Studies in Mycological Chemistry. Part XXVII.[1] Reinvestigation of the Structure of Purpurogenone, a Metabolite of *Penicillium purpurogenum* Stoll

By **John C. Roberts*** and **D. J. Thompson**, Department of Chemistry, The University, Nottingham NG7 2RD

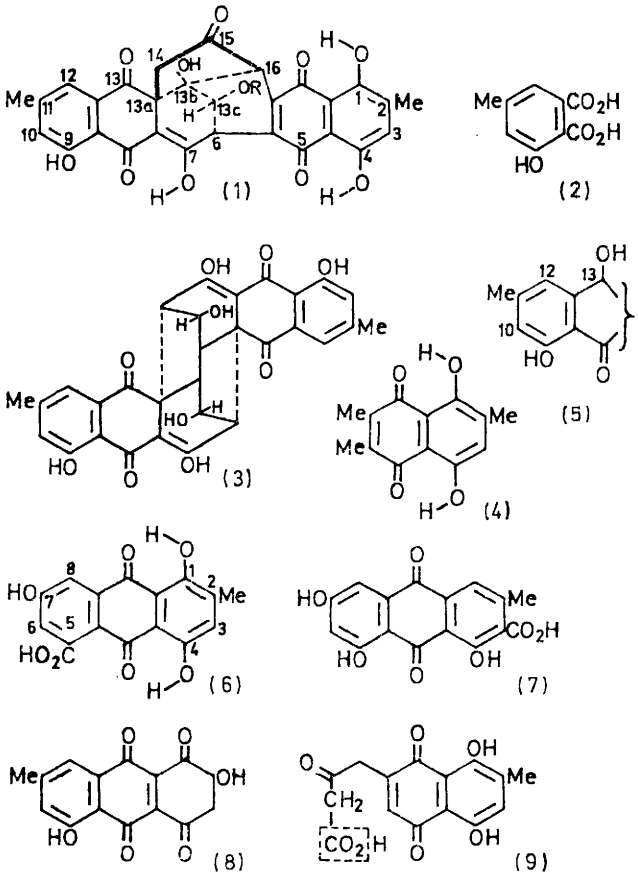
The spectral and other properties of purpurogenone, a metabolite of *Penicillium purpurogenum* Stoll, whose structure has been recently elucidated as (1; R = H) by *X*-ray crystallographic analysis of its monobromoacetate (1; R = OC·CH_2Br), are reported. A previously suggested structure is thus disproved.

The isolation and preliminary investigation of purpurogenone, the chief colouring matter of some strains of *Penicillium purpurogenum* Stoll, were recorded[2] in 1955. The pigment forms crimson prisms (from acetone), m.p. 310° (decomp.). Elemental analysis and a cryoscopic molecular weight determination indicated the molecular formula $C_{14}H_{12}O_5$ (*M* 260). More recently, we were able to determine the molecular weight of purpurogenone by mass spectrometry and found a value of 544 or 546. The previously recorded molecular formula, and suggested structural formula,[2] are therefore incorrect. We now report our re-examination of this problem.

Our original strain of the mould[2] had been lost. Of ten newly acquired strains, only one (C.B.S., 257·37) produced the required pigment. This particular strain, however, enabled us to obtain sufficient of the metabolite for our projected re-investigation.

We established the molecular formula, $C_{29}H_{20}O_{11}$ (*M* 544), for the pigment and found that crystals which had separated from a solution in acetone tenaciously retained a variable amount of solvent. (This observation probably accounts for the incorrectness of the molecular weight previously recorded.[2]) We also found that purpurogenone separates from chloroform as crimson prisms, m.p. *ca.* 300° (decomp.), with the constant composition $C_{29}H_{20}O_{11}$,$CHCl_3$. Hydrogenation of purpurogenone (Adams catalyst in glacial acetic acid) was accompanied by the uptake of two mol. equiv. of hydrogen and the fading of the colour of the solution from deep red to pale yellow. Exposure of the solution to air rapidly restored the red colour, and the product (dark red prisms) had the composition $C_{29}H_{22}O_{11}$. We concluded that purpurogenone contained two readily reducible functions, one of them being probably a quinone group which, after reduction, was easily re-oxidised by air. The i.r. spectrum of the dihydropurpurogenone was similar to that of purpurogenone but lacked a band at 1694 cm^{-1}, indicating that a carbonyl group in purpurogenone had been reduced. This interpretation was confirmed by the observation that purpurogenone formed a hexa-acetate whereas dihydropurpurogenone yielded a hepta-acetate.

