CCCLXXII.—*Experiments on the Synthesis of Anthocyanins. Part VI. A Synthesis of Chrysanthemin Chloride.*

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THE method (Part V; J., 1928, 1460) employed for the synthesis of callistephin chloride (3-β-glucosidylpelargonidin chloride) has now been extended to the synthesis of 3-β-glucosidylcyanidin chloride and careful direct comparisons have established the identity of the salt with chrysanthemin chloride.

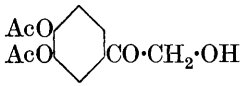
This well-characterised anthocyanin was first isolated by Willstätter and Bolton (*Annalen*, 1916, **412**, 136) from the flowers of the

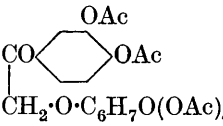
deep-red chrysanthemum (*Chrysanthemum indicum*, Linn.) and later obtained by the regulated hydrolysis of the cyanidin diglucoside, mecocyanin (Willstätter and Weil, *ibid.*, p. 231). There is, moreover, reason to believe that asterin (Willstätter and Burdick, *ibid.*, p. 149) is identical with chrysanthemin, the slight recorded divergences being the effects of small proportions of admixed foreign substances (compare Willstätter and Robinson, *Ber.*, 1928, **61**, 2503).

The sodium salt of ω : 3 : 4-trihydroxyacetophenone,

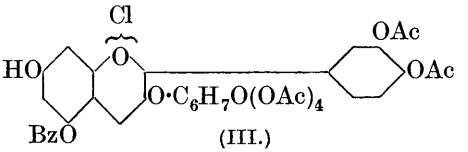
$$C_6H_3(HO)_2\cdot CO\cdot CH_2\cdot OH,$$

was prepared in the known manner (Voswinkel, *Ber.*, 1909, **42**, 4651), dissolved in water, and shaken with a solution of acetic anhydride in benzene. The resulting *ω-hydroxy-3 : 4-diacetoxyacetophenone* (I) could fortunately be separated from any triacetate, that might be formed simultaneously, by taking advantage of the separation of a *compound* with calcium chloride. This interesting substance can be crystallised from chloroform–light petroleum and on treatment with water yielded the pure diacetoxybenzoylcarbinol. The latter was converted into the acetylated β-glucoside (II) by the action of *O*-tetra-acetyl-α-glucosidyl bromide and silver carbonate on the substance in chloroform solution.

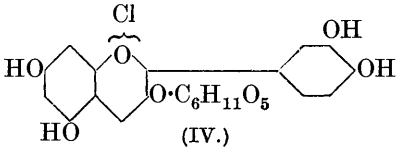
(I.) 


(II.)

As in the analogous synthesis of callistephin chloride, the flavylium salt was prepared by condensation of the substituted phenacylglucoside with *O*-benzoylphloroglucinaldehyde and hydrogen


(III.)

chloride in chloroform–ether solution and the product, presumed in the present case to be (III), was hydrolysed by means of aqueous sodium hydroxide. The pyrylium nucleus was thus modified and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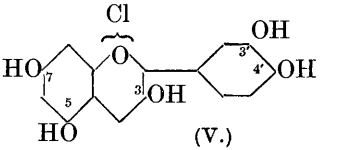
(V.)

reconstituted when the mixture was acidified with hydrochloric acid. The resulting 3-β-glucosidylcyanidin chloride (IV) was isolated in a

pure condition; it gave cyanidin chloride * (V) on hydrolysis and was found to be identical with chrysanthemin chloride in every respect. We are deeply indebted to Geheimrath Professor R. Willstätter for very kindly sending us a fine specimen of chrysanthemin chloride from the deep-red chrysanthemum.

This proof of the 3-glucoside constitution of chrysanthemin chloride has some important consequences; for example, mecocyanin chloride must now be regarded as a 3-(glucosidylglucosidyl)cyanidin chloride, since its colour reactions are closely similar to those of chrysanthemin chloride and the bioside yields the monoglucoside on partial hydrolysis. It is probable that keracyanin and prunicyanin, which resemble mecocyanin, are also 3-biosides. The behaviour of cyanin chloride, however, differs so widely from that of mecocyanin chloride that the attachment of the carbohydrate residue to position 3 cannot be assumed and, since the strong ferric chloride reaction proves that positions 3′ and 4′ likewise bear hydroxyl groups, cyanin chloride must be 5- or 7-(glucosidylglucosidyl)cyanidin chloride if it is a bioside.

