172. *Experiments on the Synthesis of Anthocyanins. Part XVIII. Cyanidin 3-Xyloside and 3 : 7- and 5 : 7-Diglucosides.*

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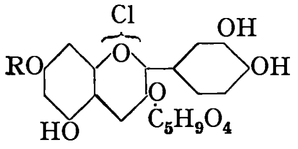
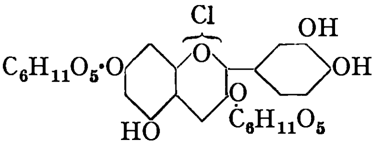
Although pentoseglucosides and, more frequently, methylpentoseglucosides have been recognised among natural anthocyanins, no monopentosides or rhamnosides have yet been isolated. It seemed of interest to examine the properties of a typical xyloside, and cyanidin 3-xyloside (I, R = H) has accordingly been synthesised. The *picrate* was purified and analysed, but an attempt to prepare the chloride from it gave a product, containing cyanidin chloride [compare Part XX (this vol., p. 817) for the similar behaviour of malvidin 3-xyloside] owing to the great tendency of the xyloside to undergo hydrolysis. The similarity in colour reactions of the xyloside and chrysanthemin could, however, be established and the distribution number could be determined. These data may be of service in recognising either the occurrence of the substance in nature or its formation by partial hydrolysis of a more complex saccharide.

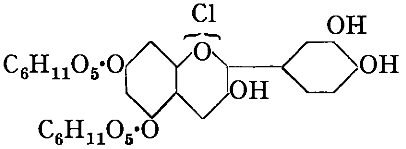
(I.)





(II.)

(III.)



The constitution of cyanin as cyanidin 3 : 5-diglucoside has been proved by synthesis (Part XVII ; J., 1932, 2488), but in order to complete the evidence it was thought desirable to examine the properties of the 3 : 7- and the 5 : 7-diglucoside now described.

The method employed in the former case was the condensation of 2-*O*-benzoyl-4-*O*-β-tetra-acetylglucosidylphloroglucinaldehyde (Part VII; J., 1931, 2672) with ω-tetra-acetyl-β-glucosidoxy-3 : 4-diacetoxyacetophenone (Part VI; J., 1931, 2665) in dry ethyl acetate under the influence of hydrogen chloride, followed by hydrolysis of the product and reconstitution of the flavylium salt.

Cyanidin chloride 3 : 7-*diglucoside* (II) is similar to cyanin in many of its properties, but is nevertheless readily differentiated from that anthocyanin, especially by its colour reactions.

A well-crystallised *cyanidin chloride* 3-*xyloside*-7-*glucoside* (I, R = $C_6H_{11}O_5$) was obtained in a like manner by the use of the appropriate components.

Cyanidin chloride 5 : 7-*diglucoside* (III) was obtained by coupling together the acetylated diglucoside of phloroglucinaldehyde (Robinson and Todd, J., 1932, 2302) and ω : 3 : 4-tri-hydroxyacetophenone in the usual series of reactions. This characteristic substance is even more easily distinguished from cyanin by its properties than is the 3 : 7-isomeride.

Experimental.

ω-O-*Triacetyl*-β-*xylosidoxy*-3 : 4-*diacetoxyacetophenone*.—Dry silver carbonate (15 g.) was added to a solution of ω-hydroxy-3 : 4-diacetoxyacetophenone (6 g., prepared by the method of Part X; J., 1931, 2722) and triacetyl-α-xylosidyl bromide (15 g.) (Levene and Sobotka, *J. Biol. Chem.*, 1925, **65**, 463) in dry benzene (40 c.c.), and the temperature raised to 40° and, after a short time, to 60°, with shaking. After 45 minutes at 60°, the mixture was refluxed for 15 minutes, and the crude xyloside precipitated from the filtered solution by the addition of light petroleum (200 c.c.). The oily material was washed and triturated with cold water and ultimately solidified; it then crystallised from 80% methyl alcohol in slender colourless needles (4·5 g.), m. p. 162° (Found : C, 53·8; H, 5·0. $C_{23}H_{26}O_{13}$ requires C, 54·1; H, 5·1%).

This substance is moderately sparingly soluble in ether, but is readily soluble in most organic solvents with the exception of light petroleum.