Degradation of purpurogenone with aqueous alkali led to the isolation of formic acid (0·98 mol. equiv.) and a number of quinonoid compounds of which none could be satisfactorily identified. Oxidative degradation (alkaline hydrogen peroxide) of purpurogenone led to a brown gum which was shown to contain 3-hydroxy-5-methylphthalic acid (2) (*cf.* ref. 2). Numerous other



attempts to isolate identifiable degradation products from the metabolite were unsuccessful.

Since spectral data for purpurogenone (see later), in conjunction with the limited amount of evidence from the degradative work already outlined, did not lead to a structural elucidation, we undertook an *X*-ray crystallographic examination of the monobromoacetate (1; R = OC·CH_2Br). This work, a preliminary account of which has already been reported,[3] established the structure and absolute configuration of purpurogenone as (1; R = H) or as one of the possible tautomeric forms. We shall

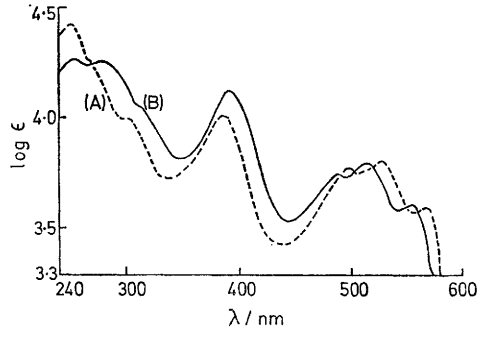
[1] Part XXVI, M. J. Rance and J. C. Roberts, *J. Chem. Soc.* (*C*), 1971, 1247.

[2] J. C. Roberts and C. W. H. Warren, *J. Chem. Soc.*, 1955, 2992.

[3] T. J. King, J. C. Roberts, and D. J. Thompson, *Chem. Comm.*, 1970, 1499.

now interpret spectral and other experimental data in terms of this formulation for the pigment.

The u.v. absorption spectrum of purpurogenone may be regarded as the sum of the spectra of two isolated chromophores. To confirm this view we secured two compounds [rugulosin [4] (3) and 2,6,7-trimethylnaphthazarin [5] (4)] which together contained chromophores similar to the two isolated chromophores of purpurogenone. A ' summed spectrum ' of the two compounds and the spectrum of purpurogenone are given in the Figure.*



U.v. spectrum of purpurogenone (A) and a ' summed spectrum,' (B) (for details see text); solutions in chloroform

The overall agreement is considered to be satisfactory, discrepancies (more marked at shorter wave-lengths) being attributed to conformational differences in the molecules concerned.

dihydropurpurogenone had strong bands at **3452**, **1755**, **1616**, and **1578** cm^{-1}. Structure (**5**; R = H) is therefore assigned to dihydropurpurogenone.

The ^1H n.m.r. spectrum of purpurogenone (chloroform solvate) possessed signals which were interpreted as shown in the Table. The signal for the 7-hydroxy-group was not observed. The spectrum for dihydropurpurogenone (see Table) showed signals corresponding to twenty-two protons. The spectrum of the monobromoacetate of purpurogenone is also given. Some of the assignments are, of necessity, equivocal, *e.g.* the values allocated to HO-1 and HO-4 could be interchanged.

Only a few crystalline derivatives of purpurogenone were obtainable. The hexa-acetate, although chromatographically homogeneous and analytically pure, was never obtained crystalline. The monoacetate (**1**; R = Ac), bromoacetate (**1**; R = $OC{\cdot}CH_2Br$), and formate (**1**; R = OCH) all crystallised readily. Dihydropurpurogenone separated from chloroform solution to give solvent-free prisms (*cf.* purpurogenone).

