494. *The Chemistry of Fungi. Part XXI.* Asperxanthone and a Preliminary Examination of Aspergillin.*

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Isolated from several varieties of *Aspergillus niger*, the black amorphous pigment aspergillin has been obtained free from iron and nitrogenous material. On oxidation with hydrogen peroxide aspergillin gave small yields of acetaldehyde, oxalic acid, and mellitic acid; the two acids were also formed with potassium permanganate. Nitric acid gave the same three products along with a little picric acid.

From the mycelium of several strains of *A. niger* there have been isolated small amounts of a new 1-hydroxydimethoxymethylxanthone, asperxanthone $C_{13}H_5O_3(OMe)_2Me$, which on demethylation gave nor-rubrofusarin. Comparison of the ultra-violet absorption spectrum of asperxanthone with the spectra of a number of synthetic polyhydroxyxanthones did not assist in the location of the substituents in the new xanthone.

An examination of the well-known black pigment aspergillin produced by varieties of *Aspergillus niger* was undertaken as part of a comprehensive study of melanin-like pigments of plants and animals in progress in these laboratories. The first recorded isolation of this substance appears to have been made by Linossier (*Compt. rend.*, 1891, **112**, 489) who extracted the spores of *A. niger* with aqueous ammonia, obtaining the pigment by acidification of the extracts. On slender evidence, chiefly because he obtained ferric oxide on ignition of this product, which he named aspergillin,† Linossier concluded that it was analogous in nature and function to hæmatin, a view which he reaffirmed later (*ibid.*, 1891, **112**, 807; 1910, **151**, 1075) without presenting further experimental evidence. Hugouenq and Florence (*Bull. Soc. Chim. biol.*, 1920, **2**, 133) isolated aspergillin with aqueous sodium hydroxide and re-precipitated it from ammonia, obtaining a product which gave the following analytical results: C, 47·3; H, 7·2; N, 11·4; Ash, 5·0%. On the basis of its physical characteristics Bortels (*Biochem. Z.*, 1927, **182**, 301) concluded that aspergillin was probably identical with the humic acid of peat whilst Rippel and Walter (*ibid.*, 1927, **186**, 474) considered it to be a nitrogen-containing pigment of the melanin type. In a more systematic examination of the substance Quilico and de Capua (*Atti R. Accad. Lincei*, 1933, **17**, 93, 197; *Gazzetta*, 1933, **63**, 400) obtained material containing 0·35% of iron which they suggested was a constituent part of the molecule. By oxidation of the pigment these authors obtained mellitic acid and oxalic acid and quoted examples indicating an analogy between these results and those obtained from oxypyrrole blacks, melanins, and humic acid. They noted, however, that nitrogen may not be an essential constituent and suggested that the compound was similar to humic acid and the polymeric oxidation products of phenols. The relationship to humic acid was supported by the similarity of

* Part XX, preceding paper.

† The trivial name aspergillin has been applied since to other products from *Aspergilli* by Stanley (*Australian J. Sci.*, 1944, **6**, 151; *Australian J. Exp. Med. Sci.*, 1946, **24**, 133), Korenyako and Krasil'nikov (*Microbiologia, U.S.S.R.*, 1945, **14**, 347), Soltys (*J. Path. Bact.*, 1946, **58**, 278), and Kovalev (*Veterinaviya*, 1940, **25**, 40), but since this black pigment has invariably been referred to as aspergillin and the name has precedence we propose that it should be retained (cf. Tobie, *Nature*, 1946, **158**, 709).

the *X*-ray patterns obtained from the two compounds (Quilico and Rollier, *Chem. Abs.*, 1939, **33**, 8074, 8467).

