Studies in Mycological Chemistry. Part IV. Purpurogenone, a Metabolic Product of* Penicillium purpurogenum *Stoll.*

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Isolation of the pigment, purpurogenone, from the mycelium of a strain of *Penicillium purpurogenum* Stoll, is described. Its molecular formula ($C_{14}H_{12}O_5$) has been established. From a knowledge of its general properties and of the nature of several compounds obtained by its hydrolytic and oxidative degradation, it is suggested that purpurogenone is the dihydroxychromanobenzoquinone (V). A resemblance between purpurogenone and citrinin (IX) is pointed out.

Penicillium purpurogenum Stoll is a mould belonging to the *P. purpurogenum* series which, together with other related series belonging to the Biverticillata–Symmetrica group, comprise a number of moulds which are remarkable for their abundant pigment production. They are, in general, of ubiquitous distribution and are frequently found growing on decaying organic matter, or, in hot climates, on deteriorating equipment such as canvas tents (Raper and Thom, " A Manual of the Penicillia," Baillière, Tindall and Cox, London, 1949). So far as we are aware, only one report (Brenner, *Svensk bot. Tidskr.*, 1918, **12**, 91) on the pigments of *P. purpurogenum* (*sic*) has been published. Since this report is chemically insignificant, we decided, as a first measure, to investigate in detail the pigments of one selected member of this species. This paper records the isolation of, and structural investigations on, a pigment from *P. purpurogenum* Stoll (" C.B.S.").

This mould grew readily on Raulin–Thom medium and produced a red colour in the substrate and a mycelium which was deep-green or yellow-green with a dark-red reverse. No significant amount of colouring material could be isolated from the substrate and this was not further investigated. The dried and powdered mycelium was extracted successively with light petroleum, with ether, and with acetone. The only pure material which could be isolated from the first two extracts was ergosteryl palmitate. The dark, reddish-brown acetone extract always contained the major portion of the pigments and, occasionally, some mannitol. When the acetone concentrate was mixed with aqueous mineral acid, a dark-brown, amorphous precipitate was formed. This crude pigment, after having been washed with light petroleum, was purified *via* its sparingly soluble sodium salt. Even so, the partially purified material resisted all attempts at crystallisation and a chromatographic purification was always essential. Chromatography of this material (in benzene solution) on a column of anhydrous magnesium sulphate, elution of the brilliant red band with benzene, and evaporation of the solvent gave a dark-red substance which was usually crystalline and almost pure [m. p. 306° (decomp.)]. Recrystallisation from acetone then yielded the pure pigment in small, flat prisms [m. p. 310° (decomp.)] which, in bulk, possess a magnificent crimson colour. We propose the name purpurogenone for this substance.

Analysis of purpurogenone detected no elements other than carbon and hydrogen and gave a zero result for methoxyl groups (Zeisel). The molecular weight was found by cryoscopic and crystallographic methods, and the molecular formula was determined as $C_{14}H_{12}O_5$. The molecule contained one *C*-methyl group (Kuhn–Roth) and three active hydrogen atoms (Zerewitinow). In ultraviolet and in visible light a solution of purpurogenone in chloroform showed a very high absorption over a wide range of wavelengths. It is not possible to determine any optical activity in purpurogenone since its solutions, of even moderate concentrations, are so intensely coloured. An attempt to perform an electrometric titration of purpurogenone proved abortive. The only water-miscible solvent in which purpurogenone was appreciably soluble was dioxan and it was found that the glass-electrode behaved abnormally in presence of this solvent.

