CCCLXXVI.—*Experiments on the Synthesis of Anthocyanins. Part X. Synthesis of 3-β-Galactosidylcyanidin Chloride, which is believed to be identical with Idaein Chloride, and of 3-β-Galactosidylpeonidin Chloride.*

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Willstätter and Mallison (*Annalen*, 1915, **408**, 15) isolated idaein chloride from the cranberry (*Vaccinium vitis idaea*, Linn.) and characterised the anthocyanin as a galactoside of cyanidin.

The colour reactions observed were similar to those of chrysanthemin and therefore there can be little doubt that idaein is a 3-galactosidylcyanidin (I). Employing the methods of the chrysanthemin synthesis (Part VI) but starting with *d*-galactose instead of glucose, we have obtained a product the properties of which are in agreement with those of idaein chloride as described by Willstätter and Mallison.

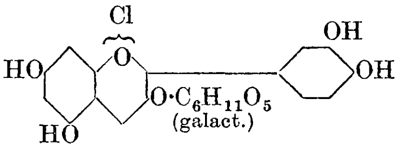
In this case the galactoside intermediate could not be crystallised, but the amorphous product, $C_6H_3(AcO)_2{\cdot}CO{\cdot}CH_2{\cdot}O{\cdot}C_6H_7O(OAc)_4$, gave satisfactory analytical figures and the anthocyanin, obtained by condensation with benzoylphloroglucinaldehyde and through subsequent stages in the usual manner, crystallised with facility.

For comparison with oxycoccicyanin chloride, 3-β-galactosidylpeonidin chloride (II) has also been synthesised. It has been found that absorption curves, in the visible region at least, do not distinguish the glucoside, chrysanthemin, from idaein or the glucoside from the galactoside of peonidin; nor do the alkali colour reactions.

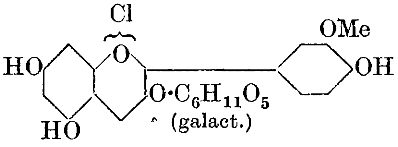
On the other hand the distribution numbers are characteristic and it is interesting to observe that within limits the sugar rather than the anthocyanidin appears to be the controlling factor. Thus the distribution constants for idaein chloride and 3-β-galactosidylpeonidin are practically identical, whereas we find the distribution of chrysanthemin chloride is similar to that of pure synthetic oxycoccicyanin chloride (see preceding communication).

A certain number of distribution experiments with mixtures of idaein and chrysanthemin chlorides (both synthetic) have been carried out and the results show that, on the association hypothesis, a certain proportion of an idaein–chrysanthemin complex is produced. We have not been able to solve the mathematical problem presented by this system.

(I.)



(II.)



EXPERIMENTAL.

ω-*Hydroxy*-3 : 4-*diacetoxyacetophenone.*—A cooled solution of acetic anhydride (20 c.c.) in chloroform (100 c.c.) was added to one of the sodium salt of ω : 3 : 4-trihydroxyacetophenone (Voswinckel, *Ber.*, 1909, **42**, 4651) (20 g.) in water (100 c.c.) and the mixture was shaken vigorously for 10 minutes. The chloroform layer was then separated and the water layer was shaken with a little chloroform, which was added to the original extract. Water (20 c.c.) was added to the combined extracts and the chloroform was evaporated under diminished pressure at 30—35°. The residue crystallised from water (charcoal) in colourless elongated plates (11·5 g.), m. p. 86—87°. This modification of the method described in Part VI has practical advantages.

ω-O-*Tetra-acetyl*-β-*galactosidoxy*-3 : 4-*diacetoxyacetophenone.*—Dry silver carbonate (15 g.) was added to a solution of ω-hydroxy-3 : 4-diacetoxyacetophenone (6 g.) and *O*-tetra-acetyl-α-galactosidyl bromide (Robertson, J., 1929, 1820) (15 g.) in dry benzene (40 c.c.) at 35°. The temperature of the mixture rose rapidly to 52° and there was a vigorous evolution of carbon dioxide. The mixture was well stirred and the temperature was maintained at 55—60° for 45 minutes, after which the solution was refluxed for 15 minutes. The crude *galactoside* was precipitated from the filtered solution by the addition of light petroleum (500 c.c.) and the resulting syrup was washed thoroughly first with hot and then with cold water. Attempts to crystallise this substance were unsuccessful, but a pure amorphous solid was obtained by repeated precipitations from methyl alcohol solutions by the addition of water. The substance is very sparingly soluble in ether, but is readily soluble in other common organic solvents (Found : C, 53·7 ; H, 5·3. $C_{26}H_{30}O_{15}$ requires C, 53·6 ; H, 5·2%).