In the present work aspergillin was isolated with alkali from several strains of *Aspergillus niger* and purified by a number of procedures. Dialysis followed by electrodialysis removed the greater part of the mineral constituents of the crude pigment, reducing the iron content from about 2% to 0·012%. Subsequently it was found that extraction of the crude aspergillin with hot water removed a mucilaginous polysaccharide and that treatment of the product with hot dilute sulphuric acid then gave a pigment free from nitrogen and iron. This was then digested with boiling methanol. When thus purified, aspergillin is a black, amorphous, acidic compound, having the properties of a cross-linked polymer and closely resembling humic acid, the inorganic constituents of which Pluguian and Hibbert (*J. Amer. Chem. Soc.*, 1935, **57**, 528) regard as impurities only. In agreement with the observations of Quilico *et al.* (*loc. cit.*) oxidation of aspergillin gave mellitic and oxalic acid but in addition small amounts of acetaldehyde were formed, whilst nitric acid also produced a little picric acid. The relationship of the purified pigment to humic acid has been further substantiated by a comparison of its infra-red spectrum with that of humic acid (Cannon and Sutherland, *Trans. Faraday Soc.*, 1945, **41**, 279).

The nitrogen content of crude aspergillin, which shows considerable variation, appears to be due to a protein-like component but whether this and the polysaccharide-like material are chemically bound to the pigment residue is not yet clear. The variation in nitrogen content and the ease with which the polysaccharide is eluted indicates that they are present as impurities.

Asperxanthone.—On extraction with methanol the defatted mycelium of *A. niger* N.R.R.L. 67, *A. niger* Van Tieghem 9029, and two strains of unknown origin gave, in addition to a little mannitol, a crystalline phenolic product in very low yield. This compound contains two methoxyl groups, a *C*-methyl group, and a hydroxyl group in the *o*-position to a carbonyl group. From the empirical formula $C_{13}H_5O_3(OMe)_2Me$ in conjunction with its general properties, including the absence of a reactive carbonyl group, the stability to warm acids and alkalis, and the formation of an unstable perchlorate and a diacetoborate, the phenol appeared to be a new 1-hydroxydimethoxymethylxanthone. On demethylation it furnished a trihydroxyxanthone; the ultra-violet absorption spectrum curve of this was practically identical with that recorded for nor-rubrofusarin, the orientation of which has not been determined (Mull and Nord, *Arch. Biochem.*, 1944, **4**, 419). With a small sample of nor-rubrofusarin kindly supplied to us by Professor Nord, the respective ultra-violet absorption spectra of the two compounds have been determined with a " Unicam " spectrograph used in this laboratory (cf. *Ann. Reports*, 1951, **48**, 347) and found to be almost identical. Further, as a mixture of the two products did not show a depression in the decomposition point it seems reasonably certain that they are identical, *i.e.*, the new xanthone is a nor-rubrofusarin dimethyl ether, which for convenience we have provisionally named asperxanthone.

As didemethylasperxanthone (nor-rubrofusarin) was not identical with the readily synthesised 1 : 5 : 6-, 1 : 6 : 7-, or 1 : 6 : 8-trihydroxy-3-methylxanthone and because the quantity of the compound available was insufficient for degradation experiments, attempts were made to deduce the position of the second and third hydroxyl groups from an examination of the ultra-violet absorption spectra of a number of hydroxyxanthones. Accordingly, the spectra of asperxanthone and nor-rubrofusarin have been compared with those of 1-, 2-, 3-, and 4-hydroxyxanthone, 1-hydroxy-5-methylxanthone, 1 : 3-, 1 : 6-, and 1 : 8-dihydroxyxanthone, 1 : 5- and 1 : 7-dihydroxy-3-methylxanthone, ravenelin (Raistrick, Robinson, and White, *Biochem. J.*, 1936, **30**, 1303), and 1 : 6 : 7- and 1 : 6 : 8-trihydroxy-3-methylxanthone. From an analysis of these results it has not been possible to establish a correlation between the ultra-violet spectra and the position of the hydroxyl groups. In this connection it would appear that the tentative structure assigned to nor-rubrofusarin by Mull and Nord (*loc. cit.*) based on the examination of the absorption spectra of a limited number of hydroxyxanthones is open to doubt.

The syntheses of 1 : 3 : 5- and 1 : 3 : 6-trihydroxyxanthone, 1 : 5-dihydroxy-3-methylxanthone, 1 : 3 : 5- and 1 : 3 : 6-trihydroxyxanthone, and 1 : 5 : 6-, 1 : 6 : 7-, and 1 : 6 : 8-

trihydroxy-3-methylxanthone have been effected by the application of Michael's method (*Amer. Chem. J.*, 1883, **5**, 81) as used by Kostanecki and his collaborators for the preparation of numerous substituted xanthones (see, *e.g.*, *Ber.*, 1891, **24**, 1896) in which equimolecular proportions of the requisite phenol and phenolic acid are heated with a dehydrating reagent, usually acetic anhydride. In the present work it has been found that optimum results are obtained when the amount of anhydride employed is that required to abstract two molecular proportions of water and when the amount of reactants employed did not exceed the 0·05-molar scale.

