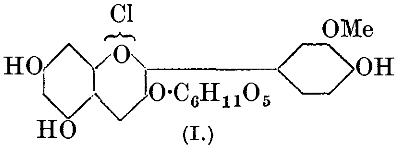
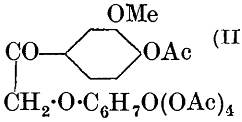
CCCLXXV.—*Experiments on the Synthesis of Anthocyanins. Part IX. Synthesis of Oxycoccicyanin Chloride. Observations on the Distribution Numbers of the Anthocyanins.*

By LEOPOLD FERDINAND LEVY and ROBERT ROBINSON.

IN a communication which has been submitted to the Biochemical Society, Miss K. E. Grove and one of us have shown that one of the pigments of American cranberries (*Oxycoccus macrocarpus*, Pers.) is 3-β-glucosidylpeonidin chloride (I), and it happened that this substance had been synthesised before it was thus identified as a natural product.



(I.)



(II.)

The present communication deals with the synthesis of the anthocyanin but not with the comparison with the natural product. The ketonic-glucoside component for the synthesis could in this instance be obtained by the method used for the callistephin intermediate, starting from the 4-hydroxy-ω-acetoxy-3-methoxyacetophenone of Nolan, Pratt, and Robinson (J., 1926, 1968). The sodium derivative of the related dihydroxymethoxyacetophenone reacted with acetic anhydride, yielding ω-hydroxy-4-acetoxy-3-methoxyacetophenone, $AcO{\cdot}C_6H_3(MeO){\cdot}CO{\cdot}CH_2{\cdot}OH$, and from this a tetra-acetyl-β-glucoside was obtained in the usual manner.

The remaining stages of the synthesis of (I) were also effected in accordance with our normal procedure and offered no difficulty.

Distribution Numbers of Anthocyanins.—It has been found that the distribution numbers of œnin chloride and of oxycoccicyanin chloride vary with the concentration of the solution in such a way that, in order to derive a distribution constant, the concentration in the amyl alcohol must be squared. Incidentally the agreement of natural with synthetic œnin chloride over a range of concentrations was very satisfactory, but the significance of the phenomenon is also of general interest. It appears to us to show that the salts are associated to double molecules in aqueous solution and occur as single molecules in amyl alcohol.

Certainly the colour of the solutions is quite different, those in amyl alcohol being much bluer-red than those in 0·5% hydrochloric acid. The concentration of chloridion is so great relatively to that of the anthocyanin that we need not consider the question of ionic dissociation; whether the oxonium kations are free in the solution or bound to chlorine ions by electrovalencies, the fact remains that association of either the salts or kations occurs in the aqueous medium.

The difficulty experienced in separating the anthocyanins and anthocyanidins by crystallisations from aqueous acid solutions is probably connected with this phenomenon.

It is also probably of the same nature as that involved in the blueing effect of tannin on œnin solutions (Willstätter and Zollinger, *Annalen*, 1916, **412**, 212); the tannin is evidently able to attach itself to the œnin molecule even in the presence of much mineral acid, but whether the effect is due to the colour of this complex or disruption of the œnin complexes is not clear.

EXPERIMENTAL.

ω-*Chloroacetovanillone.*—The method of Pratt and Robinson (J., 1923, **123**, 753) was modified and the yield greatly improved.

The following are the main points : (a) The reaction mixture was heated on the steam-bath after the second addition of aluminium chloride until evolution of hydrogen chloride ceased. (b) The ethereal mother-liquor on evaporation gave a light brown crystalline mass (30 g.), which after one crystallisation from benzene–light petroleum furnished the pure material (25 g., m. p. 102°). (c) Very crude material may be easily purified by distillation, b. p. 207—210°/22 mm.

4-*Hydroxy*-ω-*acetoxy*-3-*methoxyacetophenone*.—This substance has been prepared by a modification of the method of Nolan, Pratt, and Robinson (J., 1926, 1968).

A mixture of ω-chloroacetovanillone (10 g.), anhydrous potassium acetate (9 g.), glacial acetic acid (3 c.c.), and absolute ethyl alcohol (72 c.c.) was refluxed for 1 hour. The filtered solution was evaporated to dryness under diminished pressure, and the product dried in a vacuum over solid potassium hydroxide. The ω-acetoxy-ketone was extracted with hot benzene, from which it crystallised on concentration (yield, 6·4 g.; colourless prisms, m. p. 110°, were obtained on crystallisation from benzene–light petroleum).