3-β-*Xylosidylcyanidin Salts.*—Hydrogen chloride was passed into a solution of ω-*O*-triacetyl-β-xylosidoxy-3 : 4-diacetoxyacetophenone (2·0 g.) and 2-*O*-benzoylphloroglucinaldehyde (1·4 g.) in dry ethyl acetate (100 c.c.) until saturated at 0°. The solution was kept for 3 days in the ice-chest; the flavylium salt then precipitated from the deep red solution by means of ether (1 l.) was collected, washed, and dried (2 g.).

The whole product was dissolved in aqueous sodium hydroxide (30 c.c. of 3%) under nitrogen, after 3 hours hydrochloric acid (35 c.c. of 7%) was introduced, and the solution was heated at 60° for a few minutes and cooled. An equal volume of saturated aqueous picric acid was added, and the solution kept in the ice-chest for several days; the *picrate* was slowly and incompletely precipitated (0·7 g.). The derivative crystallised in minute, dark red needles on the addition of saturated aqueous picric acid to its alcoholic solution (Found: C, 48·5; H, 3·6; N, 6·0. $C_{26}H_{21}O_{17}N_3$ requires C, 48·2; H, 3·3; N, 6·4%). When the twice-crystallised picrate (0·2 g.) was dissolved in 5% methyl-alcoholic hydrogen chloride (10 c.c.), and the chloride precipitated by the addition of ether (100 c.c.), a certain amount of hydrolysis occurred. The salt could not be crystallised and the distribution number (8·55 mg. in 50 c.c. each of previously equilibrated *iso*amyl alcohol and 0·5% hydrochloric acid) was 53·2 for the first shaking and 48·5 for the second shaking. After a considerable interval the experiment was repeated and the distribution number was found to be 48·0 after removal of all cyanidin from a solution (10 mg. in 50 c.c. of 0·5% hydrochloric acid) by means of ether and picric acid, followed by exhaustive washing with benzene and several relatively small volumes of *iso*amyl alcohol. On the addition of sodium chloride the xyloside was completely extracted from 0·5% hydrochloric acid solution by amyl alcohol. Its colour reactions were indistinguishable from those of chrysanthemin.

Cyanidin Chloride 3-*Xyloside*-7-*glucoside*.—A solution of 2-*O*-benzoyl-4-tetra-acetylglucosidylphloroglucinaldehyde (2·4 g.) and ω-*O*-triacetylxylosidoxy-3 : 4-diacetoxyacetophenone (2·8 g.) in dry ethyl acetate (100 c.c.) was saturated with hydrogen chloride and kept at 0° for 3 days. The flavylium salt (2 g.) was precipitated by means of ether (1 l.), collected, and dissolved in aqueous sodium hydroxide (30 c.c. of 8%), air being excluded by nitrogen. After 3 hours (room temperature) hydrochloric acid (35 c.c. of 7%) was added, and the solution heated at 60° for a few minutes, cooled, and washed with *iso*amyl alcohol to remove anthocyanidin and monoside and with ether to remove *iso*amyl alcohol. The acid concentration was increased to 3% (by addition of 20 c.c. of 7% hydrochloric acid) and on keeping in the ice-chest the crude anthocyanin (0·4 g.) gradually crystallised. This material was analysed both when air-dried and when dried at 110° in a vacuum and the results were substantially as cited below. The *salt* was recrystallised in a similar fashion in flaky prismatic needles (Found in air-dried material: C, 46·5; H, 5·3; Cl, 5·6; loss at 110° in a high vacuum over phosphoric oxide, 9·0. Found in material dried as stated: C, 50·9; H, 4·8; Cl, 5·9. $C_{26}H_{29}O_{15}Cl,3H_2O$ requires C, 46·5; H, 5·2; Cl, 5·3; $3H_2O$, 8·1%. $C_{26}H_{29}O_{15}Cl$ requires C, 50·6; H, 4·7; Cl, 5·8%).

The salt is deep chocolate-brown in mass and exhibits a green reflex; viewed by transmitted light, individual crystals have a yellowish-brown colour. It is readily soluble in 0·5% hydrochloric acid to a cyanin-red solution and, as usual, in methyl alcohol to a much bluer-red solution. The colour reactions both with ordinary reagents and in a series of buffered solutions of graded p_H were identical with those described for the 3 : 7-diglucoside (below).

