

### 326. *Experiments on the Synthesis of Anthocyanins.* *Part XV. A Synthesis of Hirsutin Chloride.*

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IN 1927 Karrer and Widmer (*Helv. Chim. Acta*, **10**, 758) encountered a new diglucoside of a delphinidin trimethyl ether in the flowers of *Primula hirsuta* and found that the related anthocyanidin could be oxidised to syringic acid. Hirsutidin was thus recognised as 5- or 7-*O*-methylmalvidin. These anthocyanidins, as well as 3-*O*-methylmalvidin, were synthesised (Bradley, Robinson, and Schwarzenbach, *J.*, 1930, 793) and it transpired that hirsutidin is the 7 : 3' : 5'-trimethyl ether of delphinidin.

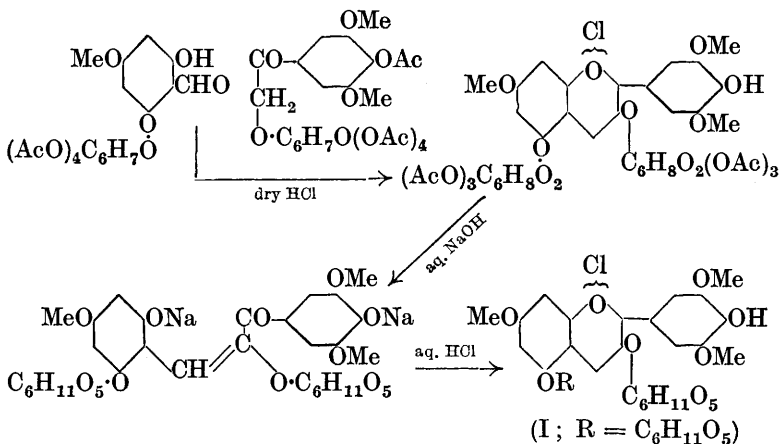
Hirsutin itself was then clearly a 3-bioside, 5-bioside, or 3 : 5-diglucoside of hirsutidin, the phenolic hydroxyl at position 4' being excluded as a possible position of attachment of a sugar residue by analogy with other anthocyanins, by the fact that hirsutin gives a violet colour-base, and by the formation of syringic acid from hirsutone by hydrolysis with alkalis (Karrer and Widmer, *loc. cit.*). Hirsutone,  $C_{30}H_{38}O_{19}$ , is a characteristic product derived from hirsutin by the action of hydrogen peroxide; its composition is that of hirsutin pseudo-base plus one atom of oxygen and thus no sugar residue is detached in the oxidation process.

Hirsutidin 5- $\beta$ -lactoside (Levy and Robinson, *J.*, 1931, 2738) does not resemble hirsutin very closely. The 3-bioside formula was put forward by Karrer and Widmer as a probable representation of the constitution of hirsutin and in order to test this view we synthesised *hirsutidin* 3- $\beta$ -glucoside (I; R = H) by combining the methods of the synthesis of cœnin (Levy, Posternack, and Robinson, *J.*, 1931, 2701) and of hirsutidin (*loc. cit.*). The reactions of this monoglucoside are not similar to those of hirsutin.

The 3 : 5-diglucoside possibility remained and the view that hirsutin chloride has the formula (I; R =  $C_6H_{11}O_5$ ) was attractive in that it brought this anthocyanin into line with pelargonin, cyanin, and malvin (see Parts VII—XII of this series). The synthesis of the anthocyanin, the first to be recorded of a naturally occurring

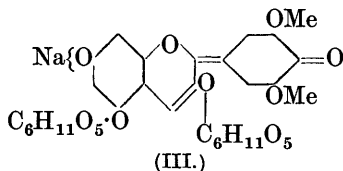
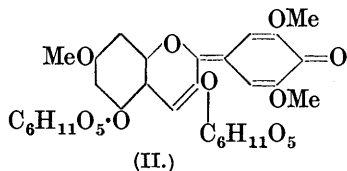
diglucoside of this group, has confirmed the correctness of these deductions.

The synthesis proceeds in accordance with the scheme :

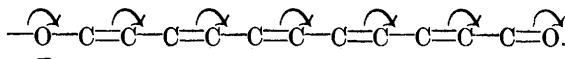


The synthetical product was identical in all respects with a specimen of hirsutin chloride which was provided by Professor P. Karrer, to whom we are greatly indebted.

