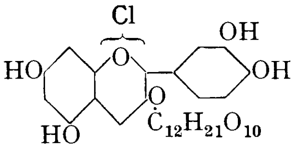
352. *Experiments on the Synthesis of Anthocyanins. Part XXIV. Cyanidin 3-Biosides and a Synthesis of Mecocyanin.*

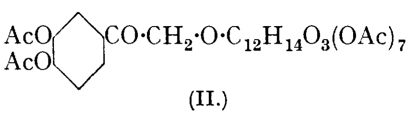
By (Miss) K. E. Grove, (the late) Mototaro Inubuse, and Robert Robinson.

Mecocyanin, a diglucoside of cyanidin isolated by Willstätter and Weil (*Annalen*, 1917, **412**, 231) from the red flowers of *Papaver Rhoeas* L., has been regarded as a cyanidin 3-bioside (Pratt and Robinson, J., 1925, **127**, 1129; Robertson and Robinson, J., 1927, 2198) as the result of a comparison of its colour reaction with those of related synthetic flavylium salts. It yields chrysanthemin chloride on part hydrolysis, and the synthesis of this monoglucoside (Murakami, Robertson, and Robinson, J., 1931, 2665) made it certain that the diglucoside must be a 3-bioside or a 3 : 7- or 3 : 5-diglucoside (positions 3′ and 4′ cannot be substituted, because the iron reaction is positive and characteristic). Recently the 3 : 5-diglucoside and the 3 : 7-diglucoside of cyanidin have been synthesised and shown to differ from mecocyanin. By the exclusion of alternatives, therefore, this colouring matter, representative of a large group, has been proved to be a cyanidin 3-bioside. There was no hint, however, of the nature of the biose except from analogy with plant products of very different chemical character.

We decided to attack the problem synthetically and have succeeded in applying the methods developed in this series of investigations to the preparation of the 3-*lactoside*, 3-*maltoside*, 3-*cellobioside* and 3-*gentiobioside* of cyanidin chloride (I). These salts differ from one another in their distribution between *n*-butyl alcohol and equilibrated 0·5% hydrochloric acid and this test indicated that mecocyanin is cyanidin 3-gentiobioside. A careful comparison of the salts confirmed this identification.

(I.)





(II.)

The second components of the type (II), which were condensed with *O*-benzoylphloroglucinaldehyde and later hydrolysed, could not be obtained anhydrous without decomposition. The substances retained water tenaciously, but this did not give rise to hydrolysis in the course of the condensations to an irretrievable extent. More or less of the monoglucoside was, however, obtained in each case.

EXPERIMENTAL.

ω-O-*Hepta-acetylcellobiosidoxy*-3 : 4-*diacetoxyacetophenone*.—The cellobiose octa-acetate used in the preparation of the hepta-acetyl bromide (Zemplén, Csürös, and Bruckner, *Ber.*, 1928, **61**, 927) was prepared from filter paper by the method of Haworth and Hirst (J., 1921, **119**, 197).

Hepta-acetylcellobiosidyl bromide (8·5 g.) was added to a solution of ω-hydroxy-3 : 4-diacetoxyacetophenone (2·0 g.) (Murakami, Robertson, and Robinson, *loc. cit.*; Grove and Robinson, J., 1931, 2722) in benzene (75 c.c.), and the mixture heated to 65° before silver carbonate (5·0 g.) was introduced. A vigorous evolution of carbon dioxide occurred and the mixture was kept at 65—70° for 30 minutes and then at 78—80° for 15 minutes. The cellobioside was precipitated as a gum from the filtered mixture by the addition of light petroleum and the precipitate was washed well with hot water and purified by repeated precipitation from methyl-alcoholic solution by the addition of water. The product was a white solid, not definitely crystalline (yield, 2·0 g.) (Found : C, 50·8; H, 5·4. $C_{38}H_{46}O_{23},1{\cdot}5H_2O$ requires C, 50·8; H, 5·5%).