Experimental

Isolation of Aspergillin.—From pilot experiments with six strains of *Aspergillus niger*, *viz.*, *A. niger* van Tieghem 9029, *A. niger* van Tieghem 330, *A. niger* van Tieghem 32, *A. niger* N.R.R.L. 67, and two strains of unknown origin it was found that *A. niger* van Tieghem 9029 gave the best yield of black pigment, and it was employed in the present work; the pigments obtained from the remaining five species were indistinguishable from aspergillin of *A. niger* van Tieghem 9029. Experiments with a number of liquid media with varying times of incubation show but little variation in the yield, although the following conditions appeared consistently to be slightly superior and were adopted. A modification of the sporulation medium described by Moyer, Wells, Stubbs, Kerrick, and May (*Ind. Eng. Chem.*, 1937, **29**, 777), *viz.*, glucose (91·3 g.), ammonium nitrate (0·45 g.), potassium dihydrogen phosphate (0·072 g.), hydrated magnesium sulphate (0·06 g.), and ferrous sulphate (0·01 g.), dissolved in water (1 l.), in standard " penicillin " flasks (in batches of 1000 flasks), each containing 1 l. of liquid medium, was inoculated with mould spores in the usual manner and incubated at 30° for 14 days. The mycelium was collected, drained for 24 hr., and then treated as follows.

(*a*) The mycelial felts from 500 flasks were kept in 1·5% aqueous sodium hydroxide (20 l.) with frequent vigorous agitation for 14 days, and the liquor was decanted through a large Buchner funnel (the slimy residue was pressed to remove as much solution as possible) and then clarified by filtration through muslin gauze and then through a sintered-glass (No. 3) funnel. Acidification of the resulting clear black liquid with 4% hydrochloric acid (8 l.) gave a black precipitate which slowly settled in the clear, yellow liquor, the greater part of which was siphoned off. On isolation the slimy product, which had been washed with water until the washings were free from chloride ion, was extracted with several portions of boiling methanol (each 1 l.) to remove small amounts of methanol-soluble products, giving a black amorphous solid (126 g.). Extraction of the mycelial felt with 2% aqueous ammonia gave a similar product.

(*b*) The air-dried, powdered mycelium (1 kg.) was extracted successively with light petroleum (b. p. 60—80°), and hot methanol, and then treated with 1·5% aqueous sodium hydroxide as in (*a*). The fine suspension obtained on acidification of the extract was collected by means of a Sharples supercentrifuge at 24,000 r.p.m., repeatedly washed until free from chloride ion, and collected in the same manner as above; the yield of air-dried product was *ca.* 10 g. The same final product was obtained when the extraction with alkali was carried out in an atmosphere of nitrogen.

After repeated extraction with alkali the mycelial residue still contained much black pigment which appeared to be insoluble.

Purification of the Pigment.—(a) *Removal of inorganic impurities.* Portions of freshly precipitated pigment were dialysed in collodion or Cellophane containers against running tap-water and then distilled water with reduction of the ash content. The product was then subjected to electrodialysis against distilled water. After 34 hr. the full working voltage of 300 v was necessary to maintain a current of 30 milliamp. and after a further period (30 hr.) the current passing, which had then fallen to 3 milliamp., was not reduced by continued application of the full voltage. The aspergillin was then collected, extracted with methanol, and dried, being obtained as a black hygroscopic powder, m. p. $>350°$. Four preparations of crude aspergillin having an average ash content of 1·995% and iron content of 0·203% had, on dialysis followed by electrodialysis, an ash content of 0·343% and an iron content of 0·030%.

(b) *Removal of nitrogen-containing compounds.* When aspergillin (3 g.) was heated under reflux with 12% hydrochloric acid (100 ml.) for 1½ hr. the cooled filtered hydrolysate reduced Fehling's and Molisch's reagents and gave a positive ninhydrin reaction but did not contain purine bases or phosphates. With phenylhydrazine it gave phenylglucosazone.