7-*Hydroxy*-5-*benzoyloxy*-3-O-*tetra-acetyl*-β-*galactosidoxy*-3′ : 4′-*diacetoxyflavylium Chloride.*—A solution of ω-*O*-tetra-acetyl-β-galactosidoxy-3 : 4-diacetoxyacetophenone (2 g.) and *O*-benzoylphloroglucinaldehyde (Robertson and Robinson, J., 1927, 1713) (1·8 g.) in dry ethyl acetate (50 c.c.) was saturated with dry hydrogen chloride at room temperature, and protected from the access of moisture. There was a slight precipitation of the sparingly soluble *O*-benzoylphloroglucinaldehyde while the hydrogen chloride was being introduced and the solution became red, the colour deepening on standing. After 3 days the solution was filtered, and dry ether (*ca.* 500 c.c.) was added in order to precipitate the pyrylium salt. This was collected and used in the preparation of the picrate without further purification (yield, 2·2 g.) [Found : C, 55·1 ; H, 4·6 ; Cl, 7·1. $C_{28}H_{25}O_{12}Cl,H_2O$ (5-*benzoyl*-3-*galactosidylcyanidin chloride*) requires C, 55·4 ; H, 4·5 ; Cl, 5·9%].

3-β-*Galactosidylcyanidin Picrate.*—8% Sodium hydroxide solution (40 c.c.) cooled to 10° was added to the finely powdered benzoyl-galactosidylcyanidin chloride (2·2 g.) in an apparatus from which the air was excluded by nitrogen. The solid gradually dissolved to a dark green, almost black, solution, which was kept at room temperature for 3 hours and then acidified with 7% hydrochloric acid (65 c.c.), whereupon it became dark red; the formation of the oxonium salt was completed by heating to 60°. After cooling, the solution was filtered, an equal volume of saturated aqueous picric acid added, and the mixture kept in the ice-chest until the separation of the precipitate was complete. The product was quite readily soluble in hot water, but was almost insoluble in the cold. It crystallised from very dilute solutions in matted, long, hair-like needles, which formed a dark red-brown powder when dried (yield, 1·2 g.).

3-β-*Galactosidylcyanidin Chloride* (*Idaein Chloride*) (I).—The picrate (3 g.) was dissolved in warm methyl alcohol (35 c.c.), 15% methyl-alcoholic hydrogen chloride (4 g.) added, and the chloride precipitated as an amorphous red solid by the addition of dry ether (*ca.* 250 c.c.). This material (1·7 g.) could be purified by the method described by Willstätter and Mallison (*loc. cit.*) for idaein, that is, by dissolution in water (0·5 g. in 50 c.c.), addition of concentrated hydrochloric acid (10 c.c.), addition of alcohol (20 c.c.) to the filtered liquid, and slow evaporation in a crystallising dish. The chloride was obtained by this method in clumps of brownish-green crystals. For further purification the chloride (1·0 g.) was dissolved in 0·5% hydrochloric acid (20 c.c.), 5% ethyl-alcoholic hydrogen chloride (60 c.c.) added, and the solution filtered and kept in the ice-chest. The chloride crystallised in microscopic red-brown prisms with a green reflex (Found: C, 49·9; H, 4·7; Cl, 7·0; loss at 105° in a high vacuum, 3·9. $C_{21}H_{21}O_{11}Cl,H_2O$ requires C, 50·1; H, 4·6; Cl, 7·0; H_2O, 3·6%. Found in anhydrous material: C, 51·8; H, 4·3; Cl, 7·4. $C_{21}H_{21}O_{11}Cl$ requires C, 52·0; H, 4·4; Cl, 7·3%).

The solubility, colour reactions, and other properties of the chloride are identical with those recorded by Willstätter and Mallison (*loc. cit.*) for idaein chloride. The 3-β-galactosidylcyanidin chloride is fairly readily soluble in water to a dark reddish-brown solution when concentrated, and an orange-red solution when dilute. In very dilute solutions the colour is discharged owing to the formation of the pseudo-base, but it is restored by the addition of acid to the solution. The chloride is easily soluble in 0·5% hydrochloric acid, less readily soluble in 3% acid, and is almost insoluble in 6% hydrochloric acid, but somewhat more readily soluble in 7% sulphuric acid. Warm solutions in the latter solvent deposit brown rhombic crystals of the sulphate on cooling.