The degradation of purpurogenone by alkaline hydrogen peroxide to the phthalic acid (**2**) is unexceptional. The liberation of 1 mol. equiv. of formic acid from the pigment (by alkaline hydrolysis, acidification, and distillation) may be rationalised as shown in the Scheme. As mentioned previously, alkaline degradation of purpurogenone gave rise to small quantities of a number

^1H N.m.r. absorptions (solutions in [$^2H_7$]dimethylformamide)

τ Values; *J* in Hz

Compound	Me-2	Me-11	H-14	H-14	H-16	H-13c	H-6	HO-13b	HO-13c
(1, R = H) [a]	7·70 3H, s	7·54 3H, s	7·30 1H, d *J*, 16	6·30 1H, d *J*, 16	5·82 1H, s	5·45 1H, d *J*, 4	5·31 1H, d *J*, 4	4·27br 2H, s	
(5, R = H) [c]	7·73 3H, s	7·64 3H, s	7·93 1H, d *J*, 15	7·49 1H, d *J*, 15	5·65 1H, s	5·51 1H, d *J*, 4	5·27 1H, d *J*, 4	4·67 1H, s	3·58br 1H, s
(1, R = $OC{\cdot}CH_2Br$) [d]	7·78 3H, s	7·52 3H, s	7·42 1H, d *J*, 14	6·38 1H, d *J*, 14	5·90 1H, s	4·16 1H, d *J*, 4	5·50 1H, d *J*, 4	4·10 1H, s	

Compound	H-3	H-12	H-10	HO-9	HO-1	HO-4	HO-7
(1, R = H) [a]	2·84 1H, s	2·78 2H, s		−1·34 1H, s	−2·67 1H, s	−2·89 1H, s	*b*
(5, R = H) [c]	2·80 1H, s	3·32br 1H, s	2·90br 1H, s	−1·50 1H, s	−2·64br 1H, s	−2·88br 1H, s	−3·42br 1H, s
(1, R = $OC{\cdot}CH_2Br$) [d]	2·85 1H, s	2·80 1H, d *J*, 2	2·74 1H, d *J*, 2	−1·18br 1H, s	−2·58 1H, s	−2·64 1H, s	*b*

[a] Chloroform solvate; an additional signal at 1·68 ($CHCl_3$) was observed. [b] No signal observed. [c] Additional signals were observed at 2·57 (1H, s) for HO-13 and 4·52 (1H, s) for H-13. [d] Solution in [$^2H_6$]dimethyl sulphoxide; an additional signal at 5·94 (2H, s) ($CO{\cdot}CH_2Br$) was observed.

The i.r. spectrum of purpurogenone showed strong bands at **3423** (OH), **1758** (C=O of strained five-membered ring), **1694** (C=O at position **13**), **1618** (hydrogen-bonded C=O groups), and **1574** cm^{-1} (C=C). (The spectrum of purpurogenone which had been crystallised from acetone also showed a band in the carbonyl stretching region at **1718** cm^{-1}.) The corresponding spectrum of

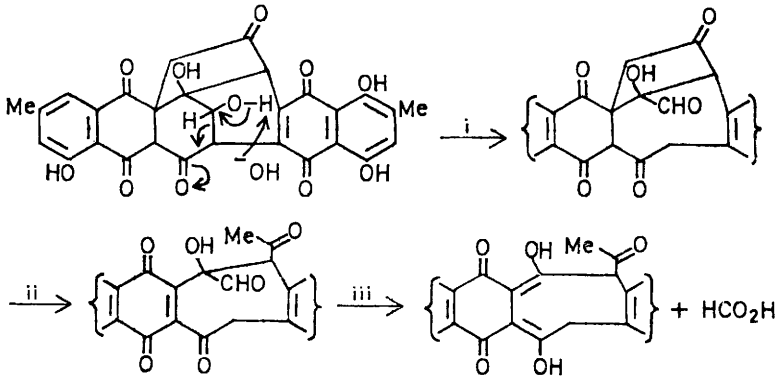
of quinone-like compounds. The production of these compounds was capricious and their purification difficult.

* In the summation, $\frac{1}{2}\varepsilon$ values for rugulosin were used.

