω-chloro-derivative, but we have been unable to follow Dreczowski's method (*Ber.*, 1909, **42**, 4651) using zinc dust and hydrochloric acid with satisfactory results. The following process is the result of a considerable number of trials. ω-Chloro-3 : 4-diacetoxyacetophenone (10 g.) was mixed with iron filings (10 g.), sodium iodide (1 g.), ethyl alcohol (90 c.c.), and 10% sulphuric acid (100 c.c.), and the whole continuously stirred at 40—50° during 1 hour. The warm solution was filtered and extracted with ether. The crude product was re-acetylated by heating on the steam-bath for 2 hours with acetic anhydride (50 c.c.) and sodium acetate (2 g.) and isolated in the usual way (yield 5·5 g.; m. p. 86—87°; after crystallisation from acetic acid, m. p. 87—88°).

5-O-*Benzoyl-luteolinidin Chloride.*—A solution of *O*-benzoylphloro-

glucinaldehyde (7·3 g.) and 3 : 4-diacetoxyacetophenone (6·0 g.) in dry ethyl acetate (200 c.c.) was saturated with hydrogen chloride at 0° during 2 hours. After 48 hours the red crystalline solid was collected (8·35 g.) and dilution of the filtrate with ether afforded an amorphous, dark red precipitate (1·45 g.), also substantially the desired substance. The crude product was dissolved in 94% alcohol, and hydrogen chloride introduced. On cooling, the *benzoyl-luteolinidin chloride* separated in crimson crystals (decomp. 182°) (Found : C, 61·2; H, 4·0. $C_{22}H_{15}O_6Cl,H_2O$ requires C, 61·6; H, 3·9%). The scarlet alcoholic solution of this salt gives with ferric chloride a dark red colour which becomes violet on the addition of water. The solution in aqueous sodium carbonate is pure blue, but in a few seconds the tone becomes more and more violet.

Luteolinidin Chloride (I).—The debenzoylation of the above-described salt was effected in the usual manner by means of 8% sodium hydroxide solution in the cold in a neutral atmosphere and by subsequent acidification of the solution. The crude product was boiled with saturated aqueous picric acid, and the resulting chocolate-brown powder crystallised twice from alcohol containing a little picric acid (Found : C, 49·7; H, 2·9. $C_{21}H_{13}O_{12}N_3,0·5H_2O$ requires C, 49·6; H, 2·7%) (decomp. above 210°). This crystallised picrate was transformed into the chloride in the usual way; the chloride was crystallised from ethyl alcohol containing hydrogen chloride and, although it was sparingly soluble, separation did not occur readily and it was necessary to concentrate the solution by distillation (Found : C, 54·1; H, 4·4. $C_{15}H_{11}O_5Cl,1·5H_2O$ requires C, 54·0; H, 4·2%).

The colour reactions (Robertson and Robinson, *Biochem. J.*, 1929, **23**, 35) in a range of buffered solutions were the following : (a) 1% hydrochloric acid, (b) 20% hydrochloric acid; the other solutions correspond to the numbers in the original paper (*loc. cit.*). (a) Golden-yellow; (b) yellow : great divergence from (a); (1) rose-red; (2) the same, a little deeper; (3) the same, a little deeper; (4) rose-red with a little violet tone; (5)—(10) the same, a little deeper in each case; (11), (12), (13), (14), (15) light Bordeaux-red.

After ½ hour : (1), (2), (3), (4), (5), (6), (7), (8), (9) form a scale decreasing in violet (permanganate) tone; (10), (11), (12), (13), (14), (15) are bright light red and do not differ.

After 4 hours : (a) and (b) no change; (1), (2), (3), (4), (5), (6) contain precipitated colour-base and the solution is almost decolorised; (7), (8), (9) are increasing red-violet (permanganate); (7) has a little precipitate; (10), (11), (12), (13), (14), (15) are unchanged.