With sodium hydroxide a solution of the chloride gives a clear blue coloration which rapidly becomes green and then yellow, but with sodium carbonate a stable violet coloration is produced. With ferric chloride in alcoholic solution it gives a blue coloration which changes to violet on dilution with water. Copper acetate gives a blue colour in alcoholic, and a red-violet precipitate in aqueous solution. Lead acetate gives a blue precipitate from an alcoholic solution. Alum produces a very stable violet colour in an aqueous solution, and bismuth nitrate a red-violet colour. The chloride melts with frothing at 210°.

Distribution Ratios.—I. *With ethyl acetate* (compare Schudel, Thesis, Zürich, 1918). 3-β-Galactosidylcyanidin chloride (5·25 mg.) was dissolved in cold saturated aqueous picric acid (50 c.c.) and shaken with pure ethyl acetate previously saturated with picric acid (50 c.c.). The ethyl acetate layer was separated, diluted with ether (300 c.c.), and extracted with 0·5% hydrochloric acid (100 c.c.). This aqueous solution was washed twice with ether (100 c.c.) and compared colorimetrically with the mother-liquor, which had also been freed from picric acid by shaking with ether. The pigment was extracted from the aqueous picric acid solution by the ethyl acetate–picric acid solution to the extent of 50·2%.

Wt. in mg.	G.-mols. $\times 10^{-6}$.	Dist.	*K*.	Log C_W.	Log C_{AA}.
3·078	6·135	17·7	0·233	0·70	0·04
3·850	7·66	15·7	0·224	0·81	0·08
5·084	10·12	13·67	0·234	0·94	0·14
7·711	15·30	11·64	0·234	1·13	0·25
10·199	20·30	10·21	0·235	1·26	0·32
15·063	29·97	8·41	0·230	1·44	0·40
For chrysanthemin chloride (natural).					
3·038	5·94	22·5	0·387	0·66	0·13
4·011	7·84	20·1	0·398	0·80	0·20
4·848	9·90	19·03	0·377	0·93	0·26

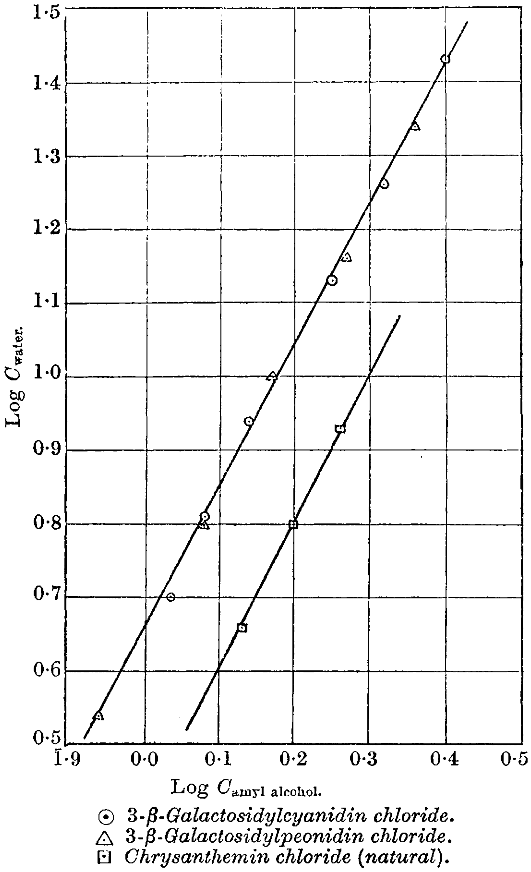
Mixtures of chrysanthemin and idaein chlorides.

Wt. in mg.		G.-mols. $\times 10^{-6}$.		Distribution No.	
Id.	Chrys.	Id.	Chrys.	1.	2.
2·980	2·985	5·930	5·836	15·37	15·49
3·902	3·887	7·765	7·599	14·41	14·53
5·066	5·060	10·080	9·892	14·04	14·50
2·408	4·810	4·792	9·404	14·95	14·41
4·992	2·435	9·934	4·760	15·96	15·96
6·026	2·039	11·990	3·986	12·92	13·04

II. *With amyl alcohol.* Willstätter and Bolton (*Annalen*, 1916, **412**, 136) give the distribution ratio for idaein chloride as 16·9 for the first shaking and 14·9 for the second. Assuming that the high distribution in the first case is due to the presence of a small amount of cyanidin, and that the second shaking is therefore correct, this figure agrees very closely with that obtained for a similar concentration of 3-β-galactosidylcyanidin chloride, namely, 15·1.