[4] U. Sankawa, S. Seo, (Miss) N. Kobayashi, Y. Ogihara, and S. Shibata, *Tetrahedron Letters*, 1968, 5557; (Miss) N. Kobayashi, Y. Iitaka, U. Sankawa, Y. Ogihara, and S. Shibata, *ibid.*, p. 6135.
[5] H. Brockmann and K. Müller, *Annalen*, 1939, **540**, 66.

One of them was obtained as a dark orange solid, m.p. 248—250° (decomp.), which we believe (from its composition and spectral properties; see Experimental section) to be 1,4,7-trihydroxy-2-methylanthraquinone-5-carboxylic acid (6), which has not previously been described (see, however, the isolation and identification of a closely related anthraquinone from deoxypurpurogenone [6]).

Biosynthesis.—The structural formula of purpurogenone (1; R = H) suggests that a molecule of the pigment is biosynthesised from two molecules of emodin [or of its carboxylic acid, endocrocin (7)] by an undefined sequence of oxidations, condensations, decarboxylations, and reductions. The initial linking of the two molecules would be a key step in the process and probably involves a Michael condensation between two systems such as (8) and (9). If these ideas are correct, then the metabolite is of acetate–malonate origin. A preliminary investigation revealed that [2-^{14}C]acetate was indeed incorporated into the pigment and that the phthalic acid (2), isolated from the radio-active purpurogenone, had about the correct proportion of activity on the assumption that the biosynthesis outlined above was operative. Further investigations into the biosynthesis of the metabolite are in progress.



SCHEME i, Reversed aldol; ii, reversed Michael condensation; iii, hydrolysis of vinylogous β-dicarbonyl system.

EXPERIMNETAL

M.p.s were determined with a Kofler hot-stage apparatus. Mass spectra were measured with an A.E.I. MS 902 spectrometer and optical rotations with an Ericsson E.T.L.–N.P.L. automatic polarimeter, type 143A. U.v. spectra were recorded with a Unicam SP 700 spectrometer. I.r. spectra were determined, for compounds in potassium bromide discs, with a Unicam SP 200 spectrometer. ^{1}H N.m.r. spectra were recorded with a Varian HA 100 MHz spectrometer, tetramethylsilane being used as internal reference.

The magnesium sulphate used for chromatography was B.D.H. Anhydrous which had been heated at 200° for 4 h and sieved (40 mesh) before use.

Isolation of Purpurogenone.—Penicillium purpurogenum Stoll (Centraalbureau voor Schimmelcultures, Baarn, Holland, No. 257·37) was grown and extracted as previously described.[2] The crude pigment (10 g) was dissolved in benzene and applied to a dry-packed magnesium sulphate column (15 × 10 cm). Development of the chromatogram with benzene produced four fractions: (i) a rapidly moving, diffuse, yellow band; (ii) a slower moving, sharp red band; (iii) a second, diffuse, red band; and (iv) a mixed zone of brown bands which remained near the top of the column. Fractions (i) and (iv) were not further investigated. Fraction (ii) gave, on removal of the benzene, almost pure purpurogenone. Crystallisation of this residue from chloroform gave pure purpurogenone (650 mg). Fraction (iii) gave, on removal of the solvent, a red solid (200 mg) which consisted of a mixture of purpurogenone and a second, newly characterised, red pigment, deoxypurpurogenone.[6]

General Properties of Purpurogenone (1; R = H).—*Purpurogenone* separates from chloroform as crimson prisms, m.p. *ca.* 300° (decomp.), $[\alpha]_D^{22}$ +254° (*c* 0·052 in dioxan) [Found (sample dried *in vacuo* over P_2O_5): C, 54·4; H, 3·4%; *M*, 544·102. $C_{29}H_{20}O_{11}$, $CHCl_3$ requires C, 54·3; H, 3·2%; $C_{29}H_{20}O_{11}$ requires *M*, 544·101], $\lambda_{max.}$ ($CHCl_3$) 253, 271sh, 308, 388, 499, 530, and 570 nm (log ε 4·43, 4·25, 4·01, 4·02, 3·78, 3·82, and 3·60). Crystallisation of the pigment from acetone gives crimson prisms, m.p. 310° (decomp.) which, even after intensive drying, always contain some of the solvent (Found: C, 63·6; H, 3·8. $C_{29}H_{20}O_{11}$,$\frac{1}{3}C_3H_6O$ requires C, 63·9; H, 3·9%).