Hirsutin is unique among anthocyanins in that it contains but a single phenolic hydroxyl group; it thus yields a violet colour-base (II) but no blue alkali-salt of this quinone. This example shows that in the anthocyanin series the quinonoid group *per se* does not lead to the development of pure blue colorations with alkalis; a salt-forming auxochromic group is necessary in addition. This occurs, for example, in malvin, the formula (III) representing the condition of the substance in the blue solutions.



The probable explanation is that the charge of the anion in (III) is shared by the oxygen atoms in positions 7 and 4', the circumstances being entirely analogous to those of the carboxylic ion. The groups (C=O) and (O<sup>-</sup>) in (CO<sub>2</sub><sup>-</sup>) are here separated by five ethenoid units and the transmission of the charge involves the electromeric changes



## EXPERIMENTAL.

**3 : 5-Di-(O-tetra-acetyl- $\beta$ -glucosidoxy)-4'-acetoxy-7 : 3' : 5'-trimethoxyflavylium Chloride.**—A solution of 2-O-tetra-acetyl- $\beta$ -glucosidyl-4-O-methylphloroglucin-aldehyde (2.3 g.) (Karrer, Lichtenstein, and Helfenstein, *Helv. Chim. Acta*, 1929, 12, 991) and  $\omega$ -O-tetra-acetyl- $\beta$ -glucosidoxy-4-acetoxy-3 : 5-dimethoxy-acetophenone (2.8 g.) (Levy, Posternack, and Robinson, J., 1931, 2710) in dry AcOEt (40 c.c.) was saturated at 0° with dry HCl; it became deep red within 1 hr. and was kept at 0° for 48 hrs. Dry Et<sub>2</sub>O (600 c.c.) then pptd. a dark red powder, which was washed with Et<sub>2</sub>O and dried (yield, 2.4 g. or 48%).

The substance sintered at 75° and decomposed at 125—128°; it dissolved in MeOH to a reddish-violet solution, which changed to a fine blue on addition of a drop of Na<sub>2</sub>CO<sub>3</sub> aq., the original colour being restored on acidification. This behaviour suggests that the substance has lost the acetyl group in the 4'-position. The analytical data (Found: C, 51.9; H, 5.1; Cl, 3.5; MeO, 8.8%) indicate de-acetylation and hydration.

**Hirsutin Chloride** (I; R = C<sub>6</sub>H<sub>11</sub>O<sub>5</sub>).—The above flavylium salt (2.3 g.) dissolved in cold 10% NaOH aq. (20 c.c.) to a greenish-brown solution, which was kept under H for 1½ hrs. 20% HCl aq. (15.5 c.c.) was then added and the 2% acid solution thus obtained was warmed to 60° to complete formation of the pyrylium salt and allowed to cool. After 24 hrs. the deep red-violet solution deposited a felted mass of deep red needles with a dark green metallic lustre (1.6 g.). Crystn. of the crude chloride (1.1 g.) is sometimes attended with considerable difficulty and the best results were obtained when it was dissolved in hot 1% HCl aq. (13 c.c.), and 5% methyl-alc. HCl (7 c.c.) added. On cooling, the solution was filled with a mass of felted needles (0.9 g.) (Found in material dried in a desiccator over H<sub>2</sub>SO<sub>4</sub>: C, 46.4; H, 6.0; Cl, 4.7; MeO, 11.7; loss at 110°, 10.0. C<sub>30</sub>H<sub>37</sub>O<sub>17</sub>Cl.4H<sub>2</sub>O requires C, 46.4; H, 5.8; Cl, 4.6; 3MeO, 12.0; 4H<sub>2</sub>O, 9.3%). The material dried at 110° in a high vac. over P<sub>2</sub>O<sub>5</sub> gave figures [Found: C, 51.8; H, 5.4; Cl, 4.3; MeO, 12.8. C<sub>30</sub>H<sub>37</sub>O<sub>17</sub>Cl (*M*, 705) requires C, 51.1; H, 5.3; Cl, 5.0; 3MeO, 13.1%] which indicated some loss of HCl on heating. The value C, 51.75 gives *M*, 696, so that the discrepancy is 8.5 units, corresponding to a loss of 24% of the Cl contained in the hydrated salt. The obs. and the calc. values for the percentage of Cl give the loss of Cl as 15.2%. Assuming that the loss is 19%, the calc. values are C, 51.7; H, 5.3; Cl, 4.1; 3MeO, 13.3; total loss on drying, 10.2%, in good agreement with experimental results already cited.