The assumption made should be valid because the distribution number for idaein chloride must always *rise* as the solutions become less concentrated. For an explanation of the table of results on p. 2726 compare Part IX (p. 2722).

FIG. 1.



ω-O-*Tetra-acetyl-*β-*galactosidoxy-*4-*acetoxy*-3-*methoxyacetophenone.*—ω-Hydroxy-4-acetoxy-3-methoxyacetophenone (see Part IX) (7 g.) and *O*-tetra-acetyl-α-galactosidyl bromide (18·5 g.) were dissolved in dry benzene (50 c.c.) at 35°, and dry silver carbonate (20 g.) added. A vigorous reaction ensued and the temperature rose quickly to 45°; this was maintained for 45 minutes by warming in a water-bath. The solution was then refluxed for 15 minutes, filtered, and the product precipitated as a gum by the addition of light petroleum. Thus gum was washed with hot and then with cold water, dissolved in acetic anhydride (20 c.c.) and pyridine (10 c.c.), kept for 12 hours, and poured into cold water. The product was obtained as an amorphous solid by repeated precipitations from methyl-alcoholic solution by means of water (yield, 7 g.) (Found : C, 54·2; H, 5·5. $C_{25}H_{30}O_{14}$ requires C, 54·2; H, 5·4%).

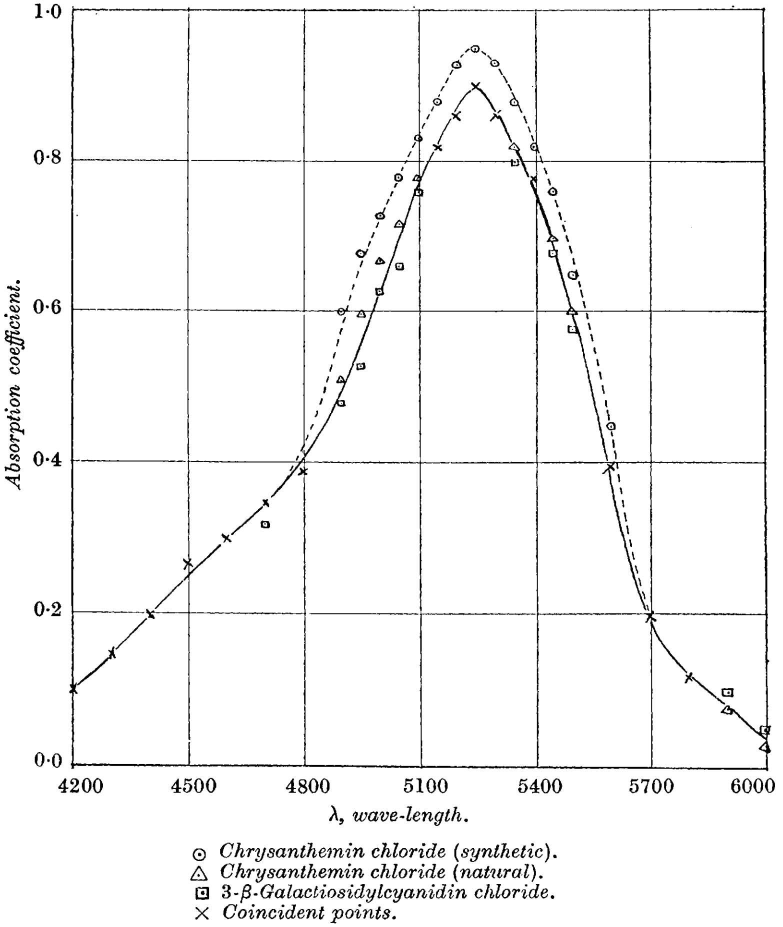
7-*Hydroxy*-5-*benzoyloxy*-3-O-*tetra-acetyl*-β-*galactosidoxy*-4′-*acetoxy*-3′-*methoxyflavylium Chloride*.—ω-O-Tetra-acetyl-β-galactosidoxy-4-acetoxy-3-methoxyacetophenone (2·0 g.) and *O*-benzoylphloroglucinaldehyde (1·6 g.) were dissolved in dry ethyl acetate (70 c.c.) and the solution was saturated with hydrogen chloride at 0° and kept in the ice-chest for 36 hours. The deep red solution was then filtered, and the product obtained as a dark red amorphous solid by precipitation with dry ether (*ca.* 750 c.c.) (yield, 2·5 g.) [Found : C, 54·7; H, 4·7; Cl, 5·8. $C_{29}H_{27}O_{12}Cl,2H_2O$ (*benzoylgalactosidylpeonidin chloride*) requires C, 54·3; H, 4·8; Cl, 5·6%].