Purpurogenone is insoluble in water and in aqueous mineral acids but dissolves in concentrated sulphuric acid to produce an intense blood-red colour. When an ethereal

* Part III, preceding paper.

solution of purpurogenone is shaken with saturated aqueous sodium hydrogen carbonate, the pigment is removed from the ethereal layer and a brown, amorphous, sparingly soluble sodium salt remains suspended in the aqueous layer. It is possible to regenerate the pigment (unchanged) from the sodium salt. The reaction of purpurogenone with aqueous caustic alkali is remarkable. If the pigment is shaken with 2N-sodium hydroxide an intense, emerald-green colour is produced which changes (within twenty seconds) to blue and later (one minute) to deep purple. When the alkaline, purple solution is warmed with zinc dust the colour disappears but it reappears when the mixture is cooled and shaken with air. At first, we thought this test indicated purpurogenone to be a hydroxy-quinone but our conclusion was premature, for it was later found that it was not purpurogenone itself but a degradation product (produced by hydrolysis and oxidation) which yielded the positive test. Hydrogenation of purpurogenone in acetic acid solution (with palladised charcoal or platinic oxide as catalyst) led to the disappearance of the brilliant red colour and uptake of one mol. of hydrogen. When the solution containing the dihydropurpurogenone was exposed to air the red colour reappeared within a few minutes.

Attempts to prepare crystalline derivatives of the pigment met with little success. Purpurogenone did not condense with 2 : 4-dinitrophenylhydrazine. Methylation by diazomethane or methyl sulphate (in presence of acetone and anhydrous potassium carbonate) yielded intractable, brown, amorphous products. The pigment appeared to condense with *o*-phenylenediamine (in glacial acetic acid) to give a product which separated in fine, red needles but different samples, prepared in apparently identical ways, did not yield consistent analytical results. Acetic anhydride (in presence of a trace of perchloric acid) readily converted purpurogenone into a yellow product (m. p. 226°, after " crystallisation " from ethanol) which was amorphous but analytically pure, giving figures corresponding to a triacetate. All attempts to obtain this compound in a crystalline state failed. We are of the opinion that it is a mixture resulting from the simultaneous acetylation of two or three tautomeric forms of purpurogenone. Triacetylpurpurogenone is optically active. Attempts at reductive acetylation were unsuccessful.

A more detailed examination of the reaction between purpurogenone and aqueous caustic alkali revealed that this reagent readily caused extensive degradation. (Lack of this knowledge had previously caused very considerable difficulties in devising a technique for isolating the pure pigment from the original acetone extracts.) Formic acid (0·5 mol. per mol. of pigment) was isolated as one of the degradation products. The involatile products were separated by conventional methods and by chromatography (on a column of anhydrous magnesium sulphate) into at least five coloured substances. One of these was almost certainly established as being a 2-hydroxy-1 : 4-naphthaquinone. It was obtained crystalline (m. p. *ca.* 220°) and gave positive results in all the colour tests for this class of compound (see Experimental section). Furthermore, it yielded a yellow acetate whose absorption spectrum indicated that it was a 1 : 4-naphthaquinone derivative. This hydroxynaphthaquinone is being further investigated.

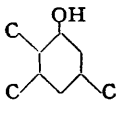
Oxidation of triacetylpurpurogenone by means of chromic acid in acetic acid–acetic anhydride (Fischer and Gross, *J. prakt. Chem.*, 1911, **84**, 372) gave a very poor yield of material which was shown (by paper chromatography) to be a mixture of two hydroxy-acids. One of these was almost certainly 3-hydroxybenzene-1 : 2 : 5-tricarboxylic acid, previously unknown (see Part III). The second acid was not identified but appeared to be identical with a product obtained as an intermediate during a graded oxidation of purpurogenone itself (see below and also Experimental section).

Purpurogenone was readily oxidised by hot nitric acid to a yellow product which was shown, by paper chromatography, to be very heterogeneous. None of the substances in the mixture could be identified.