The distribution ratios were very close to those of cyanin itself (*iso*amyl and *n*-butyl alcohols and 0·5% hydrochloric acid). On hydrolysis with boiling 10% hydrochloric acid, cyanidin chloride separated in its characteristic form as slender needles. It is curious that cyanidin chloride is hard to recrystallise in this form.

Cyanidin Chloride 3 : 7-*Di*-β-*glucoside* (II).—The preparation of this substance followed exactly that of the xyloside-glucoside described above, ω-*O*-tetra-acetyl-β-glucosidoxy-3 : 4-diacetoxyacetophenone being used instead of the related acetylated xyloside. Two grams of each of the components gave 1·8 g. of crude benzoylated and acetylated flavylium salt, and the final yield of crude crystalline *cyanidin chloride* 3 : 7-*diglucoside* was 0·4 g. The salt could be recrystallised by solution in hydrochloric acid (20 c.c. of 1%) and addition of stronger acid (20 c.c. of 5%) to the filtered solution (Found in air-dried material: C, 43·3; H, 5·6; Cl, 5·0; loss at 110° over phosphoric oxide in a high vacuum, 14·2, 12·9. Found in anhydrous material: C, 50·3; H, 4·9; Cl, 5·2. $C_{27}H_{31}O_{16}Cl,5{\cdot}5H_2O$ requires C, 43·5; H, 5·6; Cl, 4·8; $5{\cdot}5H_2O$, 13·3%. $C_{27}H_{31}O_{16}Cl$ requires C, 50·1; H, 4·8; Cl, 5·5%).

The salt is chocolate-brown in mass with a weak bronze reflex; it crystallises both in needles and in monoclinic prisms; the crystals are very fragile and appear brown in transmitted light. The distribution number, determined by the standard method using *n*-butyl alcohol (Parts XV and XVII; J., **1932**, 2296, 2492), was 4·4, 4·5, and 4·7 for 5·45, 4·25, and 3·0 mg., respectively, in 50 c.c. of the mixed solvents. Solutions of the diglucoside give a violet-blue coloration on the addition of sodium acetate, and a pure blue with sodium carbonate. This is not greenish in shade as with cyanin, nor, on the other hand, is it violet-toned as with mecocyanin. On the addition of sodium hydroxide a yellow solution is rapidly produced. The colours in a range of buffered solutions were the following (numbers and method corresponding to those given by Robertson and Robinson, *Biochem. J.*, 1929, **23**, 35) (5·07 mg. in 20 c.c. of methyl alcohol):

(1) Rose, rapidly fading; (3) bluer red, fading still more rapidly, colourless in 30 seconds; (5) cherry-red, fading to redder shade; (7) reddish-violet, moderately stable; (9) reddish-violet, more stable; (11) violet, fading; (13) blue-violet, fading rapidly; (15) blue, fading very rapidly.

After 5 minutes; (1) pale pink; (3) and (5) colourless; (7) pale violet; (9) reddish-violet; (11) very pale blue-violet; (13) and (15) colourless.

The ferric reaction of the diglucoside is identical with that of cyanin, and this also applies to the xyloside-glucoside and the 5 : 7-diglucoside described below.

Cyanidin Chloride 5 : 7-*Di*-β-*glucoside* (III).—ω : 3 : 4-Triacetoxyacetophenone did not condense smoothly with the acetylated phloroglucinaldehyde diglucoside, but the related trihydroxyacetophenone was much more reactive. In general our recent experience has been that acyl groups in the second components can be removed with advantage.

A suspension of ω : 3 : 4-trihydroxyacetophenone (0·5 g.) and 2 : 4-di-(*O*-tetra-acetyl-β-glucosidyl)phloroglucinaldehyde (2 g.) in dry ethyl acetate (45 c.c.) was saturated with hydrogen chloride at 0°; the solids gradually dissolved in the deep red-violet solution. After the whole had been kept at 0° for 48 hours, with occasional shaking, ether was added and the reddish-brown flavylium *salt* was collected, washed with ether, and dried (2 g.) (Found: C, 50·1; H, 5·1; Cl, 4·2. $C_{43}H_{47}O_{24}Cl,2H_2O$ requires C, 50·7; H, 5·0; Cl, 3·5%).

