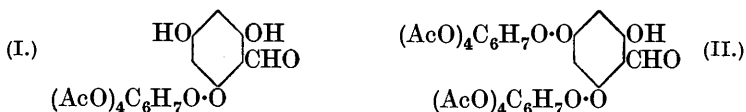


327. *Experiments on the Synthesis of Anthocyanins.*
Part XVI. A Synthesis of Malvin Chloride.

By ROBERT ROBINSON and A. R. TODD.

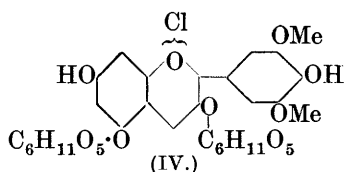
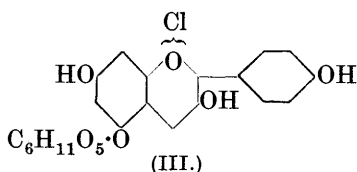
THE hypothesis that the diglucosidic anthocyanins pelargonin, cyanin, peonin, malvin and hirsutin are 3 : 5-diglucosides of the respective anthocyanidins was put forward in Parts VI—XIII (J., 1931, 2665—2742) and it has been amply justified in the case of hirsutin by the synthesis recorded in the preceding paper. The extension of synthetical confirmation of our view of their molecular structure to other anthocyanins could not be satisfactorily effected until an improved method had been worked out for the preparation of the 2-glucoside of phloroglucinaldehyde. After numerous trials of more indirect methods, which were uniformly unsuccessful, we eventually isolated a pure 2-O-tetra-acetyl- β -glucosidylphloroglucinaldehyde (I) and 2 : 4-di-(O-tetra-acetyl- β -glucosidyl)phloroglucinaldehyde (II) from the products of interaction of phloroglucinaldehyde, tetra-acetyl- α -glucosidyl bromide, and potassium hydroxide in aqueous acetonitrile solution.



The constitutions assigned to these substances are justified by the results obtained on condensing them with suitable second component ω -hydroxyacetophenones and hydrogen chloride to flavylum salts.

The acetylated monoglucoside, condensed with ω : 4-dihydroxyacetophenone and hydrogen chloride, yielded ultimately pelargonin chloride (III), the constitution of which was demonstrated by León, Robertson, Robinson, and Seshadri (J., 1931, 2685). This method of synthesis constitutes a great improvement on the

older process and it will be extended to other 5-monoglucosides of anthocyanidins.



The acetylated diglucoside can be employed for the preparation of diglucosidic flavylium salts, the description of which is reserved; this fact, however, suffices to establish the formula (II). The condensation of the glucoside (I) with ω -*O*-tetra-acetyl- β -glucosid-oxy-4-acetoxy-3 : 5-dimethoxyacetophenone (Levy, Posternack, and Robinson, J., 1931, 2701) was carried out in the usual manner in dry ethyl acetate solution by saturation at 0° with hydrogen chloride and a good yield of a partly de-acetylated product was obtained.

On hydrolysis with aqueous sodium hydroxide and reconstitution of the flavylium salt by treatment with hydrochloric acid, a solution of a substance having all the properties of malvin chloride was obtained, but only a small proportion of the material present in the liquid could be induced to crystallise. It appears that the solubility of anthocyanins in dilute hydrochloric acid is greatly increased by the presence of foreign substances; recrystallisation was very readily effected and the product was identified with malvin chloride by a careful comparison with a specimen of the pigment of natural origin.

We are greatly indebted to Professor P. Karrer for the provision of this specimen, which was exceptionally pure.

No divergences in the behaviour of the natural and the synthetic colouring matter could be detected and the synthesis (which follows the scheme given in the preceding paper for the analogous case of hirsutin) proves that malvin chloride has the constitution (IV) proposed in Parts VI—XIII.

Malvin chloride was first isolated by Willstätter and Mieg (*Annalen*, 1915, 408, 122) from the flowers of *Malva sylvestris* and recognised as a diglucoside of a delphinidin dimethyl ether chloride; references to the later developments of the structural aspect of the subject will be found in a memoir on the synthesis of cenin chloride (malvidin 3-glucoside) (J., 1931, 2701).

Analytical evidence confirming the view that the glucose residues are not contained in a bioside unit is available from the recent work of Karrer and de Meuron (*Helv. Chim. Acta*, 1932, 15, 507), who have shown that malvone, a crystalline product of oxidation of malvin by means of hydrogen peroxide, yields glucose and syringic acid on hydrolysis in alkaline solution.