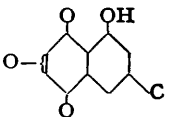
When purpurogenone was oxidised under mild conditions with alkaline hydrogen peroxide there was obtained a moderate yield of a colourless, semi-crystalline acid which gave a blue ferric reaction and was chromatographically identical with the unidentified product resulting from the chromic acid oxidation of triacetylpurpurogenone. This oxidation product was methylated and then vigorously oxidised with potassium permanganate (in alkaline solution) to yield a crystalline acid, m. p. 252°. At first, this acid was

available to us only in minute quantity (1—2 mg.) and, from its chemical properties and physical constants, we thought it might have been 3-methoxybenzene-1 : 2 : 4 : 5-tetracarboxylic acid (see Part III). However, we were later able to improve the yield in the graded degradation process, and the product was then conclusively identified as 3-methoxybenzene-1 : 2 : 5-tricarboxylic acid. This acid had previously been obtained by Posternak (*Helv. Chim. Acta*, 1940, **23**, 1046), first, as a degradation product of the anthraquinone pigment, roseopurpurin (from *P. roseopurpureum* Dierckx), and, secondly, by synthesis.

The identification of the degradation product, 3-methoxybenzene-1 : 2 : 5-tricarboxylic acid, fixes the positions of nine of the fourteen carbon atoms in the purpurogenone molecule and establishes the partial structure (I). The observation that purpurogenone, by alkaline hydrolysis and aerial oxidation, produces a 2-hydroxy-1 : 4-naphthaquinone enables us to extend this to partial structure (II). The sensitivity of purpurogenone to aqueous



(I)



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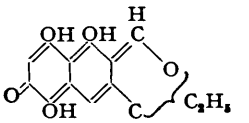
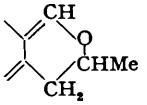
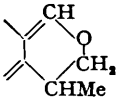


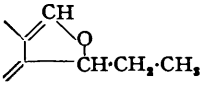
(III)



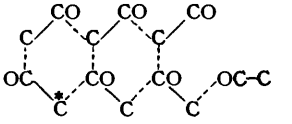
(III*a*)



(III*b*)



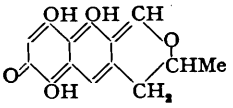
(III*c*)



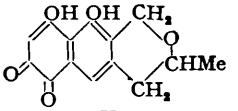
(IV)

solutions of strong alkalis and the expulsion of one carbon atom as formic acid by hydrolysis must now be taken into account and leads to partial structure (III). Finally, a *C*-methyl group has to be accommodated in the molecule. Since triacetylpurpurogenone is optically active, the *C*-methyl group in purpurogenone must be attached as in (III*a*, *b*, or *c*). The acetate rule of biosynthesis (Birch and Donovan, *Austral. J. Chem.*, 1953, **6**, 360) favours the structure (III*a*) since the complete molecule (IV) may then be regarded as resulting from the " head-to-tail " condensation of seven acetate units followed by oxidation at the position C*.

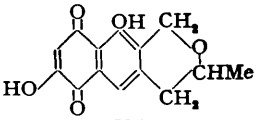
We therefore suggest that purpurogenone should be represented by structure (V) or by the tautomeric structures (V*a*) and (V*b*).



(V)

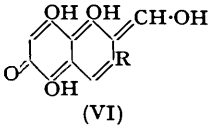


(V*a*)

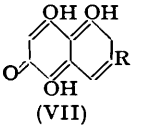


(V*b*)

Structure (V), which contains a vinylogous lactone system, readily accounts for the alkali-sensitivity of purpurogenone and for the nature of the products obtained when it is hydrolysed by aqueous alkali in presence of air. Such hydrolysis would be expected to yield initially a compound of structure (VI) which represents it as the enolic form of a vinylogous β-diketone. Further hydrolysis would lead to formic acid and a compound

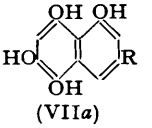


(VI)

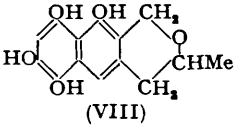


(VII)

(R = $-CH_2 \cdot CHMe \cdot OH$)



(VII*a*)

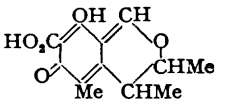


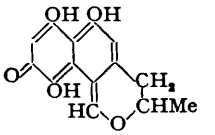
(VIII)

represented by structure (VII) or by the tautomeric structure (VII*a*). Compounds of type (VII*a*) are known to undergo aerial oxidation (under alkaline conditions) readily, to give 2-hydroxy-1 : 4-naphthaquinones (Graebe, *Annalen*, 1870, **154**, 303, 324).