Dihydropurpurogenone (5; R = H).—A solution of purpurogenone (100 mg) in glacial acetic acid (20 ml) was hydrogenated (Adams catalyst) at just over 1 atm. During the reduction the intense red colour gave way to yellow with the uptake of 2 mol. equiv. of hydrogen. On exposure to the atmosphere the solution rapidly became red again. The solution was filtered and the solvent was removed *in vacuo*. The dark red residue was washed with water and dried, and was then crystallised from chloroform to give dark red prisms of *dihydropurpurogenone* (90 mg.), m.p. >310°, $[\alpha]_D^{24}$ +299° (*c* 0·053 in dioxan) (Found: C, 63·9; H, 3·7%; *M*, 546·117. $C_{29}H_{22}O_{11}$ requires C, 63·7; H, 4·0%; *M*, 546·116), $\lambda_{max.}$ ($CHCl_3$) 279, 322sh, 338, 353sh, 499, 534, and 573 nm (log ε 3·98, 4·08, 4·09, 4·08, 3·72, 3·79, and 3·59).

Hexa-O-acetylpurpurogenone.—Two drops of a solution of aqueous 60% perchloric acid (1 drop) in acetic anhydride (1 ml) were added to a suspension of purpurogenone (100 mg) in acetic anhydride (3 ml) and the mixture was shaken for 10 min. The yellow solution was poured into water and the pale yellow precipitate was collected, washed and dried.

[6] J. C. Roberts and D. J. Thompson, following paper.

A benzene solution of this material was chromatographed on a magnesium sulphate column (10 × 2 cm). The pale yellow band was collected and, after removal of the solvent *in vacuo*, the residue was ' crystallised ' from ethanol to give pale yellow granules of the *acetyl derivative* (80 mg), m.p. 226°, $[\alpha]_D^{24}$ +106° (*c* 0·056 in dioxan) (Found: C, 61·6; H, 4·5. $C_{41}H_{32}O_{17}$ requires C, 61·8; H, 4·3%), $\lambda_{max.}$ (EtOH) 243, 280sh, 312sh, and 347sh nm (log ε 4·58, 4·13, 3·90, and 3·64), $\nu_{max.}$ 1778, 1760sh, 1702, 1664, and 1610 cm^{-1}.

Hepta-O-acetyldihydropurpurogenone.—This was prepared from dihydropurpurogenone (100 mg) by a method similar to that described in the preceding paragraph. ' Crystallisation ' from ethanol gave the *acetyl derivative* as pale yellow granules (85 mg), m.p. 238—240°, $[\alpha]_D^{24}$ +120° (*c* 0·064 in dioxan) (Found: C, 61·6; H, 4·3. $C_{43}H_{36}O_{18}$ requires C, 61·6; H, 4·3%), $\lambda_{max.}$ (EtOH) 222, 277infl, and 339sh nm (log ε 4·48, 4·25, and 3·70), $\nu_{max.}$ 1770, 1760sh, 1750sh, 1702, 1662, and 1618 cm^{-1}.

Mono-O-bromoacetylpurpurogenone (1; R = OC·CH_2Br).—Purpurogenone (100 mg) and bromoacetic acid (2 ml) were heated at 110° for 18 h. The cooled solution was poured into water and the brown precipitate was collected, washed, and dried. A benzene solution of this material was chromatographed on a column of magnesium sulphate (10 × 2 cm). Development with benzene produced a sharp red band which was eluted. Removal of the solvent gave a product which crystallised from n-bromobutane to give thin, red plates of the *bromoacetate* (60 mg), m.p. 271—274° (decomp.) (Found: C, 55·7; H, 3·3%; *M*, 664·023 and 666·024. $C_{31}H_{21}BrO_{12}$ requires C, 55·9; H, 3·2%; *M*, 664·022 and 666·021), $\lambda_{max.}$ ($CHCl_3$) 254, 273sh, 308, 385, 501, 531, and 563sh nm (log ε 4·47, 4·29, 4·10, 4·06, 3·90, 3·94, and 3·72), $\nu_{max.}$ 1752, 1724, 1684, 1624, and 1566 cm^{-1}.