3-β-*Galactosidylpeonidin Picrate*.—The finely powdered, crude benzoylgalactosidylpeonidin chloride (2·5 g.) was dissolved in 8% aqueous sodium hydroxide (50 c.c.) in an atmosphere of nitrogen, and the solution kept at room temperature for 3 hours. Sufficient 7% hydrochloric acid to make the solution 2% acid was then added and the solution was heated to 60° in order to complete the formation of the oxonium salt. After cooling, an equal volume of saturated aqueous picric acid was added to the filtered solution and the mixture was kept for 12 hours. The dark red precipitate was recrystallised by dissolving it in warm ethyl alcohol, adding an equal volume of hot aqueous picric acid (saturated in the cold), and cooling. The product was obtained in microscopic, dark red-brown needles (2·0 g.) (Found : C, 46·0; H, 4·1; N, 5·6. $C_{28}H_{27}O_{19}N_3,H_2O$ requires C, 46·2; H, 4·0; N, 5·8%).

3-β-*Galactosidylpeonidin Chloride* (II).—3-β-Galactosidylpeonidin picrate (2·0 g.) was triturated with warm absolute methyl alcohol (100 c.c.), the solution filtered and acidified with 13% methyl-alcoholic hydrogen chloride (20 c.c.), and the chloride obtained as an amorphous, dark red solid by precipitation with dry ether (*ca.* 500 c.c.). This material (0·5 g.) was crystallised by dissolving it in 0·5% hydrochloric acid (9 c.c.), adding 5% ethyl-alcoholic hydrogen chloride (50 c.c.), and keeping the solution in the ice-chest for 3 days. The substance was obtained as microscopic dark red-brown needles with a dark green reflex (yield, 0·25 g.) (Found : C, 48·5; H, 5·2; Cl, 6·5; CH_3O, 5·9; loss at 105° in a high vacuum, 8·2. $C_{22}H_{23}O_{11}Cl,2{\cdot}5H_2O$ requires C, 48·6; H, 5·2; Cl, 6·5; CH_3O, 5·7; H_2O, 8·3%. Found in anhydrous material : C, 52·8; H, 4·6; Cl, 7·2; CH_3O, 6·1. $C_{22}H_{23}O_{11}Cl$ requires C, 53·0; H, 4·7; Cl, 7·1; CH_3O, 6·2%).

3-β-Galactosidylpeonidin chloride is readily soluble in water and dilute acids, giving dark brownish-red solutions when concentrated and orange-red solutions when dilute. It is readily soluble in alcohol to a bluish-red solution. Addition of sodium carbonate to its solutions or to the solid gives a stable violet coloration; sodium hydroxide gives a similar colour which rapidly becomes green and then yellow.

FIG. 2.



The distribution of 3-β-galactosidylpeonidin chloride between amyl alcohol and 0·5% hydrochloric acid was determined by the standard method with the following results :

Wt. in mg.	G.-mols. × 10^{-6}.	Dist.	K.	Log C_W.	Log C_{AA}.
2·395	4·403	20·47	0·232	0·54	0·05
4·108	7·550	16·08	0·232	0·80	0·08
4·601	8·457	14·53	0·209	0·86	0·09
6·262	11·514	13·00	0·224	1·00	0·17
9·031	16·605	11·03	0·239	1·16	0·27
13·330	24·50	9·41	0·239	1·34	0·36

Absorption Curves.—The specimen of natural chrysanthemin chloride was kindly sent to us by Professor Willstätter; it was not recrystallised. The specimen of synthetic material was specially prepared for this purpose and was exceptionally pure. Consequently the slight divergence shown in the curves is not significant.

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The micro-analyses were carried out in the laboratory of Dr.-Ing. A. Schoeller of Berlin.

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