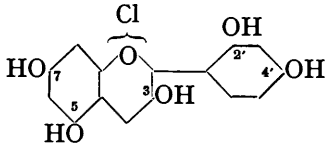
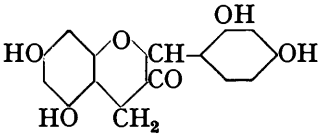
100. *A Synthesis of Pyrylium Salts of Anthocyanidin Type. Part XX. Morinidin Chloride. Observations on Cyanomaclurin.*

By E. H. Charlesworth, J. J. Chavan, and Robert Robinson.

Our main objective in applying the *O*-benzoylphloroglucinaldehyde method of synthesis of anthocyanidins (Part XV; Robertson and Robinson, J., 1928, 1526) to the preparation of morinidin chloride (I) (Willstätter and Schmidt, *Ber.*, 1924, **57**, 1945; Pratt and Robinson, J., 1925, **127**, 1137) was the elucidation of the relation of this salt to cyanomaclurin, to which A. G. Perkin (J., 1905, **87**, 715) has attributed the constitution (II) (enol form).

(I.)





(II.)

We consider that this view may yet prove to be the correct one, but it is unconfirmed and some possible alternatives are discussed below.

The condensation of *O*-benzoylphloroglucinaldehyde with fisetol (or an acylated fisetol) was carried out in ethyl acetate solution with the help of hydrogen chloride : although the desired 5-*O*-benzoylmorinidin chloride was produced, the process was not so smooth as in the case of benzoylcyanidin and difficulties detailed in the experimental section were also encountered in the hydrolysis.

Pure morinidin chloride has been characterised by means of its colour reactions in buffered solutions and by other properties; it exhibits the behaviour on reduction already noted by Pratt and Robinson (*loc. cit.*) and on which further comment must be made.

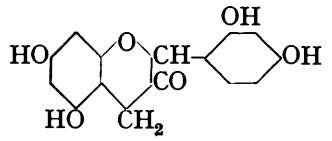
We have long been familiar with the facts that addition of zinc dust to a solution of a flavylium salt, *e.g.*, an anthocyanidin or an anthocyanin in dilute aqueous hydrochloric acid (say 0·5%), causes decolorisation and that on shaking with air the colour is regenerated. In the cases of cyanidin and cyanin we have satisfied ourselves that it is in fact the original pigment that is recovered and not, as was possible, an oxidatively coupled bis-derivative. For this purpose we used, *inter alia*, distribution-ratio methods (cf. Robinson and Robinson, *Biochem. J.*, 1931, **25**, 1687; 1932, **26**, 1647). The prolonged action of zinc dust, however, takes the reduction beyond this stage and the red colour of the anthocyanidin is not recoverable. Pratt and Robinson (*loc. cit.*) found that, if the reduction of morinidin is carried thus far, the aqueous solution contains a colourless substance that can be extracted by ether and gives a deep blue solution in cold dilute aqueous sodium hydroxide (cyanomaclurin gives a colourless solution under these conditions, but the deep blue colour is developed on heating). Thus there are two substances produced in the reduction of morinidin, one of which by analogy with cyanidin allows of re-oxidation to morinidin, and neither is identical with cyanomaclurin.

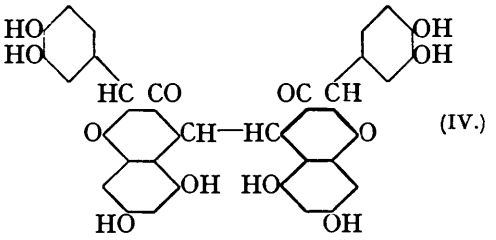
Kuhn and Winterstein (*Ber.*, 1932, **65**, 1742) have recently applied their zinc dust and pyridine method to the reduction of cyanidin and have observed the formation of a colourless hydrocyanidin which is very labile and in contact with air is re-oxidised to cyanidin. (The intense blue solution produced under these conditions appears to be due to the presence

of zinc, because the violet solution of cyanidin chloride in pyridine becomes deep blue on the addition of zinc chloride.)

The important point for the present purpose is that Kuhn and Winterstein consider that the oxidisable hydrocyanidin is dihydrocyanidin (III), and if this plausible view is justified, then cyanomaclurin can hardly be correctly represented by the expression (II). As already implied, cyanomaclurin shows no tendency to yield morinidin by facile oxidation in acid solution.