Dihydropurpurogenone is formulated as (VIII).

In that purpurogenone contains a vinylogous lactone system, it bears a resemblance to the yellow pigment, citrinin (IX) (Hetherington and Raistrick, *Phil. Trans.*, 1931,

(IX) 

 (X)

220, *B*, 269; Coyne, Raistrick, and Robinson, *ibid.*, p. 297; Brown, Robertson, Whalley, and Cartwright, *J.*, 1949, 867; Cartwright, Robertson, and Whalley, *ibid.*, p. 1563). In conclusion it should be pointed out that an alternative structure (X) for purpurogenone is in accord with all the observed facts. However, we prefer structure (V) on account of the generally accepted greater stability of the 2 : 6-naphthaquinone structure than of the 3 : 5-naphthaquinone structure.

Experimental

Paper Chromatography.—The methods employed and the significance of the terms R_F(b) and R_F(c) are described in Part III (preceding paper).

Isolation of Purpurogenone.—Penicillium purpurogenum Stoll, "C.B.S." (Centraalbureau voor Schimmelcultures, Baarn, Holland), was kept in sub-culture on Czapek–Dox agar slopes. For production of the pigment the mould was grown in surface culture on Raulin–Thom medium (see *Biochem. J.*, 1955, **59**, 480). Flat, round culture-flasks (see *ibid.*, 1944, **38**, 456), each filled with 500 c.c. of the medium, were sterilised and, after having been inoculated with a heavy, aqueous spore suspension, were kept at 28° ± 1° for *ca.* 3 weeks. The mycelium (from 140 flasks) was harvested, pressed free from surface liquid, allowed to drain for several hours, and finally dried *in vacuo* at 40°. The dry mycelium (*ca.* 250 g.) was ground to a fine, red-brown powder in a coffee-mill and was successively extracted (Soxhlet) with (i) light petroleum (b. p. 40—60°) for 48 hr., (ii) ether (48 hr.), and (iii) acetone (96 hr.).

The light-petroleum extract gave, on removal of the solvent, a liquid fat (3—5 g.), treatment of which with cold ethanol (95%; 50—75 c.c.) yielded a semi-crystalline substance which was filtered off, washed with ethanol, and then, after two crystallisations from ethanol, gave small, glistening prisms (0·65 g.), m. p. 104—106°. This material gave a strong Liebermann–Burchard test (red ⟶ violet ⟶ green) and was converted, by hydrolysis, into a sterol [fine needles, m. p. 159—161° (after two crystallisations from methanol) (Found: C, 81·3; H, 11·0. Calc. for $C_{28}H_{44}O$: C, 81·1; H, 11·1%)] and a fatty acid, m. p. 59—62° (after recrystallisation from aqueous methanol). It was thus identified as ergosteryl palmitate. Raistrick and Oxford (*Biochem. J.*, 1933, **27**, 1176) give m. p. 106—108° for this compound.

The ethereal extract contained fat, ergosteryl palmitate, and a small quantity of pigment which was not further investigated. From the dark, reddish-brown acetone extract, there occasionally separated a small quantity of long, colourless needles. These were collected, washed with acetone, and recrystallised from methanol to give mannitol (m. p. 166°; acetate, m. p. 121—122°).