Extraction of crude aspergillin (100 g.) with boiling water gave a greyish gelatinous pre-

cipitate (1 g.) which, on purification from hot water, was obtained as a white powder soluble in 2N-sodium hydroxide and in hot water. This product which did not give a colour with iodine was hydrolysed with boiling 4% sulphuric acid, giving an almost quantitative yield of a reducing sugar which was isolated as phenylglucosazone. The identity of the latter was confirmed by the preparation of phenylglucosotriazole (Hahn and Hudson, *J. Amer. Chem. Soc.*, 1944, **66**, 735), m. p. and mixed m. p. 195—196°.

After the foregoing experiments it was found that crude aspergillin (containing polysaccharide-like material *ca.* 1% and nitrogen *ca.* 3·87%) was conveniently purified thus: The freshly isolated crude pigment (20 g.) was extracted (Soxhlet) with water for 8 hr. to remove the polysaccharide-like material and then dissolved in 2% aqueous sodium hydroxide (250 ml.) and reprecipitated with 2% sulphuric acid. The pigment was collected, well washed, and heated under reflux with 20% sulphuric acid (150 ml.) for 20 hr.; the nitrogen content was thereby reduced to 1·95%. Repetition of this process gave a nitrogen-free product which was then extracted with boiling methanol (Found, in specimen dried at room temperature over phosphoric oxide for 24 hr.: C, 58·5; H, 4·1; N, nil; *C*-methyl, 3·49; Ash, 0·06; Fe, 0·007%). Thus purified, aspergillin (air-dried) is a jet-black powder which is readily soluble in aqueous sodium hydrogen carbonate. On being dried in a vacuum at room temperature it becomes hygroscopic and then dissolves slowly in aqueous sodium hydroxide or sodium hydrogen carbonate. Both undried and dried material are insoluble in the usual organic solvents, but are soluble or are dispersed in liquid ammonia, and in molten catechol, resorcinol, salicylic acid, or pyrogallol, sparingly soluble (or dispersed by) molten quinol or *p*-hydroxybenzoic acid, and insoluble in liquid sulphur dioxide, anisole, furfuraldehyde, benzyl alcohol, molten phenol, *o*-, *m*-, or *p*-cresol, guaiacol, benzoic acid, or camphor. The infra-red absorption spectrum of the pigment as a paste with liquid paraffin and hexachlorobutadiene was determined over the region 2—15 μ. As is usual with compounds of high molecular weight the absorption bands noted were not very strong. The following bands observed may be attributed with reasonable confidence as follows: 3·1 μ bonded O–H; 3·5 μ C–H; 5·9 μ C:O as in CO_2H; 6·1 μ benzenoid ring structure.

Oxidation of Aspergillin.—(*a*) Aspergillin (5 g.) was added to 5N-nitric acid (100 ml.) containing urea (2 g.), and the stirred mixture kept at 60° for 1 hr. Next day the black insoluble residue was removed, the liquor was neutralised with 2N-ammonia and treated with an excess of silver nitrate, and the precipitate (1 g.) was collected, washed, dried, and boiled with saturated methanolic hydrogen chloride (20 ml.) for ½ hr. The residue (90 mg.) left on evaporation of the filtered methanolic solution was treated with excess of ethereal diazomethane and on distillation the product gave methyl oxalate (7 mg.), b. p. 90°/30 mm., m. p. 51°, and hexamethyl mellitate (15 mg.), b. p. 160°/0·01 mm., m. p. and mixed m. p. 188°, after purification from methanol (Found: C, 51·0; H, 4·2. Calc. for $C_{18}H_{18}O_{12}$: C, 50·7; H, 4·3%).

When the oxidation was effected with 50% nitric acid and the mixture kept at 95—100°, the distillate contained acetaldehyde which was converted into the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 158° (yield, 50 mg., from 20 g. of pigment). From the acidic residue oxalic acid was isolated as calcium oxalate, and then mellitic acid as the silver salt which was converted into hexamethyl mellitate. After the removal of the silver salt the aqueous liquor was repeatedly extracted with benzene and on evaporation the dried benzene extracts left a little picric acid which was isolated as quinoline picrate in red needles, m. p. 202°, alone or admixed with an authentic specimen.