(III.)



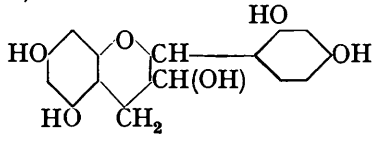


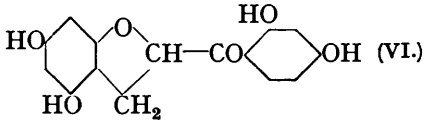
(IV.)

The loop-hole for the formula (II) is that the labile hydrocyanidin may be dihydro-bis-cyanidin (IV). We can discern no bar in theory to the assumption that ready reductive coupling may be reversed on oxidation, with the re-formation of the simple molecules. If, however, it transpires that the Kuhn–Winterstein theory is correct, then it seems probable that cyanomaclurin is, after all, $C_{15}H_{14}O_6$ and not $C_{15}H_{12}O_6$ in spite of the very impressive analytical evidence put forward by Perkin in favour of the latter formula.

The substance would then be regarded as the catechin of the morin group (V); this is supported by the general character of the substance, by its resemblance to catechin, and by the ready penta-acylation described by Perkin, but on this hypothesis the alkali colour reaction is a surprising property. Catalytic reduction of morinidin chloride has not yet afforded a crystalline substance of the formula (V) and we have also attempted the reduction of benzoylmorinidin without isolating homogeneous substances, even after full benzoylation of the products. This is doubtless due to the production of stereoisomerides (cf. Freudenberg, "Handbuch der Pflanzenanalyse," 1932, III, 392, for a review of stereoisomeric catechins).

(V.)





(VI.)

There are, of course, many possible constitutions for cyanomaclurin ($C_{15}H_{12}O_6$) other than that discussed (II); the objections to (II) apply equally to the isomeride in which the resorcyl nucleus is transferred to position 4, and, of the more remote possibilities, only (VI) would appear to be worthy of consideration in view of the closely analogous behaviour of catechin and cyanomaclurin. (VI) shares with (II) the rather serious disadvantage that it contains only four normal hydroxyl groups and the fifth hydroxyl group, detected by acetylation or benzoylation in pyridine solution, must be derived by enolisation of a ketone as suggested by Perkin (*loc. cit.*).

We are greatly indebted to Professor A. G. Perkin for specimens of cyanomaclurin and its benzoyl derivative which will enable us to continue this investigation. In the present communication we have only attempted a statement of the problem, the situation being that the weight of the analytical evidence points to the formula $C_{15}H_{12}O_6$ but the chemical evidence is strongly in favour of the recognition of cyanomaclurin as the catechin related to morin, which requires that the formula should be $C_{15}H_{14}O_6$.

Experimental.

ω-Acetoxyresacetophenone, $C_6H_3(HO)_2\cdot CO\cdot CH_2\cdot OAc$.—Contrary to the experience with phloroglucinol reported in the preceding communication, acetoxyacetonitrile and resorcinol undergo the Hoesch synthesis in a normal manner and the acetyl group is not removed under

the mild conditions of hydrolysis. A mixture of resorcinol (11·2 g., dried at 110°), acetoxyacetonitrile (10 g.), and Et_2O (80 c.c.) was sat. at 0° with HCl for 3 hr. (yield of ketimine hydrochloride, 25 g. or 98%). The salt, carefully freed from HCl by exposure to vac. over KOH, was mixed with 10 times its weight of H_2O and gently heated on a steam-bath until it passed into solution; simultaneously a clear brownish oil separated which quickly crystallised. It was recryst. from 500 c.c. of H_2O, forming needles and prismatic plates, 2—3 cm. long, m. p. 164·5° (yield, 14 g.) (Found : C, 56·9; H, 4·6. $C_{10}H_{10}O_5$ requires C, 57·1; H, 4·8%). Unlike ω : 2 : 4 : 6-tetrahydroxyacetophenone, this *substance* does not reduce Fehling's solution in the cold. On benzoylation in C_5H_5N by means of PhCOCl, it affords *ω-acetoxy-2 : 4-dibenzoyloxyacetophenone* (quantitative yield), long white needles from EtOH, m. p. 151·5° (Found : C, 68·8; H, 4·1. $C_{24}H_{18}O_7$ requires C, 68·9; H, 4·3%). On acetylation by means of boiling Ac_2O and a little C_5H_5N the acetoxyresacetophenone affords *ω : 2 : 4-triacetoxyacetophenone*, which, cryst. from EtOH, has m. p. 93·5°, unaltered by recrystn. (Found : C, 57·3; H, 4·9. $C_{14}H_{14}O_7$ requires C, 57·1; H, 4·8%).