Until recently we were of the opinion that cyanin, peonin, pelargonin and malvin were biosides, but a different view is now considered to be tenable.

The properties of fisetinin chloride (see Part XI) have rendered it probable that cyanin is cyanidin 3 : 5-diglucoside and this is supported by considerations mentioned in Part XII relating to the stability of cyanin to oxidising agents and to the partial hydrolysis of the substance. The relation of this hypothesis to the work of Karrer and his collaborators on the methylation of the anthocyanins is discussed in the following communication.

Experimental.

ω-Hydroxy-3 : 4-diacetoxyacetophenone (I).—An improvement in the preparation of ω-chloro-3 : 4-dihydroxyacetophenone (Drezkowski, *J. Russ. Phys. Chem. Soc.*, 1893, **25**, 154; *Ber.*, 1893, **26**, 589; D.R.-P. 71312) consists in the addition of an equal volume of toluene to the reactants. The reaction is complete in 1 hour (heating at

* Synthetic cyanidin chloride had been compared by two of the present authors with two specimens of natural cyanidin chloride, one from mecocyanin chloride and one of unknown origin; it has now been compared also with cyanidin chloride from cyanin chloride *ex* cornflowers and from cyanin chloride *ex* dahlias.

The comparisons were made in considerable detail and no divergences were noted.

We wish to thank Geheimrath Professor R. Willstätter for the provision of specimens of the anthocyanin from the two sources mentioned and also for a large specimen of mecocyanin chloride.

110—115° in the oil-bath) and the product is separated by steam-distillation of the toluene and crystallisation of the residue from water. The sodium salt of ω : 3 : 4-trihydroxyacetophenone (5 g.) was dissolved in water (25 c.c.) and agitated in the cold with a solution of acetic anhydride (5 c.c.) in benzene (10 c.c.). After a few minutes, during which the vessel was cooled in melting ice, the benzene layer was separated, concentrated until almost all the benzene had been removed, and then the residue taken up in chloroform. This solution was mixed with powdered anhydrous calcium chloride, shaken, gently heated, and then kept for 12 hours.

The crystalline calcium chloride *compound* of the carbinol was purified by dissolution in chloroform at room temperature and precipitation by the addition of light petroleum. The colourless prisms are stable in ordinary air and have m. p. 126—127° (Found : Ca, 6·4. $2C_{12}H_{12}O_6,CaCl_2$ requires Ca, 6·5%). The *carbinol* was regenerated from the crude double compound by treatment with water; it was collected, washed, and crystallised from alcohol, forming colourless elongated plates (3·2 g.), m. p. 86—87° (Found : C, 57·3; H, 4·6; $CH_3{\cdot}CO$, 34·3. $C_{12}H_{12}O_6$ requires C, 57·7; H, 4·8; $2CH_3{\cdot}CO$, 34·1%). The substance is sparingly soluble in cold ether but is fairly soluble in most organic solvents; it reduces Fehling's solution in the cold in the presence of a little alcohol.

ω-O-*Tetra-acetyl*-β-*glucosidoxy*-3 : 4-*diacetoxyacetophenone* (II).—Dry silver carbonate (15 g.) was added to a solution of *O*-tetra-acetyl-α-glucosidyl bromide (14·5 g.) and ω-hydroxy-3 : 4-diacetoxy-acetophenone (7·5 g.) in dry chloroform (30 c.c.) with vigorous agitation for 15 minutes, followed by heating on the steam-bath for 15 minutes under reflux. The liquid was filtered and mixed with light petroleum, giving an oil that was triturated with water until it crystallised. The substance was recrystallised from 80% methyl alcohol forming colourless, stout, prismatic needles (5 g.), m. p. 105—105·5° (Found : C, 53·7; H, 5·2. $C_{26}H_{30}O_{15}$ requires C, 53·6; H, 5·2%). The substance is readily soluble in most organic solvents, sparingly so, however, in ether, light petroleum and carbon disulphide; it is appreciably soluble in hot water.