(*b*) Hydrogen peroxide (60 ml.; 50-vol.) was added to a solution of aspergillin (1·5 g.) in 0·2N-sodium hydroxide (100 ml.) during 3 hr.; the reacting mixture was kept at below 40°. 15 Hours later the solution was neutralised with nitric acid and, after the removal of the excess of hydrogen peroxide with manganese dioxide, was treated with silver nitrate, giving a precipitate from which methyl oxalate (7 mg.) and hexamethyl mellitate (0·12 g.) were obtained in the usual manner.

Oxidation of aspergillin with hydrogen peroxide according to the procedure of Quilico and de Capua (*loc. cit.*) or a number of modifications of this method failed to yield a residue of mellitic acid on distillation of the reaction mixture. The distillate contained acetaldehyde, isolated as the 2 : 4-dinitrophenylhydrazone.

(*c*) Oxidation of aspergillin (3 g.), dissolved in 0·5N-sodium hydroxide (300 ml.), with potassium permanganate (8 g. in 150 ml. of water) on the steam-bath for ½ hr. gave an acidic product from which methyl oxalate (0·47 g.) and hexamethyl mellitate (1·2 g.) were isolated by way of their silver salts.

Asperxanthone.—The dried pulverised mycelium of *Aspergillus niger* (1 kg.) was extracted

(Soxhlet) with light petroleum (b. p. 60—80°) for 100 hr. and then with methanol for 150 hr. Concentration of the methanolic extract gave mannitol, m. p. 166°, after purification, identified by comparison with an authentic specimen. On evaporation the residual liquor then gave a black viscous product (45 g.) which was repeatedly extracted with ether. After having been washed with 0·5N-sodium hydroxide (300 ml. × 2), the combined ethereal extracts were evaporated, leaving a yellow amorphous residue (2 g.) which on repeated crystallisation from chloroform–alcohol gave *asperxanthone* in primrose-yellow needles (450 mg.), m. p. 203°, unchanged on sublimation in a high vacuum [Found: C, 67·2; H, 5·0; OMe, 21·7; *C*-Me, 5·6. $C_{13}H_5O_3(OMe)_2Me$ requires C, 67·1; H, 4·9; OMe, 21·7; *C*-Me, 5·3%]. This compound, which gives an intense green ferric reaction in alcohol, is readily soluble in alcohol or ether, insoluble in light petroleum, and dissolves with difficulty in 2N-sodium hydroxide, forming a yellow solution from which it is precipitated unchanged with acid. With warm perchloric and acetic acid asperxanthone gave an unstable perchlorate in bright yellow needles, m. p. 270° (decomp.), and with boroacetic anhydride in acetic anhydride a yellow boroacetate, m. p. 210—220° (decomp.), which, on treatment with warm water, regenerated the parent compound. Demethylated with hydriodic acid (10 ml; *d* 1·7) at 100° for 5 hr. asperxanthone (200 mg.) gave a product which separated from methanol in orange-yellow needles (100 mg.), m. p. 286—290° after darkening at 240°, undepressed on admixture with nor-rubrofusarin (*loc. cit.*); it sublimed unchanged at 200°/0·001 mm. (Found: C, 64·7; H, 4·2. Calc. for $C_{14}H_{10}O_5$: C, 65·1; H, 3·9%). This compound, which is readily soluble in alcohol and insoluble in water, gives an intense green-brown ferric reaction in alcohol and forms a deep yellow solution with aqueous sodium hydroxide which slowly becomes brown in air. Prepared by warm pyridine–acetic anhydride at 100° for 5 hr., the *triacetate* separated from acetic acid and then alcohol in deep golden-yellow needles, m. p. 210°, having a negative ferric reaction (Found: C, 62·3; H, 4·4. $C_{20}H_{16}O_8$ requires C, 62·5; H, 4·2%).