The salt had m. p. 150—153° (decomp.) after sintering at about 145°. In all superficial respects its properties were not to be distinguished from those of the specimen of natural origin and these include the appearance in mass, deep brown-violet with weak lustre, the reddish-violet smear on paper, the violet-tinged deep red solutions in MeOH, the more orange-red solutions in hot dil. HCl aq. (still much bluer-red than cyanin solutions), the appearance of the crystals in suspension and under the microscope (short, very slender, opaque needles, deep violet by reflected light), the sparing solubility even in very dil. HCl aq. and the rate of dissolution on heating, the power of crystn., and a large number of colour reactions and conditions of formation of the pseudo-base.

The colour reactions are quite characteristic; AcONa or Na<sub>2</sub>CO<sub>3</sub> added to an acid solution gives a reddish-violet coloration due to the colour-base (II) and in the former case the solution is rapidly decolorised; Na<sub>2</sub>CO<sub>3</sub> also causes

pseudo-base formation, but the solution subsequently becomes greenish-yellow. The action of NaOH is to give momentarily a violet colour, rapidly changing to yellowish-green. In all cases the pyrylium salt can be reconstituted by the addition of HCl. The distribution number (*isoamyl alcohol*) appears to be almost zero, but the pigment is extracted to a small extent from its aq. solution by *n*-butyl alcohol.

*Standard Method for the Determination of the Distribution Ratio (n-Butyl Alcohol) in the Case of Diglycosidic Anthocyanins.*—Pure *n*-butyl alcohol (1150 c.c., b. p. 116.5°) was shaken (20°) with 0.5% HCl aq. (230 c.c.), the aq. layer removed, and the liquid shaken with a further quantity (115 c.c.) of the 0.5% acid (aq. layer, 127 c.c., discarded); 0.5% HCl aq. (1000 c.c.) was shaken with the prepared butyl alcohol (250 c.c.) and the layers were separated, the butyl alcohol being returned to the stock of prepared solvent. Finally, equal vols. of the prepared butyl alcohol and prepared 0.5% HCl aq. were shaken together and the separated and filtered solutions were then employed.

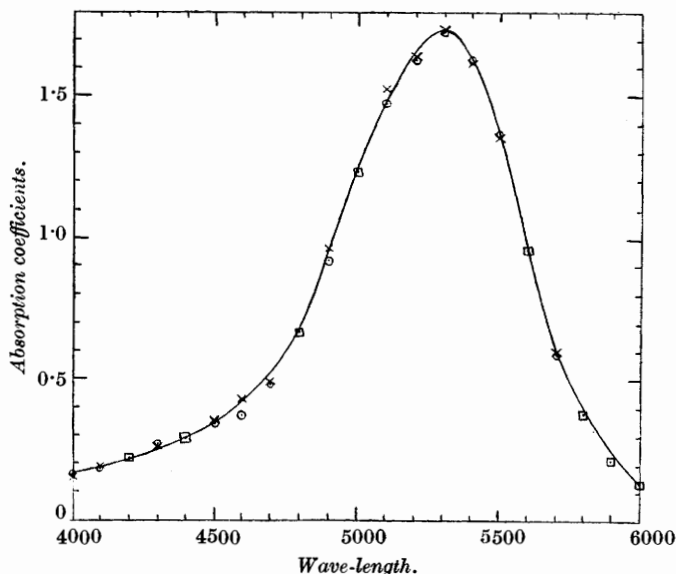
A weighed sample of the anthocyanin (about 3.0 mg.) was shaken with 25 c.c. of each solution until completely dissolved; the liquids were then separated and the upper layer was filtered. The aq. layer (5.0 c.c., carefully measured from a burette) was mixed with the prepared butyl alcohol (10 c.c.) and made up to 25 c.c. by means of EtOH (containing 1 c.c. of conc. HCl aq. in 1 l.); similarly the butyl-alc. layer (10 c.c.) was mixed with prepared 0.5% HCl aq. (5 c.c.) and made up to 25 c.c. by means of acidified EtOH. If the length (mm.) of a column of the first solution matching one of 50 mm. of the second solution is *l*, the distribution number is  $100l/(100 + l)$ . In this way the distribution number (butyl alcohol) for natural hirsutin chloride was found to be 11.4; 11.3, and for the synthetic diglucoside, 11.3; 11.3.