The filtered acetone extract (*ca.* 1 l. from 250 g. of mycelium) was concentrated to 150 c.c., acidified with 4N-hydrochloric acid, and diluted with about four times its volume of water. The diluted mixture was left at room temperature for ½ hr. and the chocolate-brown precipitate, which had formed, was collected by filtration, washed with water, and dried *in vacuo*. This crude pigment (8—14 g.) was ground to a fine powder and exhaustively extracted (Soxhlet) with light petroleum (b. p. 40—60°) for *ca.* 8 hr. The powdered residue was heated under reflux with ether (3 l.). The cooled, deep-red ethereal solution was filtered from insoluble material (*ca.* 3 g. of a black powder) and extracted with a saturated, aqueous solution (2 × 400 and 1 × 200 c.c.) of sodium hydrogen carbonate (in this extraction vigorous shaking must be avoided, since intractable emulsions may occur). An amorphous, brown, very sparingly soluble sodium salt of the pigment was formed which remained suspended in the aqueous phase. The yellow-brown ethereal solution was discarded. The combined sodium hydrogen carbonate extracts and suspensions were washed with ether (2 × 250 c.c.) and then strongly acidified with 4N-hydrochloric acid. The liberated pigment was collected in chloroform (2 × 750 and 1 × 500 c.c.), and the combined chloroform solutions were washed with water, dried (Na_2SO_4), and filtered. The sodium sulphate was washed well with chloroform to remove adsorbed pigment.

Removal of the solvent from the combined filtrate and washings (overheating in the later stages of the distillation must be avoided, otherwise darkening and resinification of the pigments ensue) yielded a dark-red residue (2—3 g.) which did not crystallise.

This material was exhaustively extracted with successive portions of boiling benzene (*ca.* 750 c.c. in all). After the combined benzene extracts had been cooled and filtered, the dissolved pigments were chromatographed on a column (15 × 5 cm.) of anhydrous magnesium sulphate (prepared from commercial " anhydrous " material which had been previously heated at 200° for 4 hr. and stored in a desiccator). Development of the chromatogram with dry benzene produced three fractions : (i) a rapidly moving, diffuse, yellow band ; (ii) a more slowly moving, vivid red band, and (iii) a mixed zone of dark-brown bands which remained near the top of the column. Continued percolation of dry benzene through the column gave eluates which contained fraction (i) (in 250 c.c.) and fraction (ii) (in 3 l.). Fraction (i) (which consisted of a very small quantity of pale-yellow material) and fraction (iii) were not further investigated. The eluate containing fraction (ii) was concentrated by distillation to 20—30 c.c., and the remaining solvent removed either by gentle heating in a stream of air or *in vacuo* with constant agitation. The residue, after having been washed with cold light petroleum (b. p. 40—60°) until the washings were almost colourless, consisted of a dark-red mass (generally crystalline) of almost pure *purpurogenone* (0·8—1·3 g.), m. p. 306° (decomp.). This material was used, without further purification, for most of the preparative and degradative work described below. For crystallisation, the pigment (50 mg.) was dissolved in boiling acetone (40 c.c.), and the solution was filtered and concentrated to 5 c.c. to give pure purpurogenone (21 mg.).

General Properties of Purpurogenone.—The pigment separated from benzene in dark-red prisms and from acetone (or ethyl methyl ketone) in small, flat, crimson prisms, m. p. 310° (decomp.) [Found : C, 64·5, 64·3, 64·7 ; H, 4·55, 4·2, 4·2 ; OMe, 0 ; *C*-Me, 6·2 ; active H, 1·3% ; *M* (cryoscopic in benzene), 273 ; *M* (calc. from density of substance, 1·525 g./c.c., and on the assumption of eight molecules per cell of volume 2326 Å^3), 266·9. $C_{14}H_{12}O_5$ requires C, 64·6 ; H, 4·65 ; 1*C*-Me, 5·8 ; 3 active H, 1·2% ; *M*, 260·2]. It was insoluble in water and light petroleum, sparingly soluble in ether, benzene, acetone, ethanol, chloroform, and glacial acetic acid, and readily soluble, even in the cold, in dioxan. Light absorption (in $CHCl_3$) : $\lambda_{max.}$ 252, 306, 387, 498, 528, and 568 mμ ; log ε 4·18, 3·73, 3·80, 3·42, 3·47, and 3·27 respectively. Addition of a trace of ferric chloride solution to its solution in ethanol produced an olive-green colour. (Other general properties have been described above.)