1 : 6 : 8-*Trihydroxy*-3-*methylxanthone*.—A mixture of *p*-orsellinic acid (Robertson and Robinson, *J.*, 1927, 2199) (2·1 g.), phloroglucinol (1·55 g.), and acetic anhydride (3 ml.) was gradually heated to 200° and the distillate discarded. The residue was then distilled in a vacuum and the fraction, b. p. 270—290°/0·5 mm., obtained as a yellow glass, was dissolved in ether. Extraction of this solution with 2N-sodium hydroxide, followed by acidification of the extract, gave a product from which 1 : 6 : 8-*trihydroxy*-3-*methylxanthone* was separated with hot ether. Crystallised from 95% alcohol this xanthone formed pale cream-coloured needles (2·6 g.), m. p. 275° (decomp.), moderately soluble in benzene, chloroform, or ether and having an intense brown ferric reaction (Found: C, 65·2; H, 3·6. $C_{14}H_{10}O_5$ requires C, 65·1; H, 3·9%). The *triacetate* separated from dilute acetic acid in colourless silky needles, m. p. 204°, with a negative ferric reaction (Found: C, 62·6; H, 4·2. $C_{20}H_{16}O_8$ requires C, 62·5; H, 4·2%).

1 : 5 : 6-*Trihydroxy*-3-*methylxanthone*.—Distillation of a mixture of *p*-orsellinic acid (2·1 g.), pyrogallol (1·55 g.), and acetic anhydride (3 ml.) yielded a primrose-yellow solid, b. p. 160—200°/0·5 mm. which, on being washed with ether and then crystallised from chloroform–alcohol, gave 1 : 5 : 6-*trihydroxy*-3-*methylxanthone* in primrose-yellow needles (0·83 g.), m. p. 243°, having solubilities similar to those of 1 : 6 : 8-trihydroxy-3-methylxanthone and an intense green ferric reaction (Found: C, 63·4; H, 3·6%). The *triacetate* formed colourless needles, m. p. 178°, from alcohol (Found: C, 62·9; H, 4·1%).

1 : 3 : 6-*Trihydroxyxanthone*.—Prepared by the standard method from β-resorcylic acid (1·54 g.), phloroglucinol (1·28 g.), and acetic anhydride (3 ml.), the fraction, b. p. 160—260°/0·5 mm., was dissolved in ether, and the solution repeatedly extracted with 2N-sodium hydroxide. Acidification of the combined extracts gave 1 : 3 : 6-*trihydroxyxanthone* which was purified from pyridine, forming mustard-yellow needles (0·5 g.), m. p. 332° (decomp.), unchanged on sublimation at 220°/0·01 mm., with a red-brown ferric reaction (Found: C, 63·7; H, 3·5. $C_{13}H_8O_5$ requires C, 63·9; H, 3·3%). This compound is almost insoluble in alcohol, acetone, benzene, dioxan, or ethyl acetate. The *triacetate* separated from acetic acid or alcohol in colourless needles, m. p. 160° (Found: C, 61·7; H, 3·9. $C_{19}H_{14}O_8$ requires C, 61·6; H, 3·8%).

1 : 5-*Dihydroxy*-3-*methylxanthone*.—An intimate mixture of *p*-orsellinic acid (830 mg.), catechol (550 mg.), and phosphoric oxide (1 g.) was kept at 200° for 5 min. and poured into water (150 ml.). Sublimation of the resulting yellow precipitate at 190°/0·001 mm. followed by recrystallisation of the sublimate from ethanol gave 1 : 5-*dihydroxy*-3-*methylxanthone* in primrose-yellow needles (300 mg.), m. p. 264°, with a green ferric reaction (Found: C, 69·8; H, 4·4. $C_{14}H_{10}O_4$ requires C, 69·4; H, 4·2%). The *diacetate* formed colourless needles, m. p. 142°, from alcohol (Found: C, 66·2; H, 4·3. $C_{18}H_{14}O_6$ requires C, 66·3; H, 4·3%).