*Absorption in the Visible Region* (see Fig.).—The observations were made with solutions of the anthocyanins (1.30 mg.) in 0.1% methyl-alc. HCl (100 c.c.) and no significant divergences between the natural and the synthetic specimens were noted.

*3-β-Glucosidylhirsutidin Chloride* (I; R = H).—A solution of 2-*O*-benzoyl-4-*O*-methylphloroglucinaldehyde (0.9 g.) (Bradley, Robinson, and Schwarzenbach, *loc. cit.*; Levy and Robinson, J., 1931, 2738) and *ω*-*O*-tetra-acetyl-β-glucosidoxy-4-acetoxy-3 : 5-dimethoxyacetophenone (2.2 g.) in dry AcOEt (25 c.c.) was saturated at 0° with HCl and kept for 48 hrs. at 0°, and the dark red product pptd. by Et<sub>2</sub>O (500 c.c.), washed with Et<sub>2</sub>O, and dried in a desiccator (yield, 0.7 g. or 33%) (Found: C, 57.4; H, 4.9; Cl, 3.5; MeO, 12.3, 12.7. C<sub>41</sub>H<sub>41</sub>O<sub>18</sub>Cl requires C, 57.4; H, 4.8; Cl, 4.1; 3MeO, 10.9%). The analyses indicate partial loss of acetyl groups and HCl with compensating hydration.

The crude acetylated flavylium salt glucoside (0.65 g.) slowly dissolved in 8% NaOH aq. (6 c.c.) under H and after 3 hrs. 20% HCl aq. (3.28 c.c.) was added to the dark greenish liquid. The red flavylium salt was produced and the reaction was completed by heating to 60°; on cooling and keeping for 12 hrs., the glucoside mixed with benzoic acid separated and the solids were washed with 2% HCl aq., dried, washed with Et<sub>2</sub>O, and again dried (0.35 g.). The small, hard, cryst. aggregates had a fine bronze lustre and gave a bluish-violet smear on porcelain; for recrystn. it was found best to dissolve the material in 1% methyl-alc. HCl (12 c.c.) and ppt. it with Et<sub>2</sub>O. The amorphous salt could then be quickly dissolved in hot 1% methyl-alc. HCl (6 c.c.), and the conc. of HCl increased to 5% in the cold filtered solution. On

keeping, the *chloride* separated in bronze leaflets and prisms with a striking lustre (0.2 g.) (Found: C, 50.7; H, 5.3; Cl, 6.8; MeO, 16.2; loss at 110°, 4.6, 3.7, 7.2.  $C_{24}H_{27}O_{12}Cl \cdot 1.5H_2O$  requires C, 50.6; H, 5.3; Cl, 6.2; 3MeO, 16.3;  $1.5H_2O$ , 4.7%. Found in material dried at 110° in a high vac. over  $P_2O_5$ : C, 53.3; H, 5.1; Cl, 6.4; MeO, 16.8.  $C_{24}H_{27}O_{12}Cl$  requires C, 53.1; H, 5.0; Cl, 6.5; 3MeO, 17.1%). The solution in  $Na_2CO_3$  aq. is dichroic, being blue in thin layers and violet in thick layers; on keeping, the solution becomes green: the same changes occur more rapidly on the addition of NaOH aq. AcONa added to an acid aq. solution develops a reddish-violet coloration and on standing the colourless pseudo-base is produced; recovery of the flavylum salt on acidification with HCl is quantitative.



○ *Hirsutin chloride* (natural).      × *Hirsutin chloride* (synthetic).  
 □ Coincident points.

The distribution number (equilibrated *isoamyl alcohol* and 0.5% HCl aq.) (5.005 mg. in 50 c.c. of the mixed solvents) was 9.1. Cenin chloride at this conc. (5.007 mg.) had distribution number 10.1 (Levy and Robinson, J., 1931, 2720).

In the colour of its acid solutions, general appearance, and in many other properties, the new glucoside resembles cenin chloride, of which it is a methyl ether.