Catalytic Hydrogenation of Purpurogenone.—The pigment was dissolved in glacial acetic acid and hydrogenated at atmospheric pressure in presence of either palladised charcoal (5%) or Adams's platinic oxide. In three experiments 0·95, 1·1, and 1·0 mol. of hydrogen were absorbed. During the reduction the intense red colour gave way to pale yellow but rapidly returned when the solution was exposed to air.

Triacetylpurpurogenone.—Two drops of a solution of aqueous 60% perchloric acid (one drop) in acetic anhydride (1 c.c.) were added to a suspension of purpurogenone (0·1 g.) in acetic anhydride (3 c.c.), and the mixture was shaken for 10 min. Complete dissolution soon occurred and the intense red colour changed to pale yellow. The amorphous material, which separated when the solution was poured on crushed ice (75 g.), was collected, washed well with water, and dried (135 mg.). This material was " recrystallised " twice from ethanol to yield *triacetylpurpurogenone* (40 mg.) in pale-yellow granules, m. p. 226°, $[\alpha]_D^{17}$ +105° (*c*, 0·418 in $CHCl_3$) [Found (in a sample dried at 100° *in vacuo* over P_2O_5) : C, 62·0 ; H, 4·6 ; Ac, 34·0. $C_{20}H_{18}O_8$ requires C, 62·2 ; H, 4·7 ; 3Ac, 33·4%]. Attempts to crystallise this substance from a number of other solvents failed. It chromatographed on a column of acid-washed alumina as a diffused, yellow band. Ultraviolet light absorption (in EtOH) : $\lambda_{max.}$ 245, 296 (infl.), and 344—350 (infl.) mμ ; log ε 4·26, 3·79, and 3·45 respectively.

Oxidative Degradation of Triacetylpurpurogenone.—Triacetylpurpurogenone (61 mg.) was dissolved in a mixture of acetic anhydride (4 c.c.) and glacial acetic acid (2 c.c.). To this solution, maintained at 70—80°, were gradually added (during ½ hr.) 2·7 c.c. of a solution which had been made by dissolving chromic anhydride (2·5 g.) in water (2 c.c.) and acetic acid (25 c.c.). After having been heated at 95° for 1½ hr., the mixture was cooled and diluted with water (40 c.c.). Extraction with ether (4 × 50 c.c.) gave a yellow extract which was evaporated to to yield a light brown solution (5 c.c.) from which the water and acetic acid were removed by gentle heating *in vacuo*. The brown gum, so obtained, was warmed for 1 hr. on the steam-bath with N-sodium hydroxide (4 c.c.). The solution was cooled, saturated with carbon dioxide, and extracted with ether (20 + 10 c.c.) to remove any phenolic compounds. The aqueous layer was acidified (2N-hydrochloric acid) to pH 2 and extracted with ether (20 + 15 + 10 c.c.).

The solvent was evaporated from the dried (Na_2SO_4) ethereal solution to give a coloured residue (*ca.* 30 mg.). This was dissolved in hot water, and the solution was decolorised with Norite and filtered. From the filtrate there was isolated, by ether-extraction, a partly crystalline material (*ca.* 2 mg.) which gave a pink ferric reaction in water. Paper chromatography revealed the presence in this material of two hydroxy-acids (A and B). The acid, A, gave a reddish-violet spot on paper when sprayed with ferric chloride solution and had $R_F(b)$ 0·62 and $R_F(c)$ 0·02 and was, therefore, almost certainly 3-hydroxybenzene-1 : 2 : 5-tricarboxylic acid. The acid, B, proved identical, in its ferric reaction and in its chromatographic behaviour, with the hydroxy-acid obtained when purpurogenone itself was oxidised with an alkaline solution of hydrogen peroxide (see below).