Malvin is probably a widely distributed anthocyanin; it has been isolated in substance from the flowers of *Primula viscosa* by Karrer and Widmer (*Helv. Chim. Acta*, 1927, **10**, 32), and an examination of flower pigments according to a system described by (Mrs.) G. M. Robinson and one of us (*Biochem. J.*, 1931, **25**, 1687) has shown that a substance having the properties of malvin occurs in species of *Althaea*, *Armeria*, *Clarkia*, *Dierama*, *Dodecatheon*, *Epilobium*, *Fuchsia*, *Geranium*, *Gladiolus*, *Lathyrus*, *Lythrum*, *Nigella*, *Petunia*, *Phlox* and *Silene*; during the present season this list is being greatly extended.

EXPERIMENTAL.

2:4-Dicarbethoxyphloroglucinaldehyde.—This substance was first obtained by Dr. A. Robertson in the course of early attempts to prepare phloroglucinaldehyde-2-glucoside. 5% NaOH aq. (32 c.c.) was added drop-wise with constant stirring to a solution of phloroglucinaldehyde (6 g.) and ethyl chloroformate (4 c.c.) in Me₂CO (30 c.c.) at 0°, and the mixture was then stirred at 0° for 30 mins. NaHCO₃ aq. pptd. a reddish oil, which slowly solidified (1.5 g.) after being washed with H₂O, and crystallised from aq. MeOH in clusters of colourless needles, m. p. 76° (Found: C, 52.7; H, 4.6; OEt, 30.6. C₁₃H₁₄O₈ requires C, 52.3; H, 4.7; OEt, 30.2%).

The constitution of this *dicarbethoxy*-compound is analogous to that of *O*-dibenzoylphloroglucinaldehyde and follows from the fact that it condenses with *ω*-hydroxyacetophenone derivatives to give flavylum salts. The yield was not improved by increasing the amount of ethyl chloroformate used, as this led to the formation of much gummy material. All attempts to obtain a monocarbethoxy-derivative were unsuccessful.

The introduction of a glucose residue into the *dicarbethoxy*-compound was investigated and doubtless succeeds, but the method was abandoned when the direct process described below was discovered. The partial hydrolysis of *O*-dibenzoylphloroglucinaldehyde by means of HCl aq. (5% and 10%) in Me₂CO solution yielded phloroglucinaldehyde only. Attempts to prepare a naphthoylphloroglucinaldehyde and to introduce the triphenylmethyl group in various ways were also unsuccessful.

Glucosidation of Phloroglucinaldehyde.—A solution of phloroglucinaldehyde (9.1 g.) and *O*-tetra-acetyl- α -glucosidyl bromide (24.9 g.) in acetonitrile (90 c.c.) was cooled in melting ice, and 10% KOH aq. (35 c.c.) added in small portions with vigorous shaking, the temp. being kept between 5° and 10°. The mixture, which had separated into two layers, was then agitated for 24 hrs., filtered, and evaporated almost to dryness under diminished pressure on the steam-bath. The dark gummy residue was shaken with CHCl₃ (ca. 300 c.c.); a portion dissolved to a dark red solution, and the residue was mainly unchanged phloroglucinaldehyde (ca. 5 g.) and KBr. The CHCl₃ solution was dried, and the solvent removed in vac. and replaced by Et₂O (300 c.c.). After filtration from insol. flocculent material, the ethereal extract was shaken twice with 5% Na₂CO₃ aq., which removed the monoglucosidic fraction, leaving the diglucoside in the ethereal solution (A).

2-O-Tetra-acetyl- β -glucosidylphloroglucinaldehyde (I).—The Na₂CO₃ extract (above) was immediately acidified with HCl aq., the gummy ppt. taken up

in Et_2O , the extract washed with H_2O and dried, and the solvent distilled. The dark red syrup obtained was triturated with a little MeOH ; crystn. was greatly facilitated by seeding and after being kept in the ice-chest for a day the crystals were collected and washed with a little MeOH (yield, 1.6 g.; a further 0.3 g. separated from the mother-liquor). The substance separated from 80% MeOH in small, pale yellow crystals, m. p. 78—80° after sintering at 75° (Found: C, 51.3; H, 5.1%).