Mono-O-acetylpurpurogenone (1; R = Ac).—Purpurogenone (100 mg), fused zinc chloride (200 mg), and glacial acetic acid (15 ml) were heated under reflux for 16 h. The red crystalline solid which separated on cooling was collected, washed with water, and recrystallised from glacial acetic acid to give dark red needles of the *monoacetate* (50 mg), m.p. *ca.* 300° (decomp.) (Found: C, 63·8; H, 3·9%; *M*, 586·1115. $C_{31}H_{22}O_{12}$ requires C, 63·5; H, 3·8%; *M*, 586·1111), $\lambda_{max.}$ ($CHCl_3$) 253, 292sh, 306sh, 385, 500, 522, and 567 nm (log ε 4·32, 4·23, 3·99, 3·89, 3·72, 3·78, and 3·58), $\nu_{max.}$ 1756, 1686, 1624, and 1570 cm^{-1}, τ ($CDCl_3$) −2·80 (1H, s, HO-4), −2·54 (1H, s, HO-1), −1·20 (1H, s, HO-9), 2·84 (1H, d, *J* 1 Hz, H-10), 2·94 (1H, d, *J* 1 Hz, H-12), 2·99 (1H, s, H-3), 4·20 (1H, d, *J* 4 Hz, H-13c), 4·27 (1H, s, HO-13b), 5·36 (1H, d, *J* 4 Hz, H-6), 5·64 (1H, s, H-16), 6·48 (1H, d, *J* 15 Hz, H-14), 7·35 (1H, d, *J* 15 Hz, H-14), 7·48 (3H, s Me-11), 7·61 (3H, s, Me-2), and 7·88 (3H, s, OAc).

Mono-O-formylpurpurogenone (1; R = OCH).—Purpurogenone (120 mg) and formic acid (95%; 20 ml) were heated under reflux for 18 h. The excess of formic acid was removed by evaporation *in vacuo* and the residue, having been washed with water and dried, was dissolved in benzene and chromatographed on a magnesium sulphate column (10 × 2 cm). The fast-running red band was collected, the solvent was removed, and the residue was crystallised from acetone to give scarlet-red prisms of the *monoformate* (55 mg), m.p. 280° (decomp.) (Found: C, 63·0; H, 3·9%; *M*, 572·096. $C_{30}H_{20}O_{12},\frac{1}{3}C_3H_6O$ requires C, 62·9; H, 3·8%; $C_{30}H_{20}O_{12}$ requires *M*, 572·097), $\lambda_{max.}$ ($CHCl_3$) 254, 275sh, 309, 384, 504, 532, and 563 nm (log ε 4·43, 4·24, 4·03, 3·99, 3·86, 3·89, and 3·68), $\nu_{max.}$ 1754, 1734, 1698, 1620, and 1578 cm^{-1}, τ ($CDCl_3$) −2·86 (1H, s, HO-4), −2·62br (1H, s, HO-1), −1·23br (1H, s, HO-9), 2·02 (1H, s, O·COH), 2·83 (1H, s, H-10), 2·96 (1H, s, H-12), 3·03 (1H, s, H-3), 4·10 (1H, d, *J* 4 Hz, H-13c), 4·20 (1H, s, HO-13b), 5·32 (1H, d, *J* 4 Hz, H-6), 5·62 (1H, s, H-16), 6·50 (1H, d, *J* 16 Hz, H-14), 7·36 (1H, d, *J* 16 Hz, H-14), 7·50 (3H, s, Me-11), 7·64 (3H, s, Me-2), and 7·78 (2H, s, Me_2CO).