Nierenstein, Wang, and Warr (*J. Amer. Chem. Soc.*, 1924, **46**, 2551) ascribe the m. p. 129° to a substance which they supposed to be ω : 2 : 4-triacetoxyacetophenone.

The *phenylhydrazone* of ω-acetoxyresacetophenone was prepared in aq. solution from the ketone, $PhNH{\cdot}NH_2$,HCl, and KOAc. The orange-yellow crystals from H_2O had m. p. 152° (Found : N, 9·4. $C_{16}H_{16}O_4N_2$ requires N, 9·3%).

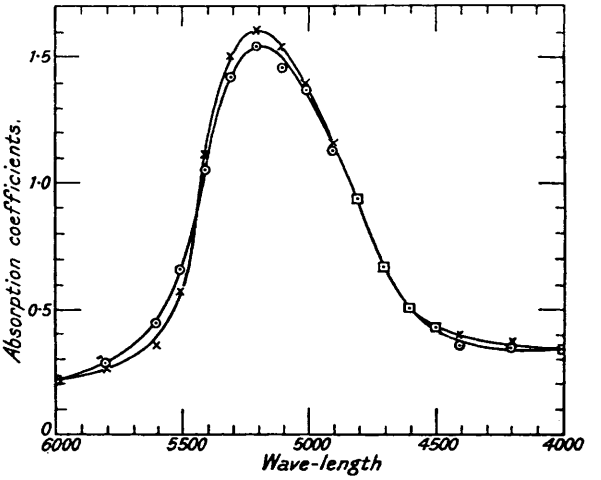
ω : 2 : 4-Trihydroxyacetophenone (Fisetol).—The monoacetate (5 g.), when heated on the steam-bath with Na_2CO_3 aq. (100 c.c. of 5%) for 3 hr., gradually disappeared and, on cooling, prisms of fisetol (1·6 g. or 35%) separated. After recrystn. (charcoal) a colourless product, m. p. 189°, was obtained (Found : C, 56·9; H, 4·9. Calc. for $C_8H_8O_4$: C, 57·1; H, 4·8%). This work was resumed after a considerable interval and the various available methods have been compared. That now described using acetoxyacetonitrile is the most satisfactory, but the hydrolysis of the monoacetate is best effected by the following method. 25 G. in 250 c.c. of 10% NaOH aq., kept for 2 hr. at room temp. and then acidified, gave 18·3 g. of fisetol, m. p. 190° to a red liquid (Found : C, 56·7; H, 4·9%). This m. p. was attributed to the substance by Sonn and Falkenheim (*Ber.*, 1922, **55**, 2975), by Nierenstein, Wang, and Warr (*loc. cit.*), as well as by Karrer and Biedermann (*Helv. Chim. Acta*, 1927, **10**, 441), who employed glycollonitrile in a Hoesch synthesis with resorcinol. On the other hand the acetylation of fisetol with boiling Ac_2O and a little C_5H_5N has furnished the ω : 2 : 4-triacetoxyacetophenone mentioned above, obstinately, m. p. 93·5°, and identical in all respects with the analysed specimen. Hydrolysis of the triacetate, m. p. 94°, by means of 2*N*-NaOH gives back fisetol, m. p. 189—190°. Again, all three groups of authors report the existence of a phenylhydrazone, m. p. 109°, but we have not encountered this compound and have obtained only the *osazone*, yellow needles from MeOH, m. p. 204·5° (Found : N, 15·6, 15·6, 15·8. $C_{20}H_{18}O_2N_4$ requires N, 16·2%). In this case there was probably a divergence in the conditions : we employed a boiling aq. solution of $PhNH{\cdot}NH_2$,HCl together with KOAc or alternatively $PhNH{\cdot}NH_2$ and AcOH aq. on the steam-bath.