Closer examination of these crystals showed that two distinct substances were present, but the separation presented some difficulty, as the best solvent for crystn. proved to be 80% MeOH , in which both components were easily soluble. By repeated fractional crystn. from this solvent we obtained the more sparingly sol. constituent in small, hard, faintly yellow prisms which rapidly became opaque on exposure to air owing to loss of solvent of crystn. After drying in a desiccator it had an indefinite m. p. 77—80°. This material retained H_2O which was only removed on drying at 100° in a high vac. (Found: C, 51.3; H, 5.2; loss at 100° in a high vac., 1.5. $\text{C}_{21}\text{H}_{24}\text{O}_{13} \cdot 0.5\text{H}_2\text{O}$ requires C, 51.1; H, 5.1; H_2O , 1.8%. Found in anhyd. material: C, 52.1; H, 5.0. $\text{C}_{21}\text{H}_{24}\text{O}_{13}$ requires C, 52.1; H, 5.0%).

The pure substance had $[\alpha]_{5461}^{19} - 47.84^\circ$ in CHCl_3 ($c = 1.442$). 2-O-Tetra-acetyl- β -glucosidyl-4-O-methylphloroglucinaldehyde has $[\alpha]_{5461}^{19} - 47.52^\circ$ in CHCl_3 ($c = 1.494$), which indicates that the configurations of the two compounds are analogous.

The mother-liquors of the crystn. of the tetra-acetyl glucoside, m. p. 77—80°, afforded colourless needles, m. p. 109—110°, after drying in a desiccator (Found: C, 50.2; H, 5.3%); this substance, which is obtained in relatively small yield, is being collected for more complete examination.

That the substance, m. p. 77—80°, is indeed 2-O-tetra-acetyl- β -glucosidyl-phloroglucinaldehyde was proved by using it to synthesise pelargonin chloride.

3 : 7 : 4'-Trihydroxy-5-O-tetra-acetyl- β -glucosidoxylavylum Chloride. — A solution of the above glucoside (0.2 g.) and ω : 4-dihydroxyacetophenone (0.1 g.) in CHCl_3 -AcOEt, when saturated with dry HCl at 0°, rapidly became reddish-brown and green-fluorescent. It was kept at 0° for 60 hrs., dry Et_2O added, and the pptd. flavylum salt washed with Et_2O . It formed a dark red-brown powder with a green reflex (0.22 g.) (Found: C, 52.9; H, 4.9; Cl, 4.9. $\text{C}_{29}\text{H}_{29}\text{O}_{14}\text{Cl} \cdot \text{H}_2\text{O}$ requires C, 53.2; H, 4.7; Cl, 5.4%).

Pelargonin Chloride (III).—The dark greenish-brown solution of the acetylated flavylum chloride (0.2 g.) in cold 10% NaOH aq. (2 c.c.) was kept under H for 90 mins., 20% HCl aq. (1.54 c.c.) added, and the 2% acid solution so obtained was warmed to 50° and allowed to cool. Pelargonin chloride soon separated as a mass of small scarlet-red needles, which were recryst. from hot 2% HCl aq. (Found in anhyd. material: C, 53.8; H, 4.8; Cl, 7.4. Calc. for $\text{C}_{21}\text{H}_{21}\text{O}_{10}\text{Cl}$: C, 53.8; H, 4.5; Cl, 7.6%). It exhibited all the characteristic properties of the anthocyanin.

2 : 4-Di-(O-tetra-acetyl- β -glucosidyl)phloroglucinaldehyde (II).—When the ethereal solution (A, above) was dried and concentrated, the diglucoside separated as a white cryst. mass; it was washed with Et_2O (yield, 1.6 g.) and recrystallised from EtOH , forming a mass of very fine matted needles, m. p. 194—195°, $[\alpha]_{5461}^{19} - 52.5^\circ$ in CHCl_3 ($c = 1.466$) (Found: C, 51.6; H, 5.3. $\text{C}_{35}\text{H}_{42}\text{O}_{22}$ requires C, 51.6; H, 5.2%). The compound condenses with ω : 3 : 4-triacetoxyacetophenone in presence of HCl to a product which

furnishes a diglucosidic flavylum salt on hydrolysis. The properties of this cyanidin diglucoside will be described later.