Oxidative Degradation of Purpurogenone.—A solution of purpurogenone (500 mg) in a mixture of a 1% solution of sodium hydroxide (80 ml) and a 3% solution of hydrogen peroxide (60 ml) was kept at room temperature for 3 days, filtered, and extracted with ether. The aqueous layer was strongly acidified (conc. hydrochloric acid) and was continuously extracted with ether. Removal of the solvent yielded a brown gum (100 mg). A solution of this gum and dimethyl sulphate (1·2 ml) in acetone (25 ml) was heated under reflux with anhydrous potassium carbonate (1·5 g) for 20 h. The acetone was evaporated off *in vacuo* and the residue was heated under reflux for 1 h with 2N-sodium hydroxide (25 ml). The solution was washed with ether, strongly acidified, and then continuously extracted with ether. Removal of the solvent gave a solid which crystallised from chloroform–ethyl acetate to yield prisms (30 mg). This material was suspended in dry ether and treated with an excess of ethereal diazomethane. Removal of the solvent gave a crystalline solid which sublimed (80° and 0·1 mmHg) to yield prisms of dimethyl 3-methoxy-5-methylphthalate (26 mg), m.p. 84—86° (Found: C, 60·6; H, 5·8. Calc. for $C_{12}H_{14}O_5$: C, 60·5; H, 5·9%), identical (m.p., mixed m.p., and u.v. and i.r. spectra) with an authentic specimen (see later).

Degradation of Purpurogenone with 2N-Sodium Hydroxide.—A solution of purpurogenone (500 mg) in 2N-sodium hydroxide (20 ml) was heated on a steam-bath for 2 h. To the cooled solution was added phosphoric acid (*d* 1·75; 1 ml) and the brown precipitate was filtered off and washed with water. The combined filtrate and washings were distilled to minimum volume. After addition of water to the residue, distillation was again carried out. This operation was repeated several times. The combined distillates were neutralised with 0·1N-sodium hydroxide (≡0·98 mol. equiv. of a monobasic acid) and evaporated to small bulk (5 ml). The solution so obtained gave positive tests (including the chromotropic acid test [7]) for formic acid. The neutralised solution was evaporated to dryness and to the solid (30 mg) so obtained were added aniline hydrochloride (150 mg) and freshly distilled aniline (300 mg). The mixture was heated under reflux for 15 min. The solution, after the addition of 2N-hydrochloric acid (1 ml), was filtered, and the filtrate was kept at 0° overnight. The product (10 mg, needles, m.p. 248—253°) was identical (m.p., mixed m.p., and u.v. and i.r. spectra) with an authentic specimen [8] of *NN'*-diphenylformamidine hydrochloride.

The air-dried, brown precipitate (400 mg) mentioned above was dissolved in chloroform and applied to an acid-washed silica [9] column (40 × 5 cm). Elution with chloroform produced three orange bands. Bands (i) and (iii) were shown by t.l.c. to be complex mixtures from which no pure compounds were obtainable. The middle band was

[7] F. Feigl, ' Qualitative Analysis by Spot Tests,' Elsevier, New York, 7th English edn., 1966, p. 451.

[8] W. B. Whalley, *J. Chem. Soc.*, 1948, 1014.

[9] I. Singh, R. E. Moore, C. W. J. Chang, R. T. Ogata, and P. J. Scheuer, *Tetrahedron*, 1968, **24**, 2969.

collected and chromatographed [benzene–ethyl acetate–acetic acid (7·5 : 2·5 : 1 v/v)] on acid-washed silica [9] plates. From the sharp orange band was obtained a dark orange solid (20 mg), m.p. 248—250° (decomp.), believed to be *1,4,7-trihydroxy-2-methylanthraquinone-5-carboxylic acid* (6) (Found: *M*, 314·0420. $C_{16}H_{10}O_7$ requires *M*, 314·0425), $\lambda_{max.}$ ($CHCl_3$) 263, 473sh, 492, and 522sh nm (log ε 4·30, 3·83, 3·86, and 3·67), $\nu_{max.}$ 3300br, 1712, 1620, and 1575 cm^{-1}, τ ([$^2H_6$]dimethyl sulphoxide), −2·92 (1H, s, HO-4), −2·56 (1H, s, HO-1), −1·40br (1H, s, CO_2H), 2·42 (1H, d, *J* 2 Hz, H-6), 2·75 (1H, s, H-3), 2·92 (1H, d, *J* 2 Hz, H-8), 3·4br (1H, s, HO-7), and 7·76 (3H, s, Me-2).