ω : 4-*Dihydroxy*-3-*methoxyacetophenone*.—4-Hydroxy-ω-acetoxy-3-methoxyacetophenone (12·5 g.) was heated with 16% aqueous sodium hydroxide (70 c.c.) on the steam-bath. The *sodium* salt began to crystallise in light yellow plates before the whole of the substance had passed into solution. The cooled mixture was submitted to filtration, and the solid washed with alcohol and dried (9 g.). It crystallised from hot water (charcoal) in almost colourless, rhombic plates (Found: Na, 9·8. $C_9H_9O_4Na,1{\cdot}5H_2O$ requires Na, 9·9%).

The sodium salt (1 g.) was heated with 2*N*-acetic acid (10 c.c.) on the steam-bath until it dissolved; on cooling, ω : 4-*dihydroxy*-3-*methoxyacetophenone* crystallised (0·7 g.). The hydrated substance crystallised from water in colourless flat needles, m. p. 158—160° after being dried at 100° (Found in dried material: C, 59·0; H, 5·6. $C_9H_{10}O_4$ requires C, 59·3; H, 5·5%). The aqueous solution of this carbinol reduces Fehling's solution immediately in the cold and gives a deep violet coloration with ferric chloride.

ω-*Hydroxy*-4-*acetoxy*-3-*methoxyacetophenone*.—The sodium salt of ω : 4-dihydroxy-3-methoxyacetophenone (14·5 g.), suspended in water (72 c.c.), was shaken with a solution of acetic anhydride (10 c.c.; 1·5 mols.) in chloroform (150 c.c.) for 15 minutes; complete dissolution then occurred. The chloroform layer was separated, and the aqueous layer extracted twice with chloroform (60 c.c.). Water (14·5 c.c.) was added to the combined chloroform extracts, which were then evaporated to dryness under diminished pressure at 25°. The colourless residue crystallised from water in colourless, voluminous, flat needles (11·2 g.), m. p. 88—90°. After being dried in a vacuum at 80°, the substance melted at 96° (Found: C, 56·9; H, 5·5; loss on heating at 100° in a vacuum, 3·9. $C_{11}H_{12}O_5,\frac{1}{2}H_2O$

requires C, 56·7; H, 5·6; $\frac{1}{2}H_2O$, 3·9%. Found in anhydrous material: C, 59·1; H, 5·4. $C_{11}H_{12}O_5$ requires C, 58·9, H, 5·4%). The *carbinol* reduces Fehling's solution quickly in the cold, is insoluble in alkalis, and does not give a ferric chloride reaction. Further acetylation by heating with acetic anhydride produced ω : 4-diacetoxy-3-methoxyacetophenone, m. p. 76° (Nolan, Pratt, and Robinson, *loc. cit.*, give m. p. 73°).

ω-O-*Tetra-acetyl*-β-*glucosidoxy*-4-*acetoxy*-3-*methoxyacetophenone* (II).—Dry silver oxide (20 g.) was added to a solution of ω-hydroxy-4-acetoxy-3-methoxyacetophenone (8·4 g.; dried at 100° in a vacuum) and *O*-tetra-acetyl-α-glucosidyl bromide (27 g.) in dry benzene (50 c.c.) at 40°. When the mixture was shaken, the temperature quickly rose to 70°. After agitation for 30 minutes the cooled mixture was boiled and submitted to filtration. The filtrate was diluted with light petroleum (300 c.c.); the tetra-acetylglucoside was then precipitated as a straw-coloured gum. After being washed with hot and with cold water, the gum was converted, by repeated dissolution in methyl alcohol (15 c.c.) and precipitation by means of water, into an amorphous colourless solid (6·0 g.), which crystallised from dry ether in colourless plank-shaped needles, m. p. 74—76° (decomp.) (Found: C, 54·0; H, 5·6. $C_{25}H_{30}O_{14}$ requires C, 54·2; H, 5·4%). The methoxyl content estimated was too high, possibly as the result of loss of acetyl groups.