7 : 4' - *Dihydroxy* - 3 : 5 - *di* - β - *glucosidyl* - 3' : 5' - *dimethoxyflavylum Chloride* (*Malvin Chloride*) (IV).—A solution of 2-*O*-tetra-acetyl- β -glucosidylphloroglucinaldehyde (1.8 g.) and ω -*O*-tetra-acetyl- β -glucosidoxy-4-acetoxy-3 : 5-dimethoxyacetophenone (2.37 g.) in dry AcOEt (32 c.c.) was saturated with dry HCl, and kept for 3 days, at 0°, dry Et₂O (500 c.c.) then added, and the bright red ppt. washed with Et₂O and dried (2.6 g.) (Found : C, 51.2; H, 5.4; Cl, 2.3; MeO, 6.5. The fully acetylated compound, C₄₇H₅₃O₂₈Cl, requires C, 52.8; H, 5.0; Cl, 3.3; 2MeO, 5.8%. These figures indicate that partial de-acetylation has occurred and that HCl was lost on drying, a behaviour which is characteristic of several anthocyanins both natural and synthetic. If the compound obtained above were a hexa-acetate, C₄₁H₄₇O₂₃Cl, 2H₂O, which lost its H₂O of crystn. and 33% of its Cl on drying, the theoretical values would be C, 51.2; H, 5.2; Cl, 2.4; 2MeO, 6.4%, in good agreement with the analyses).

The acetylated flavylum salt (2.5 g.) was hydrolysed with 10% NaOH aq. (21 c.c.) for 90 mins. into the dark greenish-brown solution. 20% HCl aq. (16.25 c.c.) was introduced, and the 2% acid solution thus obtained was heated to 60° to complete the formation of oxonium salt and allowed to cool. The conc. of HCl was then increased to 7%, and after 36 hrs. the dark red-violet solution had deposited the anthocyanin as a mass of dark reddish-brown needles with a greenish lustre. These were dissolved in warm 0.3% HCl aq., the conc. of the acid was increased in the cold to 7%, and after a few hrs. the chloride crystallised in fine needles with a beetle-green lustre; decomp. 165° without melting [Found in material dried for 18 hrs. over H₂SO₄ in a desiccator : C, 48.0; H, 5.4; Cl, 4.8; MeO, 8.9. C₂₉H₃₅O₁₇Cl, 2H₂O requires C, 47.9; H, 5.4; Cl, 4.9; 2MeO, 8.5%. Loss at 110° in a high vac. over P₂O₅, 5.9. Required loss of wt. for 2H₂O + 24% of Cl, 6.2%. Found in material dried at 110° in a high vac. : C, 51.0; H, 5.1; Cl, 3.9; MeO, 9.3. C₂₉H₃₅O₁₇Cl requires C, 50.4; H, 5.1; Cl, 5.1; MeO, 9.0%. These values indicate a partial loss of HCl on drying, and this has been recorded of malvin chloride from natural sources. In the anhyd. material, C 51.0% corresponds to *M* 682. But C₂₉H₃₅O₁₇Cl has *M*, 690.5, and, assuming the difference of 8.5 units is due to loss of HCl, we find there has been a loss of approximately 24% of Cl on drying. Similarly, the difference between the obs. and the calc. value for Cl (1.27) give the figure 24.7% as the proportion of Cl lost. C₂₉H₃₅O₁₇Cl, 2H₂O, losing 2H₂O and 24% of its content of HCl, requires C, 51.0; H, 5.1; Cl, 4.0; 2MeO, 9.1%, in good agreement with the analyses].

Willstätter and Mie \ddot{g} (*loc. cit.*) give the formula C₂₉H₃₅O₁₇Cl, 8H₂O to the air-dried substance and state that it loses all the solvent of crystn. in a desiccator; they observed loss of HCl on drying in vac. at 110°.

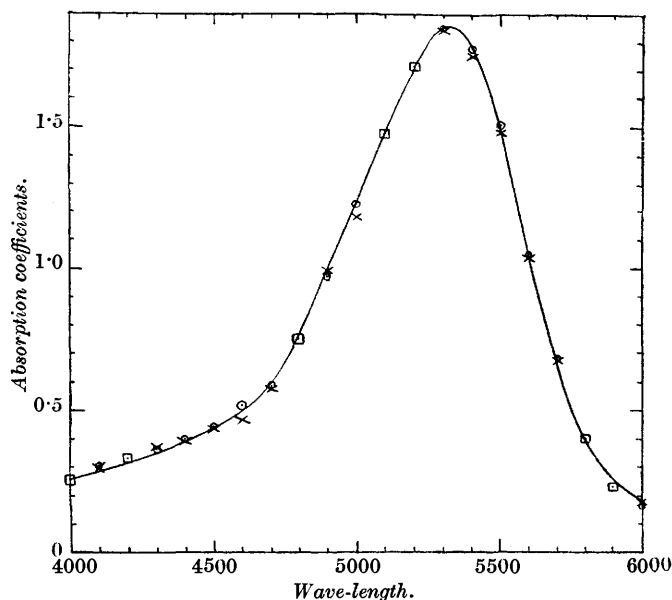
Karrer and Widmer (*loc. cit.*) dried the salt at 100°/12 mm., and under these conditions loss of HCl appears to have been prevented.