1-*Hydroxy*-6 : 7-*dimethoxy*-3-*methylxanthone*.—Distillation of a mixture of 3 : 4-dimethoxy-

phenol (Baker and Evans, *J.*, 1938, 375) (7·7 g.), *p*-orsellinic acid (9·3 g.), and acetic anhydride (15 ml.) gave a fraction, b. p. 200—250°/10 mm., which, on being washed with ether and crystallised from alcohol, furnished 1-*hydroxy*-6 : 7-*dimethoxy*-3-*methylxanthone* in colourless needles (30 mg.), m. p. 204°, with a greenish-brown ferric reaction (Found : C, 67·0; H, 4·8. $C_{16}H_{14}O_5$ requires C, 67·1; H, 4·9%). On demethylation with warm hydriodic acid this ether (20 mg.) gave 1 : 6 : 7-trihydroxy-3-methylxanthone which was purified by sublimation at 180°/0·1 mm. and obtained in primrose-yellow needles (5 mg.), m. p. >300°, with a greenish-brown ferric reaction.

1 : 3 : 5-*Trihydroxyxanthone*.—Prepared from 2 : 3-dihydroxybenzoic acid (9·24 g.), phloroglucinol (7·66 g.), and acetic anhydride (12 ml.) this *xanthone* (950 mg.) (from the fraction, b. p. 260—280°/0·1 mm.) was crystallised from dilute alcohol and sublimed at 170°/0·1 mm., being obtained in pale yellow needles, m. p. 255°, with a red-brown ferric reaction (Found : C, 63·3; H, 3·5%). The *triacetate* separated from alcohol in colourless needles, m. p. 204° (Found : C, 61·6; H, 3·8%).

5 : 8′-*Dihydroxy*-6 : 7′-*dimethylchromono*(2′ : 3′ : 2 : 3)*xanthone*.—Distillation of a mixture of quinol (5·5 g.), *p*-orsellinic acid (8·4 g.), and acetic anhydride (12 ml.) gave a distillate consisting of pale yellow needles with a little colourless oil. This was washed with ether and the yellow residue (450 mg.) purified by sublimation at 200°/0·01 mm., giving the *chromonoxanthone* in pale yellow needles, m. p. 209—210°, sparingly soluble in the usual organic solvents and having an intense green ferric reaction (Found : C, 70·7; H, 4·1. $C_{22}H_{14}O_6$ requires C, 70·6; H, 3·8%). The diacetate separated from alcohol in colourless leaflets or from acetic acid in colourless needles, m. p. 226°.

[With D. H. Johnson] 2-*Hydroxy*-6-*methoxybenzonitrile*.—The following improved methods were employed for the preparation of this nitrile required for the synthesis of ravenelin according to the method of Raistrick *et al.* (*loc. cit.*). (*a*) Methyl 3-formyl-2 : 4-dihydroxybenzoate (Shah and Laiwalla, *J.*, 1938, 1828) (2 g.) was hydrolysed with boiling 30% aqueous potassium hydroxide (40 ml.) in nitrogen for 5 hr. On isolation in the usual manner an ethereal solution of the product was washed with 2N-sodium hydrogen carbonate, dried, and evaporated, leaving γ-resorcylaldehyde (1 g.), m. p. 154°, after purification from warm water (cf. Shah and Laiwalla, *loc. cit.*, who give m. p. 155—156°). Methylation of this aldehyde (6 g.) with methyl sulphate (5·5 g.) and potassium carbonate (12 g.) in boiling acetone (40 ml.) for 6 hr. gave 2-hydroxy-6-methoxybenzaldehyde, forming pale yellow needles (5 g.), m. p. 75°, from aqueous methanol (cf. Limaye, *Rasayanam*, 1936, **1**, 1). This aldehyde was converted into 2-hydroxy-6-methoxybenzonitrile by the method of Mull and Nord (*loc. cit.*).

(*b*) When a mixture of 2-hydroxy-6-methoxyacetophenone (Baker, *J.*, 1939, 956) (1·45 g.), iodine (2·21 g.), and pyridine (10 ml.) was heated on the steam-bath for 1 hr. and then kept at 0° for 16 hr., 1-(2-hydroxy-6-methoxyphenacyl)pyridinium iodide separated in massive prisms. A solution of this in a little hot alcohol was added to 2% aqueous potassium hydroxide (75 ml.), and the mixture heated on the steam-bath in nitrogen for 1 hr., cooled, and acidified with hydrochloric acid, giving 2-hydroxy-6-methoxybenzoic acid in colourless needles (850 mg.), m. p. 135°, after purification (cf. Clewer, *J.*, 1915, **107**, 839). This acid was converted into the nitrile by way of the amide according to standard procedures.

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