7-*Hydroxy*-5-*benzoyloxy*-3-O-*tetra-acetyl*-β-*glucosidoxy*-4′-*acetoxy*-3′-*methoxyflavylium Chloride*.—Dry hydrogen chloride was passed into a filtered solution of ω-*O*-tetra-acetyl-β-glucosidoxy-4-acetoxy-3-methoxyacetophenone (6·0 g.) and 2-*O*-benzoylphloroglucinaldehyde (5·0 g.) in dry ethyl acetate (100 c.c.) cooled to 10° and protected from access of moisture. A small quantity of the aldehyde separated in the course of 2 hours. After standing for 2 days, the deep red mixture was submitted to filtration, and the filtrate diluted with dry ether (500 c.c.). The dark red precipitate of the glucosidic flavylium chloride was collected, well washed with dry ether to remove hydrogen chloride, and dried over solid potassium hydroxide (yield, 6·0 g.) [Found: C, 55·5; H, 4·7; Cl, 4·9, 5·1. $C_{39}H_{37}O_{17}Cl,1\frac{1}{2}H_2O$ requires C, 55·7; H, 4·8; Cl, 4·2%. $C_{33}H_{31}O_{14}Cl,1\frac{1}{2}H_2O$ (loss of 3 acetyl groups) requires C, 55·5; H, 4·75; Cl, 5·0%].

The salt is readily soluble in alcohol to a reddish solution with a violet tinge and it is insoluble in cold 0·5% hydrochloric acid. The alcoholic solution gives a purplish-violet coloration with sodium hydroxide. Concentrated and dilute aqueous sodium carbonate solutions give bluish-green colorations.

3-β-*Glucosidylpeonidin Picrate*.—The crude pyrylium salt (6·0 g.) was finely powdered and mixed with 8% sodium hydroxide solution

(144 c.c.) which had been cooled to 10°; air was excluded from the apparatus by hydrogen. After 3 hours, the solution was acidified with 7% hydrochloric acid (260 c.c.) to give a 2% hydrochloric acid solution and the clear, deep red solution was warmed to 60° to complete the formation of the oxonium salt. A saturated aqueous picric acid solution (400 c.c.) was added to the cooled solution; 3-β-glucosidylpeonidin picrate was then precipitated as a reddish mass (3·9 g. after drying). The crude powdered material was washed with ethereal picric acid in order to remove peonidin picrate; the derivative crystallised from aqueous picric acid in reddish-brown, microscopic, plank-shaped needles (2·9 g.), which darkened at 110° and decomposed at 165—180° (Found: C, 43·2; H, 4·6; N, 5·6; loss at 105° in a high vacuum, 9·3. $C_{28}H_{25}O_{18}N_3,4{\cdot}5H_2O$ requires C, 43·5; H, 4·4; N, 5·4; $4H_2O$, 9·3. Found in dried material: C, 47·8; H, 3·9; N, 5·7. $C_{28}H_{25}O_{18}N_3,0{\cdot}5H_2O$ requires C, 48·0; H, 3·7; N, 6·0%). It is evident that the salt tenaciously retains solvent of crystallisation.

3-β-*Glucosidylpeonidin Chloride* (*Oxycoccicyanin Chloride*) (I).—The pure crystalline picrate (2·7 g.) was dissolved in 2% methyl-alcoholic hydrogen chloride (150 c.c.). The chloride was precipitated by the addition of dry ether (1100 c.c.) as a reddish-brown flocculent mass (1·25 g.). This material was crystallised by dissolving it in 0·5% hydrochloric acid (10 c.c.), a nearly saturated solution, and then adding 5% ethyl-alcoholic hydrogen chloride (50 c.c.). The anthocyanin crystallised on stirring in dark brown, rectangular, flat needles (0·9 g.) (Found: C, 49·1; H, 5·2; Cl, 6·6; CH_3O, 5·8; loss at 110° in a vacuum, 8·1, 7·8, 7·3. $C_{22}H_{23}O_{11}Cl,2H_2O$ requires C, 49·4; H, 5·1; Cl, 6·6; CH_3O, 5·8; $2H_2O$, 6·8%. Found in anhydrous material: C, 53·1; H, 4·3; Cl, 7·0; CH_3O, 6·3. $C_{22}H_{23}O_{11}Cl$ requires C, 53·0; H, 4·6; Cl, 7·1; CH_3O, 6·2%).

3-β-Glucosidylpeonidin chloride occupies an intermediate position between callistephin chloride and œnin chloride and its colour reactions reflect this circumstance.

Callistephin is barely affected by ferric chloride, but œnin gives a noticeable deepening of the colour of the solution; glucosidylpeonidin exhibits this property but to a less degree than œnin. Callistephin dissolves in aqueous sodium carbonate to a reddish-violet solution, œnin gives a bluish-violet, and 3-glucosidylpeonidin gives a rich, full violet. As is the case with callistephin and œnin, the colour is unchanged by addition of sodium hydroxide, although fading occurs as the result of ring fission.