Rugulosin (3).—This was obtained [10] from *Penicillium rugulosum* (Commonwealth Mycological Institute, Kew, Surrey, No. 40,041), and crystallised from acetone to give pale yellow prisms, m.p. 288° (decomp.), [lit.,[10] *ca.* 293° (decomp.)], $\lambda_{max.}$ ($CHCl_3$) 253, 280sh, and 396·5 nm (log ε 4·17, 4·15, and 4·25).

2,6,7-Trimethylnaphthazarin (4).—This was prepared from 2,3-dimethylhydroquinone [11] and citraconic anhydride by the method of Brockmann and Müller.[5] It crystallised from methanol as dark red needles, m.p. 165—167° (lit.,[5] m.p. 165°), $\lambda_{max.}$ ($CHCl_3$) 291, 489, 519, and 557 nm (log ε 3·97, 3·80, 3·87, and 3·68).

3-Methoxy-5-methylphthalic Acid (2; OMe for OH).—This was prepared by the method of Raistrick *et al.*[12]

Dimethyl 3-Methoxy-5-methylphthalate.—The foregoing acid (100 mg) was suspended in dry ether (20 ml) and treated with an excess of ethereal diazomethane. Removal of the solvent and sublimation of the residue at 80° and 0·1 mmHg gave the ester as prisms (95 mg), m.p. 84—86° (lit.,[13] 86°).

Biosynthesis of Purpurogenone.—*Penicillium purpurogenum* Stoll (C.B.S., 257·37) was grown (surface culture) in fourteen conical flasks (each 250 ml), each containing Raulin–Thom medium [2] (100 ml). After 5 days at 28° a solution of sodium [2-^{14}C]acetate (25 μCi) was distributed evenly among the flasks. After a further 5 days growth, the mycelium was collected, washed, dried, powdered, and extracted as previously described.[2] A benzene solution of the crude pigment (1·4 g) was chromatographed on a magnesium sulphate column (20 × 4 cm). Development and elution with the same solvent led to two distinct red bands, which were separately collected. The solvent was removed from the faster-running band and the residual purpurogenone was crystallised to constant activity (from chloroform). This purpurogenone chloroform solvate (50 mg; $2·39 \times 10^6$ decomp. min^{-1} $mmol^{-1}$; 0·33% incorporation) was diluted with inactive purpurogenone solvate (500 mg) and crystallised to constant activity (496 mg; $2·07 \times 10^5$ decomp. min^{-1} $mmol^{-1}$).

Degradation of Labelled Purpurogenone.—The labelled material (495 mg) was degraded with alkaline hydrogen peroxide, as described previously, to give 3-methoxy-5-methylphthalic acid. This acid was sublimed (140° and 0·01 mmHg) and the sublimate resublimed to give needles of 3-methoxy-5-methylphthalic anhydride (10 mg), m.p. 166—168° (lit.,[12] 168°) ($6·8 \times 10^4$ decomp. min^{-1} $mmol^{-1}$; 32·8% of activity of purpurogenone).

Degradation of 3-Methoxy-5-methylphthalic Anhydride.—The anhydride (7·6 mg) was oxidised by the modified Kuhn–Roth procedure.[14] The acetic acid produced required 3·84 ml of 0·01N-sodium hydroxide (≡0·97 mol. equiv. of acetic acid) for neutralisation. The sodium acetate solution was evaporated to dryness and the residue was treated with 0·1N-hydrochloric acid (2 drops) and counted in the usual way ($1·37 \times 10^4$ decomp. min^{-1} $mmol^{-1}$; 6·6% of activity of purpurogenone).

Radioactive Assay.—Compounds were counted, *via* liquid scintillation spectrometry [Nuclear Enterprises Spectrometer (type NE 8310)] by use of a dioxan-based scintillator (NE 250). Efficiencies were calculated by means of [^{14}C]-toluene internal standards. Since purpurogenone is intensely coloured, the solution for assay was prepared as follows. Purpurogenone (1 mg) was treated with a solution (0·5 ml) made from hydrogen peroxide (30%; 2 ml), aqueous sodium hydroxide (10%; 1 ml) and dioxan (7 ml); the reaction mixture was left at room temperature for 12 h and then diluted with dioxan-based scintillator (9·5 ml).

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