Degradation of Purpurogenone with 2N-*Sodium Hydroxide.*—A solution of purpurogenone (0·13 g.) in 2N-sodium hydroxide (5 c.c.) was heated, with free access of air, on the steam-bath for 2 hr., the water lost by evaporation being replaced from time to time. To the cooled solution was added phosphoric acid (*d* 1·75; 1 c.c.), and the chocolate-brown precipitate produced was filtered off and washed with water. The combined filtrate and washings were distilled to mimimum volume. After addition of water (5 c.c.) to the residue, distillation was again carried out. This operation was repeated several times. The combined distillates (which had a pungent odour) required 2·41 c.c. of 0·1N-sodium hydroxide (≡ 0·48 mol. of a monobasic acid) for neutralisation to phenolphthalein as external indicator. The neutralised distillates were evaporated to small volume (3—4 c.c.). The solution so obtained (i) reduced a cold, acidified solution of potassium permanganate, (ii) reacted with a warm, neutral solution of mercuric chloride to produce a strong, white turbidity, and (iii) gave a positive result in the chromotropic acid test for formic acid (Feigl, " Qualitative Analysis by Spot Tests," Elsevier, New York, Third English Ed., 1947, p. 397).

The air-dried, chocolate-brown precipitate, mentioned above, was extracted with boiling ether (5 × 50 c.c.), leaving a black, amorphous residue. The combined ethereal solutions were extracted with (i) a saturated solution of sodium hydrogen carbonate (20 + 20 + 10 c.c.) and (ii) 2N-sodium hydroxide (20 + 10 c.c.). The latter extracts, when acidified, yielded a dark-red powder (0·7 mg.) which gave a positive test for a hydroxyquinone when its solution in 2N-sodium hydroxide was warmed with zinc dust. The sodium hydrogen carbonate extracts, when acidified (concentrated hydrochloric acid), gave a copious, dark red, amorphous precipitate which was filtered off (giving filtrate F, see below) and dried (40 mg.). This material was repeatedly extracted with boiling benzene (175 c.c. in all), and the benzene solution of the pigments, after having been left overnight at room temperature, was filtered and poured on a column (8 × 4·5 cm.) of anhydrous magnesium sulphate. The chromatogram, when developed with dry benzene, showed, in order from the top, (i) a brown zone, (ii) a violet band, and (iii) an orange band. The last two bands were eluted from the column with benzene–ether (9 : 1 v/v).

Evaporation of the solvents from the eluate containing the orange band yielded orange-red needles (3—4 mg.), m. p. *ca.* 220° (decomp.) after considerable previous darkening. A further quantity (2 mg.) of this same material was obtained by extraction of filtrate F (see above) with ether, evaporation of the solvent, and crystallisation of the residue from benzene. This material was readily soluble in ether but sparingly soluble in water. Its aqueous solution gave a violet ferric reaction and its solution in 2N-sodium hydroxide gave a positive test for a hydroxyquinone. It dissolved in aqueous solutions of the following reagents to give the colours indicated: (*a*) mineral acid (yellow); (*b*) sodium acetate (orange-red); (*c*) sodium hydrogen carbonate (deep-red); (*d*) sodium hydroxide (intense purple). It dissolved in concentrated sulphuric acid to give an intense crimson colour. The remainder of this pigment was acetylated, a very small quantity of an amorphous, yellow acetate being obtained which, in ethanolic solution, showed maximum absorption in ultraviolet at 255 and 343 mμ [cf. the peak absorption of triacetylflaviolin (Part I, *J.*, 1953, 3302) at 253 and 349 mμ]. There is little doubt that this pigment is a derivative of a 2-hydroxy-1 : 4-naphthaquinone.