After 20 hours : (a) Golden-yellow with a little precipitate; (b)

golden-yellow, a little weaker; (1), (2), (3), (4), (5), (6) colour-base; (7) red-violet (permanganate) with some precipitate; (8) deeper red-violet; (9) deeper red-violet; (10), (11), (12), (13), (14), (15) bright, light red.

Fisetinidin Chloride (II).—A slow stream of hydrogen chloride was passed for 1 hour through an ice-cold solution of β-resorcylaldehyde (3 g.) and ω : 3 : 4-triacetoxyacetophenone (6 g.) in ethyl acetate (85 c.c.); after 5 days the crystalline precipitate (7·2 g.) was collected. The crude product (5 g.) was recrystallised from 0·5% hydrochloric acid (400 c.c.) (2·25 g. separated) and then from aqueous-alcoholic hydrochloric acid by concentration of the solution. The red crystals had a green lustre (Found: C, 55·4; H, 4·3. $C_{15}H_{11}O_5Cl,H_2O$ requires C, 55·5; H, 4·0%). An alcoholic solution develops a pure blue coloration on the addition of potassium acetate, and other colour-reactions were observed in the standard manner (Robertson and Robinson, *loc. cit.*). Dilute methyl-alcoholic solutions of fisetinidin are more violet than those of cyanidin, which preserve a redder shade.

(a) Red-orange; (b) weaker red-orange (cyanidin is much bluer red); (1) rose, weaker and fading more quickly than cyanidin; (2) a little bluer; (3) cherry-red, fading; (4) still bluer (cyanidin deposits colour base at this concentration); (5) violet-red; (6) darker violet-red; (7) the same; (8) similar to (6) but fisetinidin is now brighter than cyanidin; (9) similar; (10) very bright red-violet (cyanidin is blue-violet); (11) a little bluer; (12) deeper, similar colour; (13) violet; (14) sharp change to violet-blue; (15) blue, bright tone. After 20 hours: (a) and (b) identical orange-red (cyanidin still red); (1) weak violet with precipitate of colour base; (2) similar, a little redder; (3), (4), and (5) violet-red with little colour-base (cyanidin gives ample precipitate); (6) and (7) red-violet, no precipitate; (8) deeper violet; (9) still more intense violet; (10), (11), (12), and (13) identical, somewhat more intense violet; (14) pale salmon-pink; (15) the same (cyanidin is yellow).

Comparison of Some Anthocyanidins and Anthocyanins related to Cyanidin and Malvidin in Respect of their Stability to Ferric Chloride.—The 5-β-glucosidylmalvidin chloride and 5-β-glucosidylcyanidin chloride used in this comparison were obtained by applying the methods of the pelargonenin synthesis (Part VII, p. 2695), the ω : 4-dihydroxyacetophenone being replaced by ω : 4-dihydroxy-3 : 5-dimethoxyacetophenone (Bradley, Robinson, and Schwarzenbach, J., 1930, 793) and ω : 3 : 4-trihydroxyacetophenone, respectively. Both anthocyanins will be described in greater detail in a subsequent communication; they were purified through their picrates and were quite free from anthocyanidin, as was proved by the fact that ether

extracted nothing from an aqueous solution containing picric acid. In addition the 5-β-glucosidylcyanidin chloride (cyanenin chloride) has been obtained *in solution* by partial hydrolysis of cyanin chloride.