A solution of this salt (1·97 g.) in aqueous sodium hydroxide (16·5 c.c. of 10%) was kept under hydrogen at room temperature for 90 minutes, concentrated hydrochloric acid (7·1 c.c.) added, the solution (containing 2% HCl) heated to 50° and cooled, and the acid concentration increased to 6%. When kept for 24 hours, the solution deposited reddish-brown needles (0·9 g.). This material was readily soluble in 0·5% hydrochloric acid and could not be readily recrystallised; from 6% acid it separated in crystalline form and on drying became a greenish flaky mass (Found: C, 42·1; H, 5·5; Cl, 8·4; loss at 110° in a high vacuum over phosphoric oxide, 18·8, 19·6. Found in dried material: C, 51·7; H, 4·3; Cl, 9·0%). In a second experiment the crystals separating after the hydrolysis were washed and dried for 18 hours over sulphuric acid in a desiccator (Found: C, 34·4, 34·5; H, 4·4, 4·4; Cl, 8·0; loss at 110° as above, 11·5, 14·7. Found in dried material: C, 39·4; H, 3·7; Cl, 8·0%). This oxonium salt has, therefore, a remarkable tendency to separate from solutions containing sodium chloride in crystal aggregates containing that salt as well as hydrogen chloride (16·078 mg. gave 3·965 mg. Na_2SO_4).

Ultimately it was found possible to remove the inorganic contamination by solution of the crude colouring matter in a large volume of 0·5% ethyl-alcoholic hydrogen chloride and increase of the acid concentration to 2·5% in the cold. Three crystallisations in this way gave microscopic reddish-brown needles with a weak green lustre (Found: loss on drying over phosphoric oxide at 110° in a high vacuum, 5·5, 6·2. Found in dried material: C, 50·5; H, 5·0; Cl, 5·4. $C_{27}H_{31}O_{16}Cl,2H_2O$ requires H_2O, 5·5%. $C_{27}H_{31}O_{16}Cl$ requires C, 50·1; H, 4·8; Cl, 5·5%). This *diglucoside* is readily soluble in 0·5% hydrochloric acid and almost insoluble in cold alcohol. The distribution to *iso*amyl alcohol from 0·5% hydrochloric acid is very low and the distribution number with *n*-butyl alcohol (standard method; 3·5 mg. in 50 c.c. of the mixed solvents) was 14·9.

The colour reactions in buffered solutions of graded p_H were the following (5·14 mg. in 20 c.c. of methyl alcohol): (1) Rose-red, fading; (3) bluer red, fading; (5) weak reddish-violet, fading; (7) rich reddish-violet; (9) the same; (11) the same; (13) violet; (15) blue with violet tinge, fading to pure blue. After 5 minutes, (1), (3) and (5) very pale-coloured, almost colourless; (7) and (9) weak permanganate, (9) was the more persistent; (11) violet; (13) blue-violet; (15) greenish-blue. The range of colours when first produced was throughout much redder in tone than those produced by cyanin (3 : 5-) or cyanidin 3 : 7-diglucoside.

The methyl-alcoholic solutions of the 3 : 7- and the 5 : 7-diglucoside of cyanidin, also a methyl-alcoholic solution of cyanin chloride of the same concentration, were used for a further comparison of ease of oxidation. Each solution (5 c.c.) was mixed with 1% hydrochloric acid (5 c.c.) and made up to 150 c.c. with water; aged ferric chloride (10 c.c. of 0·125%) was then added. The solutions became dull reddish-violet and no considerable change occurred in the cases of the **3** : 7- and the **3** : 5-diglucoside; the colour of the 5 : 7-diglucoside solution was almost completely discharged in 5 minutes and after 10 minutes the pigment was destroyed and not regenerated on the addition of concentrated hydrochloric acid. The **3** : 7-diglucoside and the **3** : 5-diglucoside (cyanin) remained largely unaltered and the colours faded to about half the original intensity in the course of 2 hours. This experiment with the three isomeric cyanidin diglucosides emphasises the value of this method of diagnosis of a free hydroxyl in position **3**.

The authors thank the Royal Commissioners for the Exhibition of 1851 for a Senior Studentship awarded to one of them (A. R. T.).

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