In the ferric chloride oxidation test described in Part VII (p. 2697) 3-glucosidylpeonidin and peonin exhibit great stability (1·90 mg. of the former in 50 c.c.; the peonin and peonidin solutions were matched with this), whereas the peonidin solution is decolorised in 10 minutes. The distribution number of the anthocyanidin is mentioned below and colour reactions in buffered solutions and absorption measurements are described elsewhere.

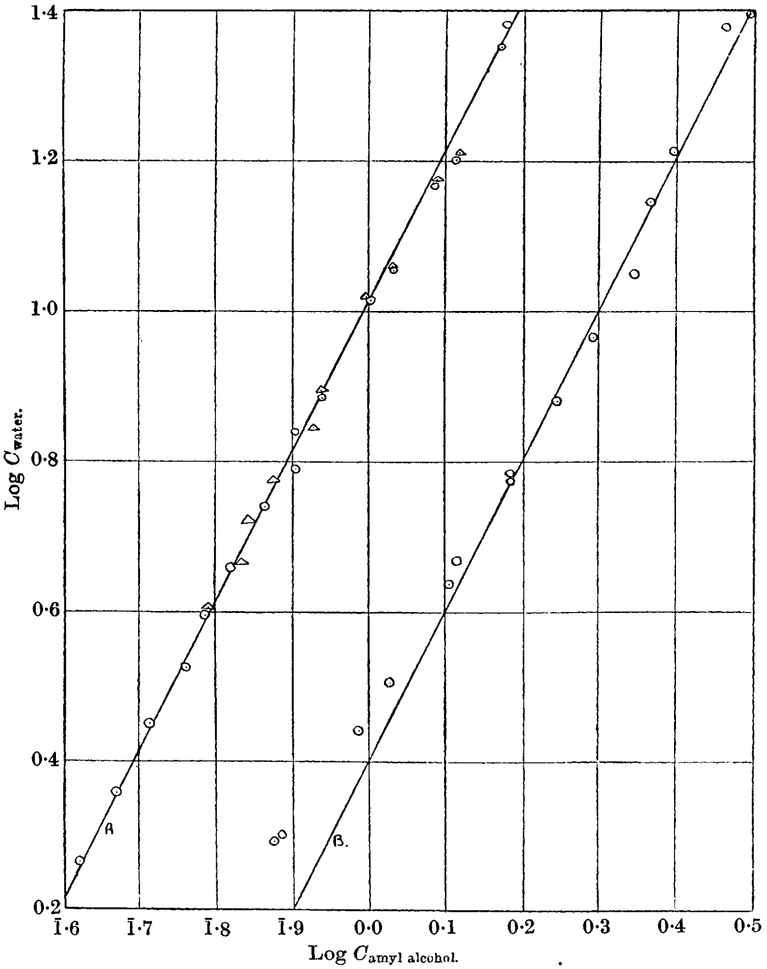
TABLES OF DATA.