3-O-*Cellobiosidylcyanidin Chloride*.—A solution of hepta-acetylcellobiosidoxydiacetoxyacetophenone (2·0 g.) and 2-*O*-benzoylphloroglucinaldehyde (1·5 g.) in dry ethyl acetate (75 c.c.) was protected from the access of moisture, saturated with dry hydrogen chloride at 0°, and kept in the ice-chest for 48 hours. The dark red solution was then filtered from a small precipitate of benzoylphloroglucinaldehyde, and the product precipitated as a dark red, amorphous powder by the addition of dry ether (yield, 2·0 g.) (Found : C, 49·1; H, 5·4; Cl, 5·2. $C_{34}H_{35}O_{17}Cl,4H_2O$ requires C, 49·6; H, 5·2; Cl, 4·4%). This and similar analyses are only quoted as providing indications of composition, but it is apparent that the crude product consists essentially of hydrated 3-*cellobiosidoxy*-7 : 3′ : 4′-*trihydroxy*-5-*benzoyloxyflavylium chloride*, in which a part of the water is replaced by hydrogen chloride; the degree of de-acetylation is uncertain.

The crude salt (2·0 g.) dissolved in 8% sodium hydroxide (25 c.c.) in the cold in an atmosphere of nitrogen to a violet solution, which became greenish-brown and finally almost black. After being kept for 3 hours at room temperature, the solution was acidified with 7% hydrochloric acid (36 c.c.) and heated to 60° on a steam-bath to complete the formation of the oxonium salt. The cooled filtered solution could not be precipitated with picric acid, picrolonic acid, perchloric acid, or flavianic acid. A precipitate was obtained with potassium ferrocyanide, but this was not found to afford a satisfactory method of purification. The solution was evaporated to dryness in a vacuum desiccator, the residue extracted with absolute methyl alcohol, and the salt precipitated from the acidified solution by the addition of dry ether, as a dark red, amorphous powder. After a rather long interval (in which deterioration probably occurred) this material was found to contain monoglucoside; it was dissolved in 1% hydrochloric acid and extracted twice with *n*-butyl alcohol and then fifteen times with twice its volume of *iso*amyl alcohol, the original volume being maintained by the addition of 1% hydrochloric acid. The dark red solution was then mixed with acetic acid (3 volumes), and the salt precipitated by means of ether; the syrupy deposit was dissolved in 0·1% methyl-alcoholic hydrogen chloride and again precipitated with ether in a solid form. The salt then crystallised from 2% ethyl-alcoholic hydrogen chloride in dark red needles with a green reflex (Found : C, 45·1; H, 5·2; Cl, 5·2. $C_{27}H_{31}O_{16}Cl,4H_2O$ requires C, 45·1; H, 5·4; Cl, 5·0%). On drying at 110° in a high vacuum over phosphoric oxide, the salt lost hydrogen chloride as well as water.

The properties of this salt were closely similar to those of the other cyanidin 3-biosides. Thus in buffered solution of graded p_H the reactions were identical with those of pure mecocyanin already recorded by Robertson and Robinson (*Biochem. J.*, 1929, **23**, 35); a direct comparison was made.

The distribution number for equilibrated *n*-butyl alcohol and 0·5% hydrochloric acid was found to be 10·9, 10·8 for 3·2 mg. of the pigment in 50 c.c. of the mixed solvents (compare Robinson and Todd, J., 1932, 2296, 2492).

The ferrocyanide crystallises from aqueous solutions in microscopic needles but no other sparingly soluble salt could be obtained.

ω-O-*Hepta-acetyl-lactosidyloxy*-3 : 4-*diacetoxyacetophenone*.—The quantities used were: silver carbonate (10 g.), ω-hydroxydiacetoxyacetophenone (3 g.), hepta-acetyl-lactosidyl bromide (9·9 g.) (E. Fischer and H. Fischer, *Ber.*, 1910, **43**, 2521), and dry benzene (40 c.c.). After the first period at 60° the mixture was refluxed for 30 minutes. The working-up was like that of the cellobioside, but ultimately the derivative crystallised from benzene–light petroleum in colourless needles (3·5 g.), m. p. 78—80°, very sparingly soluble in ether and light petroleum but readily soluble in benzene and alcohol (Found : C, 51·7; H, 5·3. $C_{38}H_{46}O_{23},0·5H_2O$ requires C, 51·8; H, 5·2%).