ω : 2 : 4-Tribenzoyloxyacetophenone is readily obtained by the benzoylation of fisetol (Ph·COCl and C_5H_5N); it crystallises from EtOH in slender colourless needles, m. p. 140° (Found : C, 72·4; H, 4·5. $C_{29}H_{20}O_7$ requires C, 72·5; H, 4·2%).

5-O-Benzoylmorinidin Chloride.—For the prepn. of this salt it is necessary to use pure 2-*O*-benzoylphloroglucinaldehyde and as second component we have employed fisetol, ω-acetoxyresacetophenone, or ω : 2 : 4-triacetoxyacetophenone; the acetyl groups are removed by hydrolysis and apparently the same salt is produced in each case. Perhaps fisetol itself gives the best results, but in all expts. there was a considerable formation of by-products. A typical expt. is the following :—A solution of *O*-benzoylphloroglucinaldehyde (10·4 g., m. p. 200°) and fisetol (7·7 g.) in EtOAc (250 c.c.) and EtOH (10 c.c.) was satd. with HCl at 0—5° and kept for 3 days in a stoppered vessel. The dark red microcryst. ppt. was collected (8·7 g.), washed with EtAc + Et_2O and then Et_2O, and recrystallised as follows. The crude salt (5 g.) was dissolved in hot MeOH (80—90 c.c. containing 4 c.c. 20% HCl,MeOH), and the filtered solution concentrated to 20 c.c. On keeping, the benzoylmorinidin chloride crystallised in fine red needles (6 operations afforded 22·9 g.) (Found : C, 57·3; H, 4·1; Cl, 7·0; loss at 110° in high vac. over P_2O_5, 9·9. $C_{22}H_{15}O_7Cl,2H_2O$ requires C, 57·1; H, 4·1; Cl, 7·7; H_2O, 7·8%). The loss at 110° is partly HCl, because the dried product has Cl, 3·5%. Benzoylmorinidin chloride is almost insol. in hot 1% HCl aq. and is rather sparingly sol. in EtOH to a bluish-red solution. In Na_2CO_3 aq. it gives a blue-violet solution, becoming pure blue on dilution. In $C_5H_{11}{\cdot}OH$ it gives a permanganate-coloured solution, blue on addition of Na_2CO_3 and violet with NaOAc; no ferric reaction

was observed. From the mother-liquors of the condensation, a dark reddish-brown powder was pptd. by addition of Et_2O; it weighed approx. 75% of the crude benzoylmorinidin chloride. On the hypothesis that the formation of the by-product might be due to dehydration between hydroxyls at 2′ and 3, a specimen was refluxed with 5% HCl,EtOH for 6 hr. and on addition of conc. HCl aq. to the filtered solution the substance separated in micro-cryst. form; it was recrystallised in the same way (Found : C, 60·2; H, 4·4; Cl, 3·8; loss at 110° in high vac. over P_2O_5, 8·5. Found in dried material: C, 64·5; H, 3·8; Cl, 3·7%). The substance does not resemble morinidin in its general properties; it dissolves in Na_2CO_3 aq. to a red-violet solution. Similar products were obtained in the course of some early abortive attempts to prepare morinidin from benzoylmorinidin.

Morinidin Chloride. (I)—(A) Benzoylmorinidin chloride (5·6 g.) was gradually dissolved in 95% EtOH (350 c.c. containing about 1% HCl) and the filtered solution concentrated to about 50 c.c. by distillation. Conc. HCl aq. (20 c.c.) was added to the hot solution and, on keeping, a crop (0·82 g.) separated as a red cryst. powder; further concn. afforded a second fraction (0·83 g.) identical in properties, but the later fractions (2·1 g.) were contaminated with impurities similar to those obtained in the original condensation. The bright red crystals were kept in vac. over H_2SO_4 (Found : C, 52·8; H, 4·0; Cl, 9·9; loss at 110° in high vac. over P_2O_5, 6·3. $C_{15}H_{11}O_6Cl,H_2O$ requires C, 52·8; H, 3·8; Cl, 10·4; H_2O, 5·3%). Willstätter and Schmidt (*loc. cit.*) described hydrates with $3H_2O$, $2H_2O$, and H_2O, but the monohydrate lost H_2O in a desiccator and the dihydrate lost 1·5 H_2O. Pratt and Robinson (*loc. cit.*) obtained a dihydrate (dried in a desiccator).