The eluate corresponding to the violet band yielded 2 mg. of material (tiny, violet prisms) which, from its properties and colour reactions, appeared to be a hydroxylated quinone or possibly a hydroxylated diquinone.

After the elution of bands (ii) and (iii), the column was extruded and the materials in the brown zone were extracted (Soxhlet) with ether. Evaporation of the solvent yielded an intractable reddish-brown gum (16·5 mg.).

Chromatography of various residues, obtained in the work described in this section, indicated the presence of minute quantities of two other pigments.

Oxidative Degradation of Purpurogenone.—A solution of purpurogenone (0·5 g.) in a mixture

of a 1% solution of sodium hydroxide (80 c.c.) and a 3% solution of hydrogen peroxide (60 c.c.) was kept at room temperature for 3 days, filtered, and extracted with ether (50 c.c.). The aqueous layer was strongly acidified (concentrated hydrochloric acid) and extracted with ether (60 + 4 × 50 c.c.). The combined ethereal extracts were washed with water and dried (Na_2SO_4). Evaporation of the solvent yielded a brown gum (*ca.* 120 mg.). Paper chromatography indicated the presence in this gum of only one hydroxy-acid, which gave with ferric chloride a violet "spot" [R_F(b) 0·78; R_F(c) 0·29]. This hydroxy-acid was not identified but it is probably identical with the hydroxy-acid (*B*) produced by chromic acid oxidation of triacetylpurpurogenone.

A solution of the above acid (120 mg.) and of methyl sulphate (1·2 c.c.) in acetone (25 c.c.) was heated under reflux with anhydrous potassium carbonate (1·5 g.) for 19 hr. The mixture was filtered and the acetone removed from the filtrate. The residue, together with the potassium salts, was heated under reflux for 1 hr. with 2N-sodium hydroxide (25 c.c.). After filtration and extraction with ether (20 c.c.)—to remove non-acidic material—the solution was strongly acidified (7·5 c.c. of concentrated hydrochloric acid) and the product collected by ether-extraction to give a partly crystalline, pale-brown material (80 mg.) which had a negative ferric reaction in water.

The solution of this material in 2N-sodium carbonate (3 c.c.) was heated on the steam-bath, and a 4% solution of potassium permanganate (7—8 c.c.) was gradually added. The slight excess of permanganate was destroyed by the addition of a few drops of methanol. The manganese dioxide was filtered off and thoroughly extracted with hot water. The combined filtrate and extracts were strongly acidified (concentrated hydrochloric acid) and extracted with ether (4 × 40 c.c.). The ethereal extract was washed with water (2 c.c.) and dried (Na_2SO_4). Evaporation of the solvent yielded a pale yellow, semi-crystalline material (30—40 mg.). This recrystallised from hot water (2·5 c.c.) as almost colourless, slender, shining rods which, when dried (14·2 mg.), broke up and became opaque [Found: C, 49·9; H, 3·65; OMe, 12·9%; equiv., 79·8. Calc. for $C_9H_5O_6 \cdot OMe$: C, 50·0; H, 3·35; OMe, 12·9%; equiv. (as a tribasic acid), 80·0], m. p. 253°, re-melting (after the temperature had been raised to 260° and then lowered to 200°) at 252°, R_F(b) 0·76. [A further quantity (8 mg.) of this material was obtained by spontaneous evaporation of the mother-liquor and washings from the first crop of crystals.] A very dilute, aqueous solution of this acid gave a strong yellow ferric reaction. It gave a positive result in the fluorescein reaction for an *o*-phthalic acid. This substance was virtually identical with synthetic 3-methoxybenzene-1 : 2 : 5-tricarboxylic acid (Posternak, *loc. cit.*), R_F(b) 0·76, m. p. 252° unaltered by admixture with the degradation product.

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