3 - β - *Glucosidoxy* - 5 : 7 : 3′ : 4′ : *tetrahydroxyflavylium Picrate* (Picrate related to IV).—A solution of ω-*O*-tetra-acetyl-β-glucosidoxy-3 : 4-diacetoxyacetophenone (1·0 g.) and *O*-benzoylphloroglucinaldehyde (0·5 g.) in chloroform (10 c.c.) and dry ether (85 c.c.) was saturated with hydrogen chloride at room temperature; the condensation proceeded rather slowly and after a week the dark red precipitate was collected, and a further quantity obtained by the addition of ether to the filtrate (yield, 1·1 g.). The crude tetra-acetylglucosidoxybenzoyloxyhydroxydiacetoxyflavylium chloride

(2·0 g.) was dissolved in 10% aqueous sodium hydroxide (22 g.), the solution kept for 3½ hours at room temperature with occasional shaking, and the mixture then acidified with 10% hydrochloric acid (25 g.) and kept for 12 hours. Benzoic acid was removed by filtration and the filtrate, with washings, was thrice extracted with amyl alcohol (20 c.c., 20 c.c., 10 c.c.); this amyl alcohol layer was washed thrice with 0·005% hydrochloric acid (10 c.c. each time). The combined aqueous acid solution was extracted with ether, nearly neutralised, and the pyrylium salt converted into the lead salt and regenerated in the manner described by Willstätter and Burdick (*Annalen*, 1916, **412**, 155; compare J., 1928, 1468). The pyrylium salt (0·62 g.), purified in this way, was dissolved in the minimum of 0·01% hydrochloric acid (*ca.* 5 c.c.), and saturated aqueous picric acid (45 c.c.) added. The *picrate* that separated on keeping crystallised from hot semi-saturated aqueous picric acid as a deep brownish-red, crystalline mass exhibiting a feeble metallic glance (0·58 g.). Under the microscope the plates appear brown by transmitted light; the substance has m. p. 178° (decomp. 182°) (Found in material dried at 110° in a vacuum: C, 48·2; H, 3·6; N, 6·3. $C_{27}H_{23}O_{18}N_3$ requires C, 47·8; H, 3·4; N, 6·2%). Subsequently much larger quantities of this substance were available, especially when it was found that the original condensation could be conveniently effected by dry ethyl acetate solution. The details of this process are exactly similar to those employed for the synthesis of idaein chloride (see subsequent part of this series) and are therefore not included here. In these later experiments it was found that the pure picrate crystallised from half-saturated aqueous picric acid in microscopic quadrilateral, not quite rectangular prisms, orange by transmitted light and crimson with bronze glance in mass (m. p. 170—175° or higher according to the rate of heating; vigorous decomp., 190—193°) (Found in air-dried material: C, 43·4; H, 4·4; N, 5·3; loss at 110° in a vacuum, 9·7. $C_{27}H_{23}O_{18}N_3,4H_2O$ requires C, 43·2; H, 4·2; N, 5·6; $4H_2O$, 9·6%). The substance also crystallises in isolated needles and radiating clusters of needles and from dilute solutions it often separates in characteristic palm-leaf-shaped crystals. The latter is the form that is always obtained when saturated aqueous picric acid is added to a hot solution of *pure* chrysanthemin chloride in dilute hydrochloric acid.

This derivative was prepared from " asterin " chloride (Willstätter and Burdick, *loc. cit.*) and from chrysanthemin chloride (Willstätter and Bolton, *loc. cit.*; compare Willstätter and Robinson, *Ber.*, 1928, **61**, 2500) and by analysis and a comparison of all the properties the three specimens were found to be identical; they all crystallised with $4H_2O$.