The initial crystn. of the synthetical salt was effected with difficulty, but recrystn. was facile. An attempt to improve the process by evaporation of the 2% acid solution to dryness in vac. at 25—30° resulted in hydrolysis to a monoglucoside. This was isolated as the picrate and chloride and from its colour-reactions, distribution ratio, and other properties the salt was certainly malvenin chloride (5-glucoside) and not cænin chloride (3-glucoside). The examination of this specimen will be resumed.

Comparison of Synthetic Malvin Chloride (S) with the Specimen of Natural Origin (N).

(S) had a more brilliant reflex than (N), but when the salts were crystallised side by side under identical conditions from 5% HCl aq. the appearance of the needle crystals in mass and under the microscope was identical. The mode of pptn. and crystn. and the microscopic appearance of the respective picrates were also identical.

The solubilities of (N) and (S) in HCl aq., the colour of the aq. solutions before and after the addition of EtOH, the colour change on great dilution with MeOH, the rate of formation of pseudo-base and extent of recovery on acidification, and the slow rate of oxidation in very dil. solution in the presence



x Malvin chloride (synthetic). o Malvin chloride (natural).
 □ Coincident points.

of FeCl_3 were all examined under strictly comparable conditions without revealing divergences of behaviour. All the properties of solutions of (S) were found to be identical with those of (N) (see below for one explicable exception); addition of AcONa gave a violet coloration; Na_2CO_3 , a bright greenish-blue, and NaOH changed this to green and then yellow at the same rate for (S) and (N).

The colour reactions in buffered solutions of graded p_H (Robertson and Robinson, *Biochem. J.*, 1929, **23**, 35) were examined, the odd-numbered specified solutions being used. The results were identical for (S) and (N) and have already been recorded (*loc. cit.*) for a different specimen of malvin chloride.

The absorption in the visible region of the spectrum is illustrated (see Fig.) and the curves refer to observations with solutions of $0.18N/10^4$ in 0.1% methyl-alc. HCl.

The distribution number (*isoamyl alcohol*) of malvin chloride (S and N) was found to be almost zero, even in the presence of NaCl. It was therefore determined with *n*-butyl alcohol under the conditions prescribed in the preceding paper. (N) had distribution number (butyl alcohol) 10.4 (3.06 mg. in 50 c.c. of the mixed solvents), and on a second shaking the number rose to 11.6; this is doubtless the result of an association phenomenon.

On the other hand (S) (3.00 mg.) had distribution number 15.1, and this fell to 12.3 on the second shaking. A very small content of monoglucoside is responsible for this and in order to effect a comparison equal weights (5.80 mg.) of (S) and (N) were dissolved in 0.5% HCl aq. (50 c.c. each) equilibrated with *n*-butyl alcohol, and the solutions shaken with 3 successive vols. (50 c.c.) of butyl alcohol equilibrated with 0.5% HCl aq. The aq. solutions were then made of the same conc. (colorimeter) by slight dilution of the (N) solution with 0.5% HCl aq.; the distribution numbers were then found to be 10.8 in both cases. Examination of the butyl-alcohol extracts showed that only the first extracts exhibited any divergence, that from (S) being a little more intensely coloured. The second butyl-alc. extracts were treated with sat. AcONa aq. (15 c.c.), giving a violet aq. layer and a much paler reddish-violet butyl-alc. layer; identical appearances at first and on keeping. The third butyl-alc. extract was treated with sat. Na₂CO₃ aq. (15 c.c.); the blue-green coloration of the aq. layers slowly became greener and then yellow and the solutions (S and N) exhibited an identical green fluorescence. Thus the trace of monoglucoside is entirely removed by one extraction with butyl alcohol.

Further comparisons of (S) and (N), including the behaviour with co-pigments (see preceding paper), were made and no point of difference in the behaviour of (S) and (N) could be detected; some of the co-pigment effects of malvin will be described in another place.

The authors thank the Royal Commissioners for the Exhibition of 1851 for a Senior Studentship awarded to one of them, and Imperial Chemical Industries Limited for grants.

THE DYSON PERRINS LABORATORY,
THE UNIVERSITY OF OXFORD.

[Received, June 23rd, 1932.]