The difficulty in effecting this operation is that pure cyanin chloride is sparingly soluble even in hot hydrochloric acid of moderate concentration. An attempt to overcome this by using *crude* cyanin chloride from the cornflower (a specimen was used for which, and for the pure specimen, we are indebted to Professor Willstätter) gave certainly a monoglucoside, but, owing to the presence of bluer impurities, no colour match with the synthetic material was possible. Cyanin (0·5 g., well crystallised; from deep red dahlia flowers) was dissolved in hot 0·5% hydrochloric acid (500 c.c.) and heated on the steam-bath; hydrochloric acid (100 c.c., *d* 1·16) was then gradually added during 15 minutes, and the mixture heated for 30 minutes longer. The coloured salts, it was found on trial, then distributed themselves between the acid and amyl alcohol. The solution was diluted to 1500 c.c., saturated with picric acid, and extracted with *cyclo*hexanone (500 c.c.), further picric acid (25 g.) being added. The *cyclo*hexanone solution was separated, filtered through a large folded filter moistened with *cyclo*hexanone, shaken with 0·5% hydrochloric acid (100 c.c.), and diluted with light petroleum (2000 c.c.). The filtered aqueous acid solution was then extracted with ether containing picric acid until no further cyanidin picrate was extracted. Ether was removed from the residual aqueous solution by boiling it under diminished pressure and it was then extracted with several small volumes of diethyl ketone with the addition of picric acid in each case. The diethyl ketone extracts were separated, filtered through a filter moistened with diethyl ketone, and diluted with much benzene, and the monoglucoside taken up in 0·5% hydrochloric acid. The aqueous solution was repeatedly washed with benzene and then filtered. It had a cyanin-red colour, perhaps a shade bluer, and was entirely free from cyanidin. Judging from the distribution, it contained the pure monoglucoside. A comparison with the synthetic specimen was made in the following manner. A portion of the solution was extracted with *n*-butyl alcohol, previously brought into equilibrium with 0·5% hydrochloric acid, and it was found that the distribution number was very high. The solution was made up to 50 c.c. with the aqueous acid–butyl alcohol and matched colorimetrically with a similar solution prepared from the synthetic material; the amount of the latter available made 27·5 c.c. of solution. Four portions of 6 c.c. were measured from a burette in each case, and carefully measured volumes of benzene added (1 c.c., 2 c.c., 3 c.c., 4 c.c. to each series). Hydrochloric acid (6 c.c. of 0·5%) was then added to each of the 8

tubes and the contents were shaken; the agreement of the distribution in the two series was complete. The 5-glucoside gives a beautiful pure blue coloration with sodium carbonate and since the product of the hydrolysis of cyanin gives as bright and blue a shade as cyanin itself it is necessary to conclude that chrysanthemin is not a product of the partial hydrolysis. This is the only argument with which we are acquainted which supports the 5-bioside view of the structure of cyanin as against the 3 : 5-dimonoside hypothesis, but it is insufficient to influence our judgment, since all the other evidence points to the 3 : 5-dimonoside structure.

The test with ferric chloride was carried out exactly as described for the derivatives of pelargonidin type in Part VII (p. 2697). In the cyanidin series, cyanin chloride (2·25 mg.) was dissolved in 0·5% hydrochloric acid (50 c.c.); this constituted the standard and the other solutions were roughly matched with it (the 5-glucoside from the hydrolysis was independently tested on another occasion and gave the same result). Three minutes after the solutions were mixed, the synthetic 5-glucoside was destroyed; in 10 minutes, fisetinidin and cyanidin followed suit. Cyanin, fisetinin, and chrysanthemin were slowly oxidised at about the same rate; in 1 hour 35 minutes the colour was nearly discharged. Luteolinidin was considerably more stable and the colour lasted for a few hours. The anthocyanins of the pelargonidin and malvidin series are much more stable than the cyanidin-type derivatives, so that, in the latter case, it appears that oxidation and break-up of the molecule may be initiated at the vicinal hydroxyls of the catechol nucleus. Even here, however, the effect of the hydroxyl in position 3 is very striking and evidently cyanin does not contain this group. Mecocyanin and idæin (synthetic) show the same behaviour in this test as cyanin. In the malvidin series somewhat more dilute solutions were employed (about 1·25 mg. of œnin chloride in 50 c.c.) and malvidin and its 5-glucoside were destroyed in 5—10 minutes; malvin, œnin and reso-œnin (5-deoxyœnin) remained unaffected for 3 days and the colour gradually faded in the course of 10 days to a weak bluish-pink. In our opinion this remarkable series of consistent contrasts demands the acceptance of the view that pelargonin, peonin, cyanin, and malvin are similarly constituted and do not contain a free hydroxyl in position 3. For other reasons we know that position 5 carries a glucose residue.

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

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