3-β-*Glucosidylcyanidin Chloride* (*Chrysanthemin Chloride*) (IV).—The pure picrate (1·6 g.) was dissolved in 2% methyl-alcoholic hydrogen chloride (100 c.c.), and the *chloride* precipitated by the addition of much ether. This was collected and triturated with cold 3% ethyi-alcoholic hydrogen chloride; it then quickly crystallised. The substance was dissolved in the minimum of hot 0·5% aqueous hydrochloric acid and five volumes of 3% ethyl-alcoholic hydrogen chloride were added. The flavylium salt then separated in tiny diamond-shaped leaflets curiously uniform in size and shape (0·02—0·1 mm. long and from 2·5 to 3·5 times as long as broad) and presenting a very fine appearance on account of their brilliant bronze reflex. The crystals closely resemble those of which Willstätter and Bolton reproduced a microphotograph (*loc. cit.*, p. 146) (Found in different air-dried specimens: C, 49·4, 49·5; H, 4·8, 4·9; Cl, 7·1, 7·0; loss at 110° in a high vacuum, 4·9, 4·8. $C_{21}H_{21}O_{11}Cl,1{\cdot}5H_2O$ requires C, 49·3; H, 4·7; Cl, 6·9; $1{\cdot}5H_2O$, 5·3%. Found in material dried at 110° in a vacuum: C, 52·0; H, 4·4; Cl, 7·0. $C_{21}H_{21}O_{11}Cl$ requires C, 52·0; H, 4·3; Cl, 7·3%). In every respect this substance was found to be identical with chrysanthemin chloride from *Chrysanthemum indicum* L., a specimen of which was very kindly sent to us by Professor Willstätter; all the properties mentioned by Willstätter and Bolton (*loc. cit.*, pp. 146—147) were observed as characteristic of the synthetic specimen; the solubilities were not, however, determined quantitatively. The natural specimens could be used to induce the crystallisation of the synthetic and *vice versa*. The solubility in 7% sulphuric acid of the two specimens was shown to be identical by colorimetric comparison of the saturated solutions. The colour reactions, violet with sodium carbonate and blue with sodium hydroxide, the ferric chloride reaction under different conditions, the ease of pseudo-base formation, and many other properties were directly compared and no divergence was observed in any instance.

The distribution number of the synthetic salt was found to be 19·1 and 19·0 in a second experiment; Willstätter and Bolton found the number 19·0.

Willstätter and Schudel (Schudel, *Dissert.*, Zurich, 1918) have observed that ethyl acetate extracts chrysanthemin from aqueous solution in the presence of picric acid. We dissolved synthetic chrysanthemin chloride (5·00 mg.) in saturated aqueous picric acid (50 c.c.) and shook the solution with ethyl acetate (50 c.c.) saturated with picric acid. The lower layer was separated, mixed with much ether and light petroleum, and extracted with 1% hydrochloric acid (50 c.c.). The aqueous solution so obtained was freed from picric acid by means of ether and made up to 100 c.c. with 1% hydrochloric acid. It was colorimetrically compared with the upper layer from

the original mixture, which was also freed from picric acid and diluted to 100 c.c. with 1% hydrochloric acid. It was found that 37·5% of the pigment had passed into the ethyl acetate–picric acid layer. The natural chrysanthemin gave 37·4% and " asterin " gave 37·1% in this experiment. When ethyl phenylacetate was used in a similar way, the pigment was not extracted.

Colour reactions in a range of buffered solutions of graded p_H. The method of Robertson and Robinson (*Biochem. J.*, 1929, **23**, 35) was employed and the numbers refer to solutions of p_H 3·2 (1) to p_H 11·0 (14) and then to more alkaline solutions of unknown p_H. The natural and synthetic specimens gave identical results which were as follows: (1) salmon-red; (2), (3) bluish-red, fading; (4) violet-red; (5), (6), (7) brown-violet-red, deepening in the series; (8)—(13) brown-violet; (14) dull violet; (15) violet; (16) bluish-violet; (17) violet-blue. The appearances after various times were noted and at all stages identity was observed. In 0·2*N*-sodium hydroxide the solutions gave a pure blue colour, but this quickly became green and later yellow.

The methyl-alcoholic solutions employed for the colour reactions were used for other comparisons, for example, the following. 10 C.c. were diluted with water to 100 c.c. and a solution (5 c.c. of 0·1%) of iron alum was added. The violet solutions so obtained rapidly reddened together and in 6 minutes the colour was almost discharged.

A considerable series of comparisons has also been made between the synthetic chrysanthemin and the product of the semi-hydrolysis of mecocyanin; these include distribution ratios and colour-comparisons and as a result we have confirmed the statement of Willstätter and Weil (*Annalen*, 1916, **412**, 233) that mecocyanin yields chrysanthemin.

The chrysanthemin produced by following the method of these authors was isolated by extraction with ethyl acetate–picric acid and by removal of cyanidin as its picrate by means of ether extraction of an aqueous solution (Willstätter and Schudel, *loc. cit.*). The chrysanthemin was ultimately crystallised as picrate and chloride and identified by means of its distribution number and colour reactions.

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