⊙ *Morinidin chloride, acid hydrolysis.*
× *Morinidin chloride, alkaline hydrolysis.*
⊡ *Coincident points.*

(B) Benzoylmorinidin chloride (4·5 g.) was dissolved in EtOH (42 c.c.) and 20% NaOH aq. (30 c.c.) with exclusion of air by hydrogen, which was used to agitate the liquid for 4 hr. Conc. HCl aq. (35 c.c.) was added to the green liquid, which was then warmed at 80—90° for 45 min. and kept for 12 hr. The solids were collected and washed with 10% HCl aq. to remove NaCl; the crude salt was dissolved in EtOH (15 c.c.), and hot 10% HCl aq. added to the filtered solution, which was concentrated to incipient crystn. The slender red needles of morinidin chloride were dried in vac. over H_2SO_4 (Found : C, 51·1; H, 4·1; Cl, 10·0; loss at 110° in high vac. over P_2O_5, 12·9. $C_{15}H_{11}O_6Cl,1·5H_2O$ requires C, 51·4; H, 4·0; Cl, 10·1; H_2O, 7·7%). The large loss on heating indicates loss of HCl as well as of H_2O and this is confirmed on analysis (Found in material heated at 110° in vac. over P_2O_5 : C, 58·2; H, 3·5; Cl, 7·5. $C_{15}H_{11}O_6Cl$ requires C, 55·7; H, 3·4; Cl, 11·0%). This sample closely resembles that prepared by acid hydrolysis as under (A) in all its properties.

The absorption coefficients were measured over the range 4000—6000 Å. using $0·25N/10^4$-solutions in 0·1% HCl,MeOH. The specimens (A) and (B) vary slightly (see fig.), but the head of the band is at 5200 Å. in both cases.

The salt exhibits the solubilities and acid and alkali reactions previously described. With Na_2CO_3 aq. it gives a blue colour in dilute solutions and violet-blue to red-violet in more conc. solutions. Acidification of the decolorised solution formed by warming the dil. alc. solution does not restore the colour, the pseudo-base being subject to further change. It also gives the striking blue colour noted by Pratt and Robinson by reduction with zinc dust and addition of NaOH aq. to the ethereal extract.

The following effects are observed in a range of buffered solutions (Robertson and Robinson, *Biochem. J.*, 1929, **23**, 35), the odd-numbered solutions being employed. On mixing 1% and 20% HCl aq. light orange; (1) weak permanganate; (3) slightly bluer; (5) still bluer; (7) blue-violet; (9) slightly bluer; (11) violet-blue; (13), (15), and (17) each bluer than preceding solution and dichroism less; after 20 min. (1) and (17) exhibit slight fading; others unchanged; after

2 hr. (1) slightly more orange-tinted; (17) greenish-blue; others unchanged; after 24 hr. 1% and 20% HCl aq. unchanged; (1) very faint pink tinge; (3) colourless; (5) as (1); (7) violet-pink; (9) not as intense as (7); (11) blue-violet; (13) blue; (15) green shade just appearing; (17) light green; (7) and (11)—(15) are still fairly intensely coloured. Conc. HCl aq. fails to restore the colour in (1), (3), and (5).

Reduction.—Reference has already been made to the behaviour with Zn dust in 0·5% HCl aq. SO_2 and $TiCl_3$ were ineffectual, but in presence of Pt black in MeOH solution both morinidin chloride (1 mol.) and benzoylmorinidin chloride (1 mol.) readily absorbed 2H, absorption of a further mol. being always very slow, and indeed in some expts. the reduction stopped when 1 mol. had been absorbed. The products could not be crystallised and exhibited little resemblance to cyanomaclurin. Instead of a deep blue colour being produced in dil. NaOH aq. on heating, the coloration (morinidin expt.) was at once greenish-blue, rapidly becoming dichroic, green and red in thick layers and, on heating, brown and finally brown-orange.

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THE DYSON PERRINS LABORATORY, UNIVERSITY OF OXFORD.
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