Substance.		Distribution No.		G.-mols. $\times 10^{-6}$ for second (calc.).	Log C_W.		Log C_{AA}.		$K = \frac{C^2_{AA}}{C_W} \times 10^2$.
(in mgs.).	(in g.-mols. $\times 10^{-6}$).	First shaking.	Second shaking.		First.	Second.	First.	Second.	
Synthetic œnin chloride.									
14·8	25·41	5·90	6·23	23·86	1·3777	1·3497	0·1759	0·1716	9·42 9·86
9·96	17·10	7·57	7·67	15·81	1·1987	1·1640	0·1121	0·0835	10·60 10·07
7·246	12·44	8·67	8·85	10·78	1·0554	1·0151	0·0327	0·0023	10·23 9·76
5·007	8·59	10·1	10·4	7·71	0·8879	0·8400	$\bar{1}$·9388	$\bar{1}$·9050	9·76 9·33
4·065	6·98	11·5	11·77	6·18	0·7910	0.737	$\bar{1}$·9044	$\bar{1}$·8618	10·42 9·70
3·035	5·21	12·7	13·4	4·55	0·6578	0.5953	$\bar{1}$·8206	$\bar{1}$·7849	9·62 9·42
2·280	3·91	14·7	15·5	3·34	0·5235	0·4504	$\bar{1}$·7599	$\bar{1}$·7138	9·92 9·45
1·600	2·75	16·93	17·53	2·28	0·3579	0·2742	$\bar{1}$·6675	$\bar{1}$·6016	9·49 8·49
1·320	2·267	18·4	—	—	0·2670	—	$\bar{1}$·6200	—	9·40 —
0·975	1·674	19·95	—	—	0·1271	—	$\bar{1}$·5237	—	8·33 —
3-β-Glucosidylpeonidin chloride.									
14·96	28·00	11·11	11·70	24·89	1·3960	1·3420	0·4930	0·4643	0·389 $\times 10^2$. 0·387
10·000	18·71	13·17	14·20	16·24	1·2110	1·1445	0·3930	0·3630	0·376 0·382
7·17	13·42	18·49	17·36	11·20	1·0500	0·9663	0·3460	0·2887	0·435 0·408
5·00	9·36	18·80	20·10	7·60	0·8807	0·7838	0·2445	0·1840	0·406 0·384
4·00	7·48	20·55	21·88	5·95	0·7750	0·6672	0·1858	0·1145	0·395 0·365
3·00	5·61	22·69	24·53	4·34	0·6374	0·5053	0 1038	0·0275	0·372 0·355
2·00	3·74	26·10	27·70	2·77	0·4423	0·3014	$\bar{1}$·9878	$\bar{1}$·8850	0·339 0·295
1·45	2·71	27·36	30·56	1·96	0·2932	0.1352	$\bar{1}$·8750	$\bar{1}$·7775	0·286 0·263
Œnin chloride from Fogarina grapes (compare Part VIII).									
10·215	17·54	7·46	7·47	16·23					10·54 9·78
7·310	12·54	8·57	8·68	11·48					10·09 9·45
5·073	8·71	10·05	10·80	7·83					9·77 10·24
3·910	6·71	11·22	11·70	5·96					9·72 9·24
3·105	5·33	12·79	13·31	4·65					10·02 9·51

Distribution Numbers of Anthocyanins.—In order to avoid the preparation of a standard solution we had formed the habit of making direct comparisons between the aqueous acid and amyl-alcoholic layers; the fact that this procedure eliminated a weighing led to anomalous results and the cause of these was easily traced to the effect of varying the concentration.

We still, however, prefer the direct comparison of the two layers and in adopting this device it is necessary to make the composition of the solvent, in the two solutions compared, identical. Thus it might be desired to take M c.c of the amyl-alcoholic (or other solvent) layer and N c.c. of the acid layer; one would then make up the M c.c.

FIG. 1.



△ = *Natural substance.* ⊙ = *Synthetic substance.*
A = *Œnin chloride.* B = 3-β-*Glucosidylpeonidin chloride.*

of pigmented alcohol together with N c.c. of $\frac{1}{2}$% hydrochloric acid to 50 c.c. (say) by means of ethyl alcohol and compare this with N c.c. of the aqueous layer together with M c.c. of amyl alcohol also made up to 50 c.c. by means of ethyl alcohol. [The amyl alcohol and the acid employed must have been previously brought into equilibrium with one another; this should be done in the following manner. Amyl alcohol is shaken and washed with several changes of ½% or 1% hydrochloric acid; this amyl alcohol should then be used to saturate the acid of ½% or 1% concentration which is to be used in the experiments.] A green filter is very serviceable in colorimetric work with anthocyanins.

The tables on p. 2720 give the results of experiments on the distribution of œnin chloride, natural and synthetic, between amyl alcohol and 0·5% hydrochloric acid and similar data for 3-β-glucosidyl-peonidin chloride. The actual distribution number for œnin varies from 5·9 to 20·0 as the mg./50 c.c. varies from 15 to 1.

Notes on the Tables—' First shaking ' and ' Second shaking ' refer to the first and second extractions of the ½% hydrochloric acid solution of the pigment by means of an equal volume of amyl alcohol. The equation, $mD^2/(100—D) = K$, gives the distribution constant (K) in terms of the mass of substance taken (m) and the distribution number (D).

In order to compare different anthocyanins directly the mass has been expressed as g.-mol. × 10^{-6} and account has of course been taken of the solvent of crystallisation known as the result of analysis to be present. The curves, log C_{AA}–log C_W, are seen to be straight lines with the slope ½ and therefore $C^2_{AA}/C_W = K$. As the solutions become very dilute, the distribution number appears not to conform to the law very accurately; this may be due to experimental error in the colorimetry or to a partial dissociation of the associated complexes.

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