3-O-*Lactosidylcyanidin Chloride*.—The quantities used were: *O*-benzoylphloroglucinaldehyde (1·3 g.), the above lactoside (2·6 g.), dry ethyl acetate (30 g.), and the solution saturated at 0° with hydrogen chloride was kept for 3 days in the ice-chest (product precipitated by ether, 2·4 g.) (Found : C, 49·8; H, 5·4; Cl, 5·0%). This again indicates de-acetylation, hydration, and a slight extra content of hydrogen chloride. The crude product (2·0 g.) was added to aqueous baryta (60 c.c. of *ca.* 3% and of known titre with respect to *N*-sulphuric acid) and kept under hydrogen at room temperature for 24 hours. The barium was then exactly precipitated with sulphuric acid, and the filtered solution concentrated in a vacuum at 40°. The residue was crystallised from 2% alcoholic hydrogen chloride (yield, 1·2 g.) and was then free from monoglucoside. The microscopic, dark red needles were rather more clear-cut than those of the cellobioside and maltoside (Found : C, 47·9; H, 5·3; Cl, 5·3; loss at 110° in a high vacuum over phosphoric oxide, 3·5. $C_{27}H_{31}O_{16}Cl,1·5H_2O$ requires C, 48·1; H, 5·2; Cl, 5·3; $1·5H_2O$, 4·0%. Found in anhydrous material : C, 50·2; H, 5·0; Cl, 5·7. $C_{27}H_{31}O_{16}Cl$ requires C, 50·1; H, 4·8; Cl, 5·5%). The ferrocyanide is precipitated from aqueous solution, but other salts are readily soluble. The colour reactions were identical with those of mecocyanin as shown by a series of direct comparisons.

The distribution number was found to be 20·7 (*n*-butyl alcohol–0·5% hydrochloric acid; 3·0 mg. in 50 c.c. of the mixed solvents) and almost zero for *iso*amyl alcohol.

ω-O-*Hepta-acetylmaltosidyloxy*-3 : 4-*diacetoxyacetophenone*.—This was prepared like the acetylated lactoside by substituting *O*-hepta-acetylmaltosidyl bromide (9·9 g.) (Fischer and Armstrong, *Ber.*, 1902, **35**, 3153) for the lactose derivative; the product (yield of pure substance, 2·5 g.) crystallised eventually from aqueous alcohol in colourless needles, m. p. 88° (Found : C, 51·6; H, 5·1. $C_{38}H_{46}O_{23},0·5H_2O$ requires C, 51·8; H, 5·2%).

3-O-*Maltosidylcyanidin Chloride*.—This salt was prepared in the same way as the lactoside (Found in the crude condensation product : C, 52·1; H, 4·0; Cl, 4·9. Diacetate, $1H_2O$ requires C, 52·1; H, 5·0; Cl, 4·3%).

The hydrolysed salt (from the evaporation in a vacuum at 40°) was too soluble in ethyl-alcoholic hydrogen chloride for successful crystallisation, but it separated from 1% propyl-alcoholic hydrogen chloride containing 5% of *iso*propyl ether in short prismatic needles, black in mass but deep brown-red by transmitted light. Satisfactory analytical results from the air-dried material could not be obtained and when dried at 110° the salt lost hydrogen chloride. Ultimately it was dried at 50° in a vacuum for 2 hours, and then to constant weight at 105° in a high vacuum over phosphoric oxide (Found : C, 50·3, 50·2; H, 4·9, 5·0; Cl, 5·4, 5·5. $C_{27}H_{31}O_{16}Cl$ requires C, 50·1; H, 4·8; Cl, 5·5%). The colour reactions of this salt were found on comparison to be identical with those of mecocyanin.