*Colour Reactions in a Range of Buffered Solutions of Definite  $p_H$  and Notes on Co-pigments.*—Under the conditions prescribed by Robertson and Robinson (*Biochem. J.*, 1929, 23, 35) the natural and the synthetic specimens of hirsutin chloride gave identical results: (1) Rose, almost decolorised in 30 secs.; (3) reddish-violet, fading rapidly to a colourless solution; (5) rich violet, fading but less rapidly, becomes redder and is almost colourless after 2 mins.; (7), (9), (11) rich violet, (11) fades to a pale violet, (7) fades much less rapidly,

(9) is the most stable; (13) violet, fading to pale blue in 5 mins.; (15) violet, at once becoming pale blue and then more slowly becoming greenish-blue, greenish-yellow, and colourless in 5 mins.; later a yellow coloration develops.

After about 10 mins. (11), (13), (15) are respectively pale violet, pale blue, pale yellow.

After 24 hrs. : (1) just perceptible pink; (3), (5), (7), (9), (11) colourless; (13) greenish-yellow; (15) yellow. Addition of conc. HCl aq. restores the red coloration due to hirsutin chloride in all cases; this is quite characteristic behaviour, since in the case of most anthocyanins the pigment suffers destruction in the more alkaline solutions employed in these tests.

The methyl-alc. solutions of the natural and the synthetic colouring matter were diluted and on the addition of FeCl<sub>3</sub> a stable orange coloration was produced in both cases; this faded very slowly and was intense after 24 hrs.

The colour of solutions of hirsutin chloride in dil. HCl aq. is deepened and rendered bluer in shade by the addition of many substances (co-pigments: compare Willstätter and Zollinger, *Annalen*, 1916, **412**, 195; Robinson and Robinson, *Biochem. J.*, 1931, **25**, 1687). Thus tannin, 2-hydroxyxanthone glucoside, rutin, quercitrin, vanillin, and quinaldine have been found to exhibit the phenomenon in a similar fashion with the natural and the synthetic specimen. Tannin and quercitrin appear to exert the most powerful action and if an acid solution of hirsutin chloride is boiled and quercitrin added there is little change of colour; on cooling, however, the colour becomes much deeper and changes to a rich violet. The colour is the same as that of acid aq. extracts of deep purple violas and the alternation between red and purple which can be effected by heating and cooling is similar to that observed in the hirsutin-quercitrin solutions.

There is little doubt that the phenomena are basically identical and that the solutions from the violas contain a violanin-flavonol glycoside complex.

On addition of AcONa to the natural and the synthetic co-pigmented hirsutin solutions, the colorations observed were deeper and bluer than that from hirsutin itself. Especially in the presence of tannin, hirsutin gives a deep violet-blue opalescence and coloration with AcONa in dil. solution; the rate of formation of pseudo-base is also greatly diminished. A flower colour based on hirsutin might therefore have a much bluer tone than can be obtained with the pure hirsutin colour-base. The phenomenon accounts for the fact that at certain values of  $p_H$  the violet solutions of hirsutin become bluer, and doubtless the blue shade is due to a combination of the colour-base with decomposition products of the phenolic ketone class.

Hirsutidin 3- $\beta$ -glucoside chloride gave the following: (1) Rose-red, fading but much less rapidly than in the case of hirsutin; (3) cherry-red, fading rapidly and colourless in 30 secs.; (5) reddish-violet, more intense than (3), fading in the course of 5 mins.; (7) blue-violet, fading slowly; (9) stable royal-blue; (11) similar; (13) similar, fading but more slowly than (7); (15) initially royal-blue, rapidly fading to pale blue, pale green and finally yellow. After 24 hrs. : (1) rose; (3), (5), (7) colourless; (9) pale blue; (11) cornflower-blue; (13) greenish-yellow; (15) yellow.

On addition of conc. HCl aq., recovery of anthocyanin occurred in (1), (3), (5), (7), and (9); (11) showed some loss of pigment, (13) was very weakly coloured, (15) was colourless.

The colour of the acid solutions of the monoglucoside is deepened and rendered bluer by the addition of the co-pigments mentioned above. In

this respect its behaviour was found to be closely parallel to that of cœnin chloride. It may be noted that as cœnin, malvin, and hirsutin occur in various *Primulæ*, it seems very probable that this monoglucoside, which bears the relation to hirsutin that cœnin bears to malvin, will be found ultimately in some species of the same genus; a search is in progress.

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