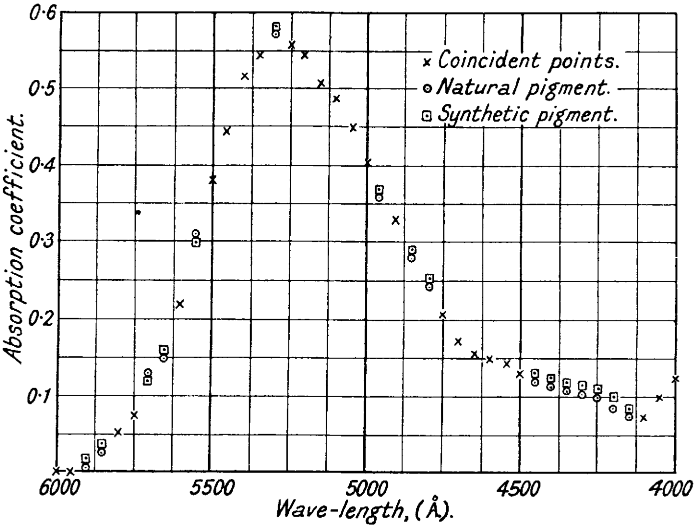
The distribution number (*n*-butyl alcohol–0·5% hydrochloric acid, 3·1 mg. in 50 c.c.) was found to be 19·0.

ω-O-*Hepta-acetylgentiobiosidyloxy*-3 : 4-*diacetoxyacetophenone*.—The method was again the same as that for the lactoside, but *O*-hepta-acetylgentiobiosidyl bromide (9·9 g.) was used (compare Bergmann and Freudenberg, *Ber.*, 1929, **62**, 2785; Zemplén, *Ber.*, 1924, **57**, 702).

The product crystallised from benzene–light petroleum in colourless needles (3·0 g.), m. p. 70—72° (Found : C, 51·8; H, 5·4. $C_{38}H_{46}O_{23},0{\cdot}5H_2O$ requires C, 51·8; H, 5·2%).

3-O-*Gentiobiosidylcyanidin Chloride* (Mecocyanin Chloride).—The preparation was carried out exactly as in the case of the lactoside (Found in crude condensation product : C, 51·1; H, 5·1; Cl, 5·3%). After the hydrolysis and evaporation in a vacuum at 40° the product crystallised from 2% ethyl-alcoholic hydrogen chloride in microscopic, dark red needles exhibiting a green reflex (Found : C, 45·7; H, 5·5; Cl, 5·2; loss at 110° in a high vacuum over phosphoric oxide, 8·9. $C_{27}H_{31}O_{16}Cl,3{\cdot}5H_2O$ requires C, 45·7; H, 5·4; Cl, 5·0; $3{\cdot}5H_2O$, 8·3%. Found in anhydrous material : C, 50·2; H, 5·0; Cl, 5·6. $C_{27}H_{31}O_{16}Cl$ requires C, 50·1; H, 4·8; Cl, 5·5%).

Absorption spectra of natural and synthetic mecocyanin chloride.



The distribution number using *n*-butyl alcohol and 0·5% hydrochloric acid under the standard conditions (3·0 mg. in 50 c.c. of mixed equilibrated solvents) was found to be 17·0, 17·0, 16·9.

A fine specimen of mecocyanin chloride was kindly supplied by Geheimrath Professor Willstätter, and this crystallised from 2% ethyl-alcoholic hydrogen chloride in dark red needles with a green reflex, microscopically indistinguishable from those of the gentiobioside (Found : C, 45·7; H, 5·6; Cl, 5·1%). The distribution number (*n*-butyl alcohol–0·5% hydrochloric acid) was found to be 16·7, 16·7, 16·8. The colour reactions in solutions of graded p_H of the natural and synthetic specimens were absolutely identical at every stage during 48 hours. Miss J. C. Bell kindly compared the absorption spectra of the specimens (Fig.). A saturated solution of mecocyanin chloride in 2% ethyl-alcoholic hydrogen chloride rapidly dissolved traces of the lactoside, cellobioside, or maltoside but did not dissolve a trace of the gentiobioside.

The cellobioside and the maltoside are much more difficult to crystallise than are the lactoside and the gentiobioside, the habit of crystallisation of which is the same as that of mecocyanin; there is, of course, no possibility that mecocyanin, proved to be a diglucoside, can be the lactoside.

THE DYSON PERRINS LABORATORY, OXFORD UNIVERSITY.

[*Received, August 